

Determination of histamine in fresh and smoked fish commercially available in Poland

Marzena Pawul-Gruba, Mirosław Michalski, Jacek Osek

Department of Hygiene of Food of Animal Origin
National Veterinary Research Institute, 24-100 Pulawy, Poland
mmichal@piwet.pulawy.pl

Received: October 21, 2013 Accepted: June 6, 2014

Abstract

The high performance liquid chromatography with diode array detection was used for the study. The histamine was detected in 14.6% and 17.8% of the samples of fresh and smoked fish respectively. The highest concentrations of the compound were found in smoked herring and smoked sprat (17.7 mg/kg and 24.1 mg/kg respectively). Histamine concentration in fresh and smoked fish did not exceed the allowable limit, indicating that they are safe for consumers.

Key words: fresh fish, smoked fish, histamine, HPLC-DAD.

Introduction

Recent trends in food safety are promoting an increasing search for compounds that can affect negatively human health. Biogenic amines (BA), including histamine belong to the group of these substances. (4, 11). This heterocyclic amine naturally occurs in many species of fish with dark meat, especially of the families: *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryfenidae*, and *Pomatomidae* (5). Histamine formation in fish is related to the free histidine content of the fish muscle, the presence of bacterial histidine decarboxylases and certain environmental conditions. The production of histamine is largely induced by post-harvest exposure of fish to high temperatures that enhance the multiplication of histidine decarboxylase producing bacteria (6, 12, 15).

Histamine high concentration in fish and fish products may cause food poisoning in humans. Scombrototoxic fish poisoning (SFP) is associated with the consumption of contaminated fish of the *Scombridae* family, for example: tuna, mackerel, herring. This intoxication causes cardiovascular, gastrointestinal, and neurological symptoms, such as skin rashes, urticaria, oedema, local inflammation, nausea, vomiting, diarrhoea, cramping, hypotension, headache, palpitation, and oral burning. The symptoms usually resolve within 24 h (1, 9, 14, 16). During the food intake process in the human gut, low amounts of

histamine are metabolised to a physiologically less active degradation product. However, upon intake of a great amount of histamine with food, the detoxification system is unable to eliminate histamine sufficiently (2, 10, 11).

On November 15, 2005, the European Union established Commission Regulation No. 2073/2005 on the microbiological criteria for foodstuffs (5). The histamine concentration in fishery products from the fish species associated with the high amount of histamine is considered satisfactory if among nine samples tested, the observed concentrations are ≤ 100 mg/kg, a maximum of two samples have a concentration between 100 and 200 mg/kg and in none sample histamine exceeds the limit of 200 mg/kg (5).

The study aimed at determination of the concentration of histamine in fresh and smoked fish available for human consumption in Poland using the high performance liquid chromatography with diode array detection (HPLC-DAD). This assay is the reference method in accordance with Commission Regulation No. 2073/2005 (5).

Material and Methods

Samples. A total of 93 samples of fresh and smoked fish were collected during two years (2012–2013) from various local shops in Pulawy, from south-

eastern region of Poland. Samples of fish were investigated during four seasons of each year. The specimens included 48 fresh fish and 45 smoked fish, such as mackerel, herring, sprat, cod, salmon, trout, flounder, and bream (Table 1). All fishes were processed by hot smoking. After purchasing, the samples were immediately delivered to the laboratory at low temperature. They were in the shelf-life time. Before preparing the samples for analysis, skin and bones were removed.

Table 1. Samples used for histamine detection

Species of fish	Number of samples		Total number of samples
	Fresh fish	Smoked fish	
Mackerel (<i>Scomber scombrus</i>)	5	10	15
Herring (<i>Clupea harengus</i> membrans)	15	10	25
Sprat (<i>Sprattus sprattus</i>)	NT	5	5
Cod (<i>Gadus morhua</i>)	5	6	11
Salmon (<i>Salmo salar</i>)	3	7	10
Brown trout (<i>Salmo trutta m. fario</i>)	4	3	7
Bream (<i>Abramis brama</i>)	4	1	5
Flounder (<i>Platichthys flesus</i>)	3	1	4
Roach (<i>Rutilus rutilus</i>)	3	NT	3
Other *	6	2	8

NT - not tested

* Fresh fish: pike (*Esox lucius*), redfish (*Sebastes marinus*), saithe (*Pollachius virens*), pollock (*Gadus chalcogrammus*), haddock (*Melanogrammus aeglefinus*), hoki (*Macruronus magellanicus*); smoked fish: blue warehou (*Seriola lalandi*) and grenadier (*Coryphaenoides rupestris*)

Reagents. Histamine dihydrochloride and 1-octanesulfonic acid were obtained from Acros Organics (Belgium). Trichloroacetic acid (TCA), potassium dihydrogen phosphate, orthophosphoric acid and methanol were obtained from POCh (Poland). SPE cartridges Polimeric Weak Anion (200 mg, 3 mL) was from Phenomenex (USA). Nylon filters (0.45 µm) were from Agilent Technology (USA). All chemicals were HPLC or analytical grade. Water was purified using Milli-Q system.

Standard solution. The stock standard solution of histamine dihydrochloride (1000 mg/L) was prepared by weighing 414 ± 0.1 mg of standard substance and dissolving it in 250 mL of water. The solution stored at 2-8°C was stable for one month. Working standard solutions for calibration curve were prepared on the day

of analysis by appropriate dilutions of the stock in phosphate buffer (0.01 M, pH 3.0).

Extraction of fish samples and clean-up. Fish sample (10 ± 0.01 g) was weighed into 50 mL polypropylene centrifuge tube. Twenty five millilitres of TCA (0.2 M) was added and the sample was mixed for 1 min, centrifuged for 10 min at $4000 \times g$ at 15°C, and kept at $\leq -18^\circ\text{C}$ for 30 min. The upper lipid layer was discarded. Five millilitres of the supernatant was then transferred into 10 mL Erlenmeyer flask and filled up of water. The sample was cleaned up by the Solid Phase Extraction (SPE) apparatus (J.T. Baker) and Polimeric Weak Anion SPE cartridges (200 mg, 3 mL). The cartridges were conditioned with 5 mL of methanol, followed by 10 mL of phosphate buffer (0.01 M, pH 3.0). Seven millilitres of the extract was transferred to SPE column and passed through the cartridge into 10 mL Erlenmeyer flask. Afterwards, SPE column was washed with 2 mL of phosphate buffer. Filtered extract was complemented to 10 mL with phosphate buffer in Erlenmeyer flask.

HPLC-DAD analysis. The instrumental analysis was performed using Varian ProStar HPLC equipped with quaternary pump, autosampler, column thermostat, and diode array detector, controlled by Galaxie Workstation software. Separation was conducted on Unisol C18 150 $\times$ 4.6 mm, 3 µm with precolumn 10 $\times$ 3 mm, 3 µm (Agela Technologies, USA). The mobile phase consisted of methanol and 0.1 M potassium dihydrogen phosphate with 1.6 mM 1-octanesulfonic acid (180:820, v:v) and was applied in isocratic conditions. The column oven temperature was maintained at 25°C and a flow rate of 0.5 mL/min was used. The injection volume was 20 µL. The UV detection was monitored at 215 nm. The range of method was 3.6-420 mg/kg, whereas the limit of detection and quantification was 1.3 mg/kg and 1.8 mg/kg respectively.

Results

Fresh fish. The results of analysis of histamine in fresh fish samples are shown in Table 2. Histamine was detected in seven samples (14.6%): herring (one sample), cod (two samples), salmon (two samples), bream (one sample), and roach (one sample). The concentration of histamine ranged between 1.4 and 5.2 mg/kg. The maximum amount of histamine (5.2 mg/kg) was found in the bream.

Smoked fish. The results of analysis of histamine in smoked fish samples are shown in Table 3. No histamine was detected in 37 (82.2%) samples. Histamine was found in the remaining eight samples (17.8%): mackerel (one sample), herring (two sample), cod (one sample), sprat (three samples), and salmon (one sample). The concentration of histamine in these samples ranged between 1.8 and 24.1 mg/kg. The

maximum amount of histamine (24.1 mg/kg) was found in the sprat.

Table 2. Content of histamine in fresh fish

Fish species	Number of samples tested	Number of samples with histamine	Content of histamine (mg/kg)
Mackerel	5	0	< LOD
Herring	15	1	1.4
Cod	5	2	1.8, 3.2
Salmon	3	2	1.5, 1.5
Brown trout	4	0	<LOD
Bream	4	1	5.2
Flounder	3	0	<LOD
Roach	3	1	2.6
Other	6	0	<LOD
LOD	1.3		

LOD - limit of detection

Table 3. Content of histamine in smoked fish

Fish species	Number of samples tested	Number of positive samples	Content of histamine (mg/kg)
Mackerel	10	1	4.9
Herring	10	2	1.8, 17.7
Cod	6	1	4.3
Sprat	5	3	3.5 ÷ 24.1
Salomon	7	1	2.7
Brown trout	3	0	<LOD
Flounder	1	0	<LOD
Bream	1	0	<LOD
Other	2	0	<LOD
LOD		1.3	

LOD - limit of detection

Discussion

Histamine is one of the most important and toxic biogenic amine in food. It is produced during bacterial decarboxylation of the histidine present in fish muscles. The fundamental actions prevent from scombrototoxin food poisoning are adequate refrigeration practices to prevent from bacterial spoilage and production of histamine. It is important to respect the time and low temperature during unloading, transportation, storage, and processing of fish (10, 14). Determination of histamine is important not only because of its toxicity for humans, but also as an indicator of the freshness of fish and fish products (11).

According to data from the United States Centers for Disease Control and Prevention (CDC) concerning

the period between 1998 and 2002, there were 463 histamine intoxication cases reported, with no deaths (9). In Japan, there were 89 incidents with 1577 cases reported with no deaths in the period between 1998 and 2002 (9). Data received from the Rapid Alert System for Food and Feed (RASFF) for the period between 2008 and 2011 showed 86 cases of histamine poisoning (17-20). The European Food Safety Authority (EFSA) reported that fish and fish products caused 71 outbreaks in the EU countries in 2011. The majority of these outbreaks were caused by histamine (56 cases, 78.9%). In 2011, crustaceans, shellfish, molluscs, and products thereof were the source of 42 outbreaks, among which 2.4% were caused by histamine (8). Due to an increase in the histamine content in fish, caused by an elevated temperature during storage and transport, there is a need for the studies ensuring consumer's health (13). For this reason, studies on the content of histamine in fish are performed in many countries.

In a survey conducted in Turkey, Erkan *et al.* (7) reported, that among 39 canned fish samples from the market, which were tested for histamine, 7.7% contained more than 1000 mg/kg of this amine. In Taiwan, Tsai *et al.* (21) analysed 61 samples of scombroid fish fillets sold in traditional retail markets, and 6.5% were found to contain histamine in the concentrations ranging from 128 to 288 mg/kg. In another Taiwan's survey, 6% of salted mackerel sold in the market had histamine content between 70.1 and 120.2 mg/kg (22), and during an incident of food poisoning due to swordfish consumption, the histamine level was 859-2937 mg/kg (3). In Greece, the average histamine concentration was from 2.7 to 220 mg/kg, while 3.2% of 55 samples were characterised as unsuitable for human consumption according to the European Commission limits. The highest histamine concentrations obtained by HPLC was measured in herring (23).

In the present study, the histamine concentration determined by HPLC-DAD ranged from 1.43 to 24.05 mg/kg. In 84% of the examined samples no histamine was found and in 16% fish tested the concentration of histamine was below the allowable limit. The maximum amount of histamine (24.05 mg/kg) was identified in smoked sprat. In fresh and smoked fish, the histamine content above the allowable limit was not found.

The study showed that the fresh and smoked fish fulfil the food safety criteria for histamine listed in the Commission Regulation (EC) No. 2073/2005 (5). The low content of histamine in fish and fish products is an indicator of freshness and shelf life, which proves that fish were fresh, as raw materials, and manufacturing process was conducted properly. The results obtained demonstrated that fish available on Polish markets are safe for the consumers. The examined fresh and smoked fish do not exceed the safety limit of 100 mg of histamine/kg.

References

1. Attaran R.R., Probst F.: Histamine fish poisoning: a common but frequently undiagnosed condition. *Emerg Med J* 2002, 19, 474–475.
2. Bodmer S., Imark C., Kneubühl M.: Biogenic amines in food: histamine and food processing. *Inflamm Res* 1999, 48, 296–300.
3. Chang S.C., Kung H.F., Chen H.C., Lin C.S., Tsai Y.H.: Determination of histamine and bacterial isolation in swordfish fillets (*Xiphias gladius*) implicated in a food borne poisoning. *Food Control* 2008, 19, 16–21.
4. Cieślak I., Migdał W.: Biogenic amines in food. *Bromat Chem Toksykol* 2011, 44, 1087–1096.
5. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.
6. Economou V., Brett M., Papadopoulou C., Frillingos S., Nichols T.: Changes in histamine and microbiological analyses in fresh and frozen tuna muscle during temperature abuse. *Food Additives Contam* 2007, 24, 820–832.
7. Erkan N., Helle N., Ozden O.: The content of biogenic amines in canned fish from Turkish market. *Berl Munch Tierarztl Wochenschr* 2001, 114, 241–245.
8. European Food Safety Authority (EFSA): The community summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in the European Union in 2011. *EFSA J* 2013, 11, 3129.
9. FAO/WHO Report: Joint FAO/WHO expert meeting on the public health risk of histamine and other biogenic amines from fish and fishery products. 2012, pp. 14–83.
10. Hungerford J.: Scombroid poisoning: A review. *Toxicon* 2010, 56, 231–243.
11. Karovicova J., Kohajdova Z.: Biogenic amines in food. *Chem Pap* 2005, 59, 70–79.
12. Kim M.K., Mah J.H., Hwang H.J.: Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Food Chem* 2009, 116, 87–95.
13. Lehane L., Olley L.: Histamine fish poisoning revisited. *Int J Food Microbiol* 2000, 58, 1–37.
14. McLauchlin J., Little C.L., Grant K.A., Mithani V.: Scombrototoxic fish poisoning. *J Public Health* 2005, 28, 61–62.
15. Punakivi K., Smolander M., Niku-Paavola M., Mattinen J., Buchert J.: Enzymatic determination of biogenic amines with transglutaminase. *Talanta* 2006, 68, 1040–1045.
16. Shalaby A.R.: Significance of biogenic amines to food safety and human health. *Food Res Int* 1996, 27, 675–690.
17. The Rapid Alert System for Food and Feed (RASFF). Annual Report 2008. Office for Official Publications of the European Communities, Luxembourg 2009, pp. 26–27.
18. The Rapid Alert System for Food and Feed (RASFF). Annual Report 2009. Office for Official Publications of the European Communities, Luxembourg 2010, pp. 36–38.
19. The Rapid Alert System for Food and Feed (RASFF). Annual Report 2010. Office for Official Publications of the European Communities, Luxembourg 2011, pp. 39–41.
20. The Rapid Alert System for Food and Feed (RASFF). Annual Report 2011. Office for Official Publications of the European Communities, Luxembourg 2012, pp. 29–30.
21. Tsai Y.H., Kung H.F., Lee G.T., Lin C.Y., Hwang D.F.: Histamine related hygiene qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. *J Food Prot* 2004, 67, 407–412.
22. Tsai Y.H., Lin C.Y., Cang S.C., Chen S.C., Kung H.F., Wei C.I., Hwang D.F.: Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiol* 2005, 22, 461–467.
23. Vosikis V., Papageoropoulou A., Economu V., Frillingos S., Papadopoulou C.: Survey of the histamine content in fish samples randomly selected from the Greek retail market. *Food Additives Contam* 2008, 1, 122–129.