Global Veterinaria 5 (5): 259-263, 2010
ISSN 1992-6197

Histamine and Heavy Metals Content of Canned Tuna Fish

[1]*Mehdi Zarei,* [2]*Alireza Mollaie,* [3]*Mohammad Hadi Eskandari,*
[3]*Sara Pakfetrat and* [4]*Shahram Shekarforoush*

[1]Department of Food Hygiene, Faculty of Veterinary Medicine,
Shahid Chamran University of Ahvaz, Ahvaz 61355-145, Iran
[2]Department of Veterinary Science, Islamic Azad University, Beyza Branch, Fars Province, Iran
[3]Department of Food Science and Technology, College of Agriculture, Shiraz University, Shiraz, Iran
[4]Department of Food Hygiene, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran

Abstract: Tuna is able to concentrate large amount of heavy metals and contain high levels of free histidine in their muscles which can convert to histamine. In this study, the levels of histamine and six heavy metals in canned tuna fish samples were determined. The histamine content of the samples was observed to be in the range of 4-236 mg/kg and 18.9 % of the samples contained more than 50 mg/kg, the allowable limit suggested by US FDA. The contents of investigated heavy metals were found to be in the range of 0.007-0.51 µg/g for lead, 0.002-0.07 µg/g for cadmium, 0.023-13.108 µg/g for tin, 0.17-8.001 µg/g for copper, 0.124-27.001 µg/g for zinc and 0.009-14.207 µg/g for iron. The results of this study indicate that the average content of histamine in canned tuna fish marketed in Iran was lower than the allowable limit suggested by FDA. Results achieved for heavy metals were in good agreement with other reported data from the literature.

Key words: Canned fish · Heavy metals · Histamine · Tuna

INTRODUCTION

Canned tuna fish are frequently and largely produced and eaten 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. Tuna, as a predator, is able to concentrate large amount of heavy metals. Some of them are used for biomonitoring of environmental contamination [1, 2]. Furthermore, tuna contain high levels of free histidine in their muscles which can convert to histamine through the proliferation of bacteria that synthesize histidine decarboxylase [3]. Therefore their chemical and toxic hazards should be of some concern to human health.

Histamine is identified by the Food and Drug Administration (FDA) as a major chemical hazard of seafood products. It is the causative agent of scombroid poisoning and is formed by time/temperature abuse of fish muscle [4]. Quality loss and histamine accumulation 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 [5]. The FDA has established the 50 mg/kg guideline for histamine and intensified its inspection efforts. Seafood products containing above this level of histamine may not be used for human consumption and are subjected to recalls [6].

There has been growing interest in determining heavy metal levels in the marine environment and attention was drawn to the measurement of contamination levels in food supplies, particularly fish [7-10]. Heavy metals can be classified as potentially toxic (aluminium, arsenic, cadmium, lead, mercury), probably essential (nickel, vanadium, cobalt) and essential (copper, zinc, selenium) [11]. Toxic elements can be very harmful, even at low concentration, when ingested over a long time period.

Levels of heavy metals in fish and canned fish samples have been widely reported in the literature [7, 12-15]. Histamine content of canned fish samples have been also documented previously [5, 10, 16]. Nevertheless, limited information on histamine and heavy

Corresponding Author: Mehdi Zarei, Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz 61355-145, Iran.
Tel.: +98-917-302-1142, Fax: +98-611-336-0807, E-mail: zarei@scu.ac.ir.

metal levels of canned tuna fish marketed in Iran has been reported. In this study, the levels of lead, cadmium, copper, zinc, tin and iron as well as the histamine content of canned tuna fish are 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 histamine and heavy metals levels.

Histamine Analysis

Apparatus: A Synergy HT Multimode Microplate Reader (BioTek Instruments) equipped with Gen 5 software was used to determine the fluorescence intensity at excitation wavelength of 350 nm and emission wavelength of 444 nm.

Reagents: All the glassware was washed with 0.1 N HCl and then rinsed with deionized water before use. All reagents used were of analytical reagent grade. Dowex 1-X8 resin, o-phthaldialdehyde and histamine dihydrochloride were purchased from Sigma (Sigma, St. Louis, MO). The standard stock solutions used for calibration were produced by dissolving 169.1 mg histamine dihydrochloride in 100 ml 0.1N HCl. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solution using 0.1 N HCl.

Sample Preparation and Histamine Extraction: Samples, 10 g, were homogenized in 50 ml 75% (v/v) methanol and incubated at 60°C for 15 min. The homogenates were filtered through Whatman No. 42 filter paper. The filtrates were then placed in volumetric flasks and 75% (v/v) methanol was added to a final volume of 100 ml. One ml of this methanol extract was subjected to ion exchange chromatography on an 80×5 mm column of Dowex 1-X8 resin, which was converted to hydroxide form by 2N NaOH. The column was washed with 35 ml of deionized water. The eluate was collected in a 50 ml volumetric flask containing 5 ml 1N HCl and the volume was adjusted to 50 ml with deionized water [17].

Preparation of the Fluorescent Histamine Derivative: Histamine standards (0.1, 0.2 and 0.3 μg/ml) and the column eluates were derivatized with o-phthaldialdehyde. Five ml of the column eluates or the standards and 10 ml of 0.1 N HCl were added into a 50 ml flask, followed by 3 ml of 1N NaOH and 1 ml of 0.1% (w/v) OPT solution, consecutively. The mixture was shaken thoroughly and after exactly 4 min, 3 ml of 3.57N H_3PO_4 was added and mixed immediately. Blank was prepared by substituting 5 ml 0.1N HCl for histamine solution. Fluorescence intensity was measured within 1.5 hours [17].

Heavy Metals Analysis

Apparatus: A Prospector GBC 932 AA atomic absorption spectrophotometer equipped with a deuterium background corrector was used for the determination of heavy metals. The operating parameters for working elements were set as recommended by the manufacturer. Lead and cadmium concentrations were determined by a graphite furnace atomic absorption spectrophotometer. Tin was determined by direct aspiration of the sample solution into the NO_2-acetylene flame and the other elements were determined in an air-acetylene flame. The blanks and calibration standard solutions were also analyzed in the same way as the sample solutions.

Reagents: All the plastic and glassware was cleaned by soaking in a 10% (v/v) nitric acid solution for 15 min and then rinsed with deionized water before use. All reagents used were of analytical reagent grade. The standard stock solutions used for calibration were produced by diluting a stock solution of 1000 mg/l of the given element supplied by Sigma-Aldrich. The working solutions were freshly prepared by diluting an appropriate aliquot of the stock solutions using 10% HNO_3.

Sample Preparation and Digestion: A sample (5 g) was placed in a porcelain crucible. The samples were ashed in a furnace for about 4 h at 550°C until a white or grey ash residue was obtained. The residue was dissolved in 10 ml of HNO_3 (10% v/v) and the mixture, where necessary, was heated slowly to dissolve the residue and then filtered through Whatman No. 42 filter paper. The solution was transferred into a 50 ml volumetric flask and made up to volume [18]. A blank digest was carried out in the same way. All metals were determined against aqueous standards.

Determination of Recovery: The recoveries of the histamine and metals were determined by spiking the samples with various concentrations of histamine or heavy metals which were then taken through the extraction or digestion procedure. These spiked samples were analyzed for histamine content or the metal concentrations in the same way as the sample solutions.

Table 1: Concentrations of histamine (mg/kg) and heavy metals (µg/g) in canned tuna samples. Data represent the mean of three replicates

	Minimum	Maximum	Average
Histamine	4	236	42.85
Lead	0.007	0.510	0.0746
Cadmium	0.002	0.070	0.0246
Tin	0.023	13.108	1.2286
Copper	0.017	8.001	2.2996
Zinc	0.124	27.001	7.3127
Iron	0.009	14.207	6.6601

RESULTS AND DISCUSSION

Samples of canned tuna fish were analyzed for lead, cadmium, copper, zinc, tin, iron and also for histamine content. The recoveries of spiked samples were in the range of 96-102 % for histamine, 93-101 % for lead, 94-99 % for cadmium, 97- 101 % for iron, 96-103 % for tin, 95- 101 % for copper and 98-102 % for zinc. These recovery values demonstrate the accuracy of the methods used.

The histamine content of the samples was found to be in the range of 4-236 mg/kg (Table 1). 18.9 % of the canned tuna samples contained more than 50 mg/kg, the allowable limit suggested by FDA. 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* [16, 19, 20]. 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.* [5], 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.

According to Tsai *et al.* [21], 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 [22]. Lopez-Sabater *et al*. [5] 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. Similar results were reported by Fernandez-Salguero and Mackie [23].

In the present study, 21.6 % of the samples contained less than 10 mg/kg histamine, but Taylor *et al.* [24] observed that only 4% of canned mackerel and tuna had histamine lower than 10 mg/kg in their study. While, Ababouch *et al*. [25] noted that 7% of canned tuna contained histamine at levels greater than 500 mg/kg, the highest level of histamine in our samples was 236 mg/kg. Histamine levels higher than 2000 mg/kg have been reported in canned sardine, mackerel and tuna [16].

The levels of investigated heavy metals are presented in table 1. All the metal concentrations were determined on a dry weight basis. Lead, cadmium, iron and tin were not detected in 10.8, 45.9, 2.7 and 5.4 % of the samples, respectively. According to our results, zinc has the highest concentration, followed by iron, copper and tin. The concentration of lead in our study varied from 0.007-0.51 µg/g. In another report from Iran, it was in the range of 0.016-0.073 µg/g [7]. Solder, used in the manufacture of cans, is a recognized source of contamination of food by lead during canning [26]. In the literature lead contents have been reported in the range of 0.0-0.03 µg/g in canned fish samples [13], 0.09-6.95 µg/g dry weight in fish species from Iskenderun Bay, northern east Mediterranean Sea, Turkey [14], 0.22-0.85 µg/g dry weight in fish samples of the middle Black Sea (Turkey) [27], 0.18-0.40 µg/g in canned tuna fish [26] and 0.076-0.314 µg/g in Turkish canned fish samples [28]. In the present study cadmium was detected in 54.1 % of the samples and it was in the range of 0.002-0.07 µg/g. It was reported previously that the canned tuna in Iran contained 0.005-0.072 µg/g cadmium [7]. Cadmium contents, in the literature, have been reported in the range 0.0-0.05 µg/g in canned fish samples [13], 0.01-4.16 µg/g dry weight in fish species from Iskenderun Bay, northern east Mediterranean Sea, Turkey [14], 0.09-0.48 µg/g dry weight in fish samples of the middle Black Sea (Turkey) [27], 0.09-0.32 µg/g in canned tuna fish [26] and 0.025-0.494 µg/g in Turkish canned fish samples [28].

While, tin was not observed in canned tuna samples by Emami Khansari *et al.* [7], this element was detected in the range of 0.023-13.108 µg/g in our samples. Tin is widely used for studding in canning and its toxicity is not as imperative as other heavy metals. Although the advances of new packaging technology, especially the use of cans with lacquered walls and mechanical seam, reduce or, in most cases, eliminate the leaching of heavy metals (lead and tin) into the food, high levels of tin in a few samples may be due to the leaching during long time storage. In this study, copper, zinc and iron were detected in all samples in the range of 0.17-8.001 µg/g, 0.124-27.001 µg/g and 0.009-14.207 µg/g, respectively. No comparative data for these elements was found in the literature from Iran. The lowest and highest copper levels in Turkish canned fish samples have been reported in the range 7.1-45.7 µg/g [28]. Furthermore, the level of this element in canned fish samples have been reported in the range of 0.01-5.33 µg/g by Ikem and Egiebor [13]. They also reported the zinc and iron content of canned fish samples in the range of 0.14-97.8 µg/g and 0.01-88.4 µg/g, respectively. According to Celik and Oehlenschlager [28], the lowest and highest zinc levels in Turkish canned fish samples were in the range 33.8-566 µg/g. Iron content in fish species from Iskenderun Bay, northern east Mediterranean Sea, Turkey, have been reported in the range of 0.82-27.35 µg/g dry weight [14]. The level of this element in fish samples of the middle Black Sea, Turkey, was found to be in the range of 9.52-32.40 µg/g [27].

In conclusion, believe that the results of the present study can provide a significant contribution to both regulatory agencies and the canning industries. Quality control programs in the tuna-canning industries need to remove poor quality and contaminated fish and improve the chemical quality of canned tuna fish. In addition, canned tuna samples should be analyzed more often in Iranian supermarkets with respect to toxic elements.

ACKNOWLEDGEMENTS

This study was supported by the Islamic Azad University of Beyza, Fars Province and the research grants provided by Shahid Chamran University of Ahvaz and University of Shiraz.

REFERENCES

1. Park, J.G. and L.R. Curtis, 1997. Mercury distribution in sediments and bioaccumulation by fish in two oregon reservoirs: point-source and nonpoint-source impacted systems. Archives of Environ. Contamination and Toxicol., 33: 423-429.
2. Schmitt, C.J. and W.G. Brumbaugh, 1990. National contamination biomonitoring program: Concentrations of arsenic, cadmium, copper, lead, mercury, selenium and zinc in US fresh water fish, 1976-1984. Archives of Environ. Contamination and Toxicol., 19: 731-747.
3. Rawles, D.D., G.J. Flick and R.E. Martin, 1996. Biogenic amines in fish and shellfish. Advances in Food and Nutrition Research, 39: 329-364.
4. Food and Drug Administration, 1998. Scombrotoxin (histamine) formation. In Fish and Fishery Products Hazards and Controls Guide, (2nd Ed.). Washington, D.C.: Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, pp: 73-90.
5. Lopez-Sabater, E.I., J.J. Rodriguez-Jerez, A.X. Roig-Sagues and M.A.T. Mora-Ventura, 1994. Bacteriological quality of tuna fish (Thunnus thynnus) destined for canning: Effect of tuna handling on presence of histidine decarboxylase bacteria and histamine level. Journal of Food Protection, 57: 318-323.
6. Food and Drug Administration, 2002. FDA's evaluation of the seafood HACCP program for fiscal years 2000/2001. Washington, D.C.: Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.
7. Emami Khansari, F., M. Ghazi-Khansari and M. Abdollahi, 2005. Heavy metals content of canned tuna fish. Food Chemistry, 93: 293-296.
8. Kalay, M., O. Aly and M. Canli, 1999. Heavy metal concentrations in fish tissues from the northeast mediterranean sea. Archives of Environ. Contamination and Toxicol., 63: 673-681.
9. Rose, J., M.S. Hutcheson, C.R. West and O. Pancorbo, 1999. Fish mercury distribution in Massachusetts, USA Lakes. Environ. Toxicol. Chem., 18: 1370-1379.
10. Tariq, J., M. Jaffar and M. Ashraf, 1991. Trace metal concentration, distribution and correlation in water, sediments and fish from the Ravi river, Pakistan. Fisheries Research, 19: 131-139.
11. Munoz-Olivas, R. and C. Camara, 2001. Speciation related to human health. In Trace element speciation for environment, food and health, Eds., L. Ebdon, L. Pitts, R. Cornelis, H. Crews, O.F.X. Donard and P. Quevauviller. The Royal Society of Chemistry, pp: 331-353.
12. Celik, U. and J. Oehlenschlager, 2004. Determination of zinc and copper in fish samples collected from Northeast Atlantic Bay DPSAV. Food Chemistry, 87: 343-347.

13. Ikem, A. and N.O. Egiebor, 2005. Assessment of trace elements in canned fishes (Mackerel, Tuna, Salmon, Sardines and Herrings) marketed in Georgia and Alabama (United States of America). Journal of Food Composition and Analysis, 18: 771-787.
14. Turkmen, A., M. Turkmen, Y. Tepe and I. Akyurt, 2005. Heavy metals in three commercially valuable fish species from Iskenderun Bay, Northern East Mediterranean Sea, Turkey. Food Chemistry, 91(1): 167-172.
15. Yilmaz, F., N. Ozdemir, A. Demirak and A.L. Tuna, 2007. Heavy metal levels in two fish species Leuciscus cephalus and Lepomis gibbosus. Food Chemistry, 100: 830-835.
16. Lopez-Sabater, E.I., J.J. Rodriguez-Jerez, M. Hernandez-Herrero, A.X. Roig-Sagues and M.A.T. Mora-Ventura, 1996. Sensory quality and histamine formation during controlled decomposition of tuna (Thunnus thynnus). Journal of Food Protection, 59: 167-174.
17. AOAC, 2000. Histamine in seafood: Fluorometric method. Method 35.1.32, Method 977.13. In Official Methods of Analysis of AOAC International, 17th ed. PA Cunniff, Gaithersburg, MD, USA: Association of Official Analytical Chemists (AOAC) International, pp: 17-19.
18. Vaidya, O.C. and R.T.T. Rantala, 1996. A comparative study of analytical methods: determination of heavy metals in mussels (Mytilus edulis) from Eastern Canada. Intl. J. Environ. Analytical Chem., 63: 179-185.
19. Kim, S.H., K.G. Field, D.S. Chang, C.I. Wei and H. An, 2001. Identification of bacteria crucial to histamine accumulation in Pacific mackerel during storage. Journal of Food Protection, 64: 1556-1564.
20. Lehane, L. and J. Olley, 2000. Histamine fish poisoning revisited. Intl. J. Food Microbiol., 58: 1-37.
21. Tsai, Y.H., H.F. Kung, T.M. Lee, H.C. Chen, S.S. Chou, C.I. Wei and D.F. Hwang, 2005. Determination of histamine in canned mackerel implicated in a food borne poisoning. Food Control, 16: 579-585.
22. Yen, G.C. and C.L. Hsieh, 1991. Simultaneous analysis of biogenic amines in canned fish by HPLC. J. Food Sci., 56: 158-160.
23. Fernandez-Salguero, E. and I.M. Mackie, 1987. Technical note: Preliminary survey of the content of histamine and other higher amines in some samples of Spanish canned fish. Intl. J. Food Sci. Technol., 22: 409-412.
24. Taylor, S.L., E.R. Lieber and M. Leatherwood, 1978. A simplified method for histamine analysis in food. J. Food Sci., 43: 247-250.
25. Ababouch, L., M.M. Alaoui and F.F. Busta, 1986. Histamine levels in commercially processed fish in Morocco. Journal of Food Protection, 49: 904-908.
26. Voegborlo, R.B., A.M. El-Methnani and M.Z. Abedin, 1999. Mercury, cadmium and lead content of canned tuna fish. Food Chemistry, 67: 341-345.
27. Tuzen, M., 2003. Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. Food Chemistry, 80: 119-123.
28. Celik, U. and J. Oehlenschlager, 2007. High contents of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets. Food Control, 18(3): 258-261.