American-Eurasian Journal of Toxicological Sciences 4 (2): 64-66, 2012
ISSN 2079-2050

DOI: 10.5829/idosi.aejts.2012.4.2.56188

Determination of Histamine in Canned Tuna Fish Using ELISA Method

[1]Ebrahim Rahimi, [2]Farnaz Nayebpour and [2]Forough Alian

[1]Department of Food Science and Technology, Faulty of Agriculture,
Islamic Azad University-Shahrkord Branch, Shahrkord, Iran
[2]Member of Young Researches Club,
Islamic Azad University-Shahrkord Branch, Shahrkord, Iran

Abstract: Histamine is a simple chemical substance created during processing of the amine acid histamine. Histamine is also an agent in inflammation and increased presence of histamine causes allergic reaction. Histamine may play a role in the increased prevalence of food in tolerances. The objective of this study was to determine of histamine contents, 43 samples of canned tuna fish produced from 5 manufactures in Iran, Analyzed by ELISA (REDASCREEN® Histamine). In 30 of 43 canned tuna fish samples (69.8%), the presence of histamine detected in concentration between 17 to 210 mg/100g. Histamine in 8 canned tuna fish samples (18.6%) from two manufactories were higher than the tolerance limit of histamine contents (50mg Histamine/100g) accepted by European countries. The values were comparable and in the range of with the literature values. The results of this study indicate that tuna fish produced and marketed in Iran have concentration bellow histamine (<50mg Histamine/100g). Further studies should be carried out to investigate the presence of these toxin in different fish and other sea food products.

Key words: Histamine · Canned Tuna Fish · ELISA · Iran

INTRODUCTION

Histamine (or scombroid) fish poisoning (HFP) is a food borne chemical intoxication caused by eating a spoiled, or bacterially contaminated fish. The fish are harmless when fresh and after they have become toxic they may still have a normal appearance and odour. No available method of preparation, including freezing, canning and smoking, will destroy the causative toxin (s) [1]. The disease is not uncomplicated histamine poisoning, but is generally associated with high levels of histamine (50 mg/100 g) in the spoiled fish [2, 3]. Symptoms usually subside spontaneously within a few hours. The most commonly encountered symptoms are tingling and burning sensations around the mouth, gastrointestinal complaint and a rash with itching [4]. The presence of histamine in foodstuffs can be used as an indicator for food hygiene, particularly for fish products.

Nausea, vomiting, headache and other symptoms are induced by consumption (ingestion) of foods containing high levels of histamine. The same symptoms can occur also after the consumption of red wine, especially in individuals with naturally reduced ability of histamine decomposition [5, 6]. Consequently, human histamine poisoning is mostly and rightly, associated with canned fish containing high levels of this amine.

Currently, there is limited information regarding the contaminant levels of histamin in canned tuna fish produced in Iran. The objective of this study was to determine histamine contents of 43 canned tuna fish samples produced from 5 manufactures in Iran, analyzed by ELISA.

MATERIAL AND METHODS

Collection of Samples: From September to November 2008 a total of 43 canned tuna fish samples were randomly selected from Isfahan, Iran. The samples were immediately transported to the laboratory in a cooler with ice packs and stored at -20°C until analysis.

Method of Analysis: To measure histamine in canned tuna fish samples, a competitive ELISA was employed using RIDASCREEN® histamine kit (R-Bipharm AG, Germany).

Corresponding Author: E. Rahimi, Department of Food Science and Technology,
Faulty of Agriculture, Islamic Azad University-Shahrkord Branch, Shahrkord-Iran.
Tel: +98 381 3361060, Fax: +98 311 6259809.

The assay was performed according to the manufacturer's recommendation. A limit of detection of this test for fresh fish and canned fish is 2.5 ppm. After the sample preparation, histamine was quantitatively converted to N-acylhistamine, 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 is ten 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 colour 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 (Stat Fax 2000, England). The amount of complexes bound to the plate and the optical density are inversely proportional to the histamine concentration of the sample.

Statistical Analysis: All statistical analyses were performed using SPSS software, version 16 (SPSS Chicago, IL, USA) and the data were expressed as mean±standard deviation (SD). Chi-square test and fisher's exact two-tailed test analysis were performed and differences were considered significant at values of $P < 0.05$.

RESULTS AND DESCUSSION

Histamine levels in canned tuna fish produced of different brands obtained from Isfahan retail markets and shown in Table 1. In 30 of 43 canned tuna fish samples (69.8%), the presence of histamine detected in concentration between 17 to 210 mg/100g. Histamine in 8 canned tuna fish samples (18.6%) from two manufactories were higher than the tolerance limit of histamine contents (50mg Histamine/100g) accepted by European countries. The values were comparable and in the range of the literature values. High levels of histamine of commercial fish in Asian countries have been reported in various FAO Fisheries reports [7-12]. There are also reports of extremely high levels of histamine in some salted, dried and fermented products in Asia (Mitchell, 1993). A survey of New Zealand smoked fish found 8/107 samples had histamine levels in excess of 5 mg/100g and of these eight only two exceeded 20 mg/100g. Another survey identified four of 91 samples containing >10 mg/100g histamine and two of the samples (smoked trevally and smoked warehou) contained 100 mg/100g or more [9]. In a study in Iran by Kamkar *et al.* reported that histamine levels in Iranian canned tuna and sardine samples ranged from 10 to 178 mg/kg for canned tuna fish and 5 to 47 mg/kg for canned sardins samples [7].

Table 1: Histamine levels in canned tuna fish produced of different brands obtained from Isfahan retail markets

No. Samples	Positive samples	Range of Histamine(mg /100gr)
43	30 (69.8%)	17-210

Nevertheless, histamine levels higher than 2000 ppm have been reported in canned sardine, mackerel and tuna [13]. Taylor *et al.* observed that only 4% of canned mackerel and tuna had histamine lower than 10 ppm in their study [14]. Ababouch *et al.* noted that 7% of canned tuna contained histamine at levels greater than 500 ppm as compared to 3.7% and 3.2% for sardine and mackerel, respectively [15]. In this study, the histamine content in canned fish products from Iran was lower than those of the above mentioned scombroid fish. The difference in histamine content in these canned fish products can be attributed to the fish species, the storage temperature of the fish and the hygienic condition of the environment for fish processing and handling.

The situation regarding a toxic dose is unclear (not least because the chemical(s) responsible is not known). Approximately 100 mg/100g histamine is considered to be toxic, but a number of incidents have involved foods containing less than 5 mg/100g histamine. A limit commonly used is 30 mg/100g, although the FDA have a limit of 50mg/100g. Another scheme states that <5mg/100g is safe to eat, 5-20mg/100g is possibly toxic, 20-100 mg/100g is probably toxic and >100 mg/100g is toxic and unsafe for human consumption [16].

Histamine production is well known to be 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, Proteus vulgaris, Klebsiella pneumoniae, Hafnia alvei, Enterobacter aerogenes, Citrobacter freundii, Serratia* spp. and *Escherichia coli* [13, 17, 18]. 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. Lopez-Sabater *et al.* demonstrated that an increase in bacterial population occurs while the frozen tuna are being defrosted and handled for processing [13]. It is recommended as follows: In general, fish should be put on

ice, in cooled seawater or brine at the temperature of 4.4°C or lower for 12 hours after death or at 10°C or lower for 9 hours after death; and Fish exposed to air or water temperature exceeding 28.3°C, should be put on ice, in cooled sea water or brine at the temperature of 4.4°C or lower for 6 hours after death [19].

Permanent control of the histamine presence in food rich in proteins and in wine should be introduced, because of the possibility of histamine development in such foodstuffs, detrimental to human health. Since the “screening" method for quantitative determination of histamine is easy to perform, the control of histamine presence should be legally regulated for the protection of human health.

RERENSSES

1. Etkind, P., M.E. Wilson, K. Gallagher and J. Cournoyer, 1987. Bluefish-associated scombroid poisoning. An example of the expanding spectrum of food poisoning from seafood. J. the American Medical Association, 258: 3409-3410.
2. Lehane, L., 2000. Ciguatera update. Med. J. Aust., 172: 176-179.
3. Leigh, L. and O. June, 2000. Histamine fish poisoning revisited. International J. Food Microbiol., 58: 1-37.
4. Mahendradatta, M., 2003. The change of histamine content in some fish-based foods during storage. Indonesian Food and Nutrition Progress, 10: 54-61.
5. Smajlović, A., A. Baković, I. Mujezinović, M. Muminović, M. Smajlović, O. Kapetanović and S. Had_ijusufović, 2008. Identification of histamine content in fish samples. Svibanj-Lipanj Br., 3: 235-238.
6. Mitchell, J.W., 1993. Scombrotoxic fish poisoning. A report prepared for the Ministry of Health. Institute of environment Health and Forensic Sciences Limited, Mt. Albert science Centre, NZ, pp: 18.
7. Kamkar, A., H. Hosseini and G. Abuhossein, 2003. A study of histamine contents of canned tuna and sardine of Iran. Pajouhesh and Sazandegi, 60: 44-50.
8. Fletcher, G.C., G. Summers, P. Veghel, R.V. Wincester and R. Wony, 1995. Histamine and histidine in Newzealand marine fish and shellfish species, particulary kahawai (Arripis trutta). J. Aquatic Food Product Technol., 4: 53-74.
9. Fletcher, G.C., G. Summers, R.V. Wincester and R. Rwony, 1998. Levels of histamine and histamine producing bacteria in Smoked fish from New Zealand markets. J. Food Protection, 6: 1064-1070.
10. Laurent, G., M. Bennasar, F. Fall and H. Lima, 1995. Histamine content in fresh and canned tara. Médecine and Nutrition, 31: 23-33.
11. Soares, V.F.M. and M.B.A. Gloria, 1994. Histamine levels in canned fish available in Belo Horizonte, Minas Gerais, Barazil. Journal of Food Composition and Analysis, 7: 102-109.
12. Veresbaranji, A., D. Kelemen and R. Curcic, 1997. Fluorimetric determination of histamine amounts of canned fish. Tehnologija Mesa, 38: 216-217.
13. 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). J. Food Protection, 59: 167-174.
14. Taylor, S.L., E.R. Lieber and M. Leatherwood, 1978. A simplified method for histamine analysis in food. J. Food Sci., 43: 246-250.
15. Ababouch, L., M.M. Alaoui and F.F. Busta, 1986. Histamine levels in commercially processed fish in Morocco. J. Food Protection, 49: 904-908.
16. Lehane, L. and J. Olley, 2000. Histamine fish poisoning revisited. International J. Food Microbiol., 58: 1-37.
17. 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. J. Food Protection, 64: 1556-1564.
18. Cemek, M., L. Akkaya, S. Bulut, M. Konuk and Y.O. Birdane, 2006. Histamine, Nitrate and Nitrite Content of Meat Products Marketed in Western Anatolia, Turkey. J. Animal and Veterinary Advances, 5: 1109-1112.
19. Food and Drug Administration, 2001. Scombrotoxin (histamine) formation. In fish and fisheries products hazards and controls guidance pp: 83-102. Washington, DC: Center for Food Safety and applied Nutrition, Office of Seafood, Food and Drug Administration.