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Histamine content in selected production stages of fish products

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Abstract

Introduction: Histamine intoxication, known as scombroid fish poisoning, is caused by the consumption of foods with high levels of histamine. This biogenic amine is formed as a result of histidine decarboxylation by bacterial decarboxylases present in food, including fish and fish products. The aim of this study was to investigate the content of histamine at different production stages of canned, marinated and smoked fish. **Material and Methods:** Raw fish, semi-finished fish products, and the final products of the same production batches were collected between 2019 and 2022 from different fish production facilities in Poland. A total of 133 raw fish samples and 76 smoked fish, 54 brined fish, 39 canned fish and 18 marinated fish final products were analysed using high performance liquid chromatography with a diode array detector. **Results:** Histamine was identified in 55 (17.2%) out of 320 tested samples, including 8 samples of raw fish with a histamine level above 100 mg/kg. However, no samples of fish products had histamine content above the European Union Commission limit. **Conclusion:** The obtained results show that fish products on the Polish market are generally safe for consumers in regard to histamine intoxication risk.

Keywords: histamine, fish products, HPLC-DAD, food safety.

Introduction

Fish is a crucial component of a healthy, well-balanced diet. There are many benefits from the consumption of fish and fish products, which derive from their high contents of protein, lipids, omega-3 fatty acids, vitamins and macronutrients. Fish can also be a source of various contaminants which are important public health threats. One of them is histamine, which is also an indicator of the freshness and suitability of foods for consumption. The production of histamine is related to the presence of free histidine in fish muscles, the activity of histidine decarboxylases and the conduciveness of the environmental conditions (9, 22). Histamine is formed from the amino acid histidine in a reaction catalysed by the diamine oxidase enzyme produced by microorganisms. Consumption of large amounts of histamine results in an intoxication called scombroid fish poisoning, which is mainly connected with fish from the *Scombridae* family (mackerel and bonito tuna) (12). Other fish species such as herrings and sardines, and their products, have also been responsible for histamine intoxication. Histamine is toxic to the circulatory, digestive and nervous systems. This amine causes vasodilation, leading to hypotension, reddening of the skin, headache and the development of anaphylaxis. Histamine can also cause smooth muscle contractions of the gastrointestinal tract, which result in abdominal pain, diarrhoea and vomiting (10, 13).

There are many factors affecting histamine formation in fish, such as the quality of raw materials, production technology, storage conditions and the presence of bacteria producing decarboxylases (9, 20, 22). Improper conditions during storage, transport and processing of fish after harvest are the most common causes of histamine production (1, 29). Compliance with hygiene rules and maintenance of the appropriate temperature at each stage of production and distribution are necessary to avoid or limit the formation of histamine (10). Many fish preservation processes, such as freezing, drying or salting can inhibit the growth of the bacteria which are responsible for histamine production (13, 25, 27). However, histamine is heat stable and can be found in the final products if it was present before mechanised processing started (11, 28).

The Commission Regulation (EC) No. 2073/2005 established maximum levels for histamine in fish and fishery products (6). In the nine samples stipulated to be taken from each batch, the mean content must not exceed 100 mg/kg, two samples may have a level higher than 100 mg/kg but lower than 200 mg/kg, and finally, no sample may have a concentration of histamine exceeding 200 mg/kg. These limits are for the fish from the *Scombridae, Scomberesocidae, Engraulidae, Clupeidae, Coryphaenidae* and *Pomatomidae* families with high concentrations of free histidine in their muscle tissue.

The aim of the present study was to investigate the content of histamine in raw fish and the change in this content as the same fish became semi-finished products (brined fish) and final products (canned and marinated fish); it also investigated the content in raw fish and its change after smoking that fish.

Material and Methods

Samples. A total of 320 samples of fish and fish products were used in the study. Samples of canned (n = 117) and marinated (n = 54) fish were collected during 39 and 18 production cycles, respectively. Each cycle included three stages: raw fish, brined fish and the final product (Table 1). The smoked fish comprised 152 samples, taken during 76 production cycles at two stages: raw fish (n = 36) and the final product (n = 36). In total, 133 samples of raw fish, 54 samples of brined fish, and 133 final fish products were tested.

The samples of canned fish were collected from various fish processing facilities located mainly in northern Poland, the marinated fish were from the south-eastern part and the smoked fish were from the north-western and eastern parts of Poland. The samples were transported to the laboratory at the National Veterinary Research Institute in Puławy under refrigerated conditions (0–4°C).

The following kinds of canned fish were tested (Table 1): mackerel in oil, sprat in oil, sardine in oil, herring in oil, mackerel in tomato sauce, sprat in tomato sauce, cod liver in its own juice, salmon in its own juice, herring with vegetables in jelly, fried mackerel in vinegar, mackerel with vegetables in jelly, and fried herring in vinegar. The marinated fish analysed were herring in light brine and herring in sauce with vegetables. The following fish species were investigated as smoked fish products: Atlantic salmon (*Salmo salar*), Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), European sprat (*Sprattus sprattus)*, Atlantic cod (*Gadus morhua morhua*), European hake (*Merluccius merluccius*), rainbow trout (*Oncorhynchus mykiss*), catfish (*Silurus glanis*), Atlantic halibut (*Hippoglossus hippoglossus*), European eel (*Anguilla anguilla*), common warehou (*Seriolella brama*) and Atlantic redfish (*Sebastes norvegicus*).

Method. Before the samples preparation for histamine testing, the skin and bones were removed from the fish. The investigation of canned and marinated fish samples began with draining of the liquid content, and a portion of approximately 50 g was homogenised using a blender. Then, 10 g of the homogenate was transferred into a 50 mL polypropylene centrifuge tube and histamine was detected in it by high performance liquid chromatography with a diode array detector, using the Pro Star system (Varian, the Netherlands) controlled by Galaxie Workstation software (Varian) as previously described (23). Briefly, histamine was extracted from samples with 25 mL of 0.2 M trichloroacetic acid, purified on Strata-X-AW-ion-exchange solid-phase extraction cartridges (Phenomenex, Torrance, CA, USA) and then filtered through a 0.45 μm nylon syringe filter. Chromatographic separation was performed on a Unisol C18 column (Agela Technologies, Torrance, CA, USA) of 150 × 4.6 mm size with pores of 3 μm, connected to a C18 precolumn of 10 × 3 mm. The mobile phase, consisting of 18% methanol in 0.1 M potassium dihydrogen phosphate (150/850, v/v) with 1.6 mM 1-octanesulphonic acid, was applied under isocratic conditions. The column oven temperature was maintained at 25°C, the flow rate at 0.5 mL/min, and the injection volume was 20 μL. Ultraviolet light detection was monitored at 215 nm wavelength. The limits of detection and quantification were 2.1 mg/kg and 3.3 mg/kg, respectively, and the range of the method was 3.3–420 mg/kg (23).

Results

Histamine was detected in 55 out of 320 (17.2%) tested samples of fish and fish products (Table 1). In the remaining 265 (82.8%) samples no detectable level of this amine was identified. Histamine was present at similar levels in raw (22 out of 133; 16.5%) and brined (9 out of 54; 16.7%) fish samples. The most contaminated final products were found among marinated fish (8 out of 18; 44.4% of samples), followed by canned fish (10 out of 39; 25.6%), whereas the final products of smoked fish were positive only in 6 out of 76 (7.9%) samples.

Taking into account all the varieties of fish products tested, the most contaminated with histamine among canned fish were cod liver (5 out of 6 samples; 83.3%) and sprat in oil (10 out of 18 samples; 55.6%) (Table 1). On the other hand, histamine was not identified in mackerel in tomato sauce, salmon in its own juice, herring with vegetables in jelly, mackerel with vegetables in jelly, fried mackerel in vinegar or fried herring in vinegar. Among marinated fish, only a few samples were positive, mainly Atlantic herring (7 out of 39 samples; 17.9%) (Table 1). In smoked fish, histamine was mainly detected in Atlantic salmon (9 out of 56 samples; 16.1%), whereas only a few samples of other fish species were positive (Table 1). No histamine was detected in the following smoked fish: Atlantic cod, European hake, Rainbow trout, catfish, Atlantic halibut, European eel, or Atlantic redfish.

The samples positive for histamine contained different levels of this amine identified in each of the production stages (Fig. 1). The most contaminated were raw fish to be smoked and canned fish and the levels of this amine were higher than those of the obtained respective final products. The highest level of histamine was found in the following raw fish: Common warehou (294.7 mg/kg), Atlantic salmon (256.1 mg/kg), Atlantic mackerel (188.2 mg/kg), and Atlantic cod liver (177.9 mg/kg). On the other hand, among marinated fish, no histamine was identified in the raw material, but the final products contained this amine in the range of 3.9–52.9 mg/kg (Table 1). The mean histamine concentrations in the final products ready for consumption was the highest in the marinated fish (30.3 mg/kg) and the lowest in the smoked fish (9.0 mg/kg) (Fig. 1).

Table. 1. Presence of histamine in the production cycles of canned, marinated, and smoked fish

	Samples tested	Number of samples tested			Number (%) of positive samples	Content of histamine in fish production stages (mg/kg)		
		Raw fish (n = 133)	Brined fish (n = 54)	Final products (n = 133)		Raw fish	Brined fish	Final products
Canned fish	Mackerel in oil	9	9	9	6 (22.2)	5.0; 45.0	6.7; 26.0	16.7; 21.1
	Sprat in oil	6	6	6	10 (55.6)	5.8–34.8	4.8–24.9	3.9–15.1
	Sardine in oil	4	4	4	1 (8.3)	<LOD	<LOD	4.6
	Herring in oil	3	3	3	2 (22.2)	4.6	3.9	<LOD
	Cod liver	3	NA	3	5 (83.3)	77.0; 177.9	NA	10.2–12.0
	Sprat in tomato sauce	2	2	2	3 (50)	15.5; 58.6	<LOD	8.0
	Other[1]	12	12	12	0	-	-	-
	Total	39	36	39	27 (23.7)	<LOD–177.9	<LOD–26.0	<LOD–21.1
Marinated fish	Atlantic herring	13	13	13	7 (17.9)	<LOD	3.9; 13.2	3.9–40.1
	Kashubian-style herring	2	2	2	1 (16.7)	<LOD	<LOD	41.4
	Herring in salsa	2	2	2	1 (16.7)	<LOD	<LOD	10.5
	Jewish-style herring	1	1	1	1 (33.3)	<LOD	<LOD	52.9
	Total	18	18	18	10 (18.5)	-	<LOD–13.2	3.9–52.9
Smoked fish	Atlantic salmon	28	NA	28	9 (16.4)	8.8–256.1	NA	4.3; 12.9
	Atlantic mackerel	17	NA	17	4 (11.8)	188.2	NA	3.8–5.2
	Atlantic herring	7	NA	7	1 (7.1)	6.8	NA	<LOD
	European sprat	4	NA	4	3 (37.5)	4.9; 4.9	NA	23.1
	Common warehou	1	NA	1	1 (50)	294.7	NA	<LOD
	Other[2]	19	NA	19	0	-	-	-
	Total	76	NA	76	18 (11.8)	4.9–256.1	-	<LOD–12.9
	All-sample total	320			55 (17.0)	<LOD–256.1	<LOD–26.0	<LOD–52.9

LOD – limit of detection (2.13 mg/kg); NA – not applicable; [1] – mackerel in tomato sauce, salmon in its own juice, herring with vegetables in jelly, mackerel with vegetables in jelly, fried mackerel in vinegar, fried herring in vinegar; [2] – Atlantic cod (*Gadus morhua morhua*), European hake (*Merluccius merluccius*), rainbow trout (*Oncorhynchus mykiss*), catfish (*Silurus glanis*), Atlantic halibut (*Hippoglossus hippoglossus*), European eel (*Anguilla anguilla*) Atlantic redfish (*Sebastes norvegicus*)

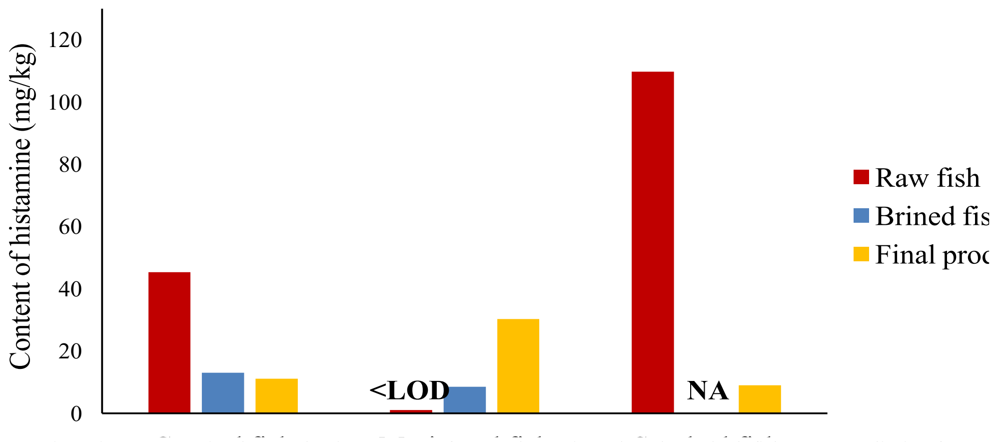

Fig. 1. Histamine content in the production stages of canned, marinated and smoked fish. LOD – limit of detection (2.13 mg/kg); NA – not applicable

Discussion

Because of the toxic effect of histamine, it is important that food does not contain this amine above the legal limits (6). In order to prevent histamine formation, the shortest practical time and continuously controlled temperature during the processing and distribution of fish and fish products should be assured. In a study performed in southern Italy, the histamine contents in fresh fish were significantly higher than those in processed samples (canned, marinated, smoked, salted and precooked fish) (4). Among the examined samples, 7.41% (342 out of 4,615) were histamine positive, with mean values of 77.67 ± 431.84 mg/kg and 32.21 ± 168.48 mg/kg for fresh fish and processed fish products, respectively. The highest histamine concentrations were found in fresh tuna fish and anchovies. In the present study, the highest histamine content was also identified in different raw fish (common warehou, Atlantic salmon, Atlantic mackerel and Atlantic cod liver). This may be due to improper storage conditions before and during transportation of fish to manufacturers, since there is a greater risk of microbial growth and histamine production in raw fish (19). In previous studies conducted in Poland during 2014–2018, histamine was detected in 21.9% of samples of fish and fish products (24). In marinated fish products (Atlantic herring) this amine was identified in all but one of 16 samples tested, in canned fish in 11 out of 50 samples (22.0%), and in raw fish in 35 out of 248 samples (14.1%). Those data were confirmed in the present studies in which histamine was detected mainly in marinated fish (44.4%), less in canned fish (25.6%), and much less in raw fish (16.5%).

According to the Rapid Alert System for Food and Feed report from 2020, of the 40 notifications of food poisoning traceable to one source, four had histamine as their cause. (26). However, in the previous years (2014–2018), this number was much higher at 79 notifications of histamine levels above the regulatory limit in fish and fish products (24). In May 2017 more than 150 people in Spain and 40 in France were affected by histamine intoxication after the consumption of yellowfin tuna (10). Furthermore, between January 2015 and August 2020, histamine was detected in different types of fish products, mainly in chilled and canned fish (48 and 40 samples, respectively), but also in marinated and smoked fish (5 and 2 samples, respectively) (28).

Several factors may have an influence on histamine formation during the preparation of fish products, some impacting it early when the fish is raw material and the final ones causing changes even as fish nears being a ready-to-eat product. In the present study, differences in histamine concentrations in raw material, semi-finished products and final products were detected. The reason for them may be not only the influence of line processes but also the different distribution of histamine content throughout fish muscle. Histamine reaches different concentrations in different fish species, in one species it may also be present at uneven levels, and even within one individual fish different body parts can evidence difference histamine content strengths. The variability can be as high as a factor of ten (14). Many other factors such as the fish catch season, age of fish, method of catching and conditions of storage before freezing may change the histamine content in some species of fish.

The histamine level at various stages of fish processing including canning, was determined in studies conducted by Ganowiak *et al.* (16). In that experiment, a low content of histamine was found in the raw material and in samples collected from all subsequent stages of processing. This proves that when sound principles are strictly applied throughout line processing, there is no histamine formation in the meat tissue of fish. During the present study no adverse effect of automation was observed in the production of canned fish, and in fact the level of this amine was even lower in the final product than in the raw fish. In another study, no statistically significant differences between the content of histamine in the raw material and the content in marinated fish fillets were found (8). On the other hand, the results obtained by Ganowiak *et al.* (16) showed a seven-fold higher content of histamine in the semi-finished and finished products than in the raw fish material used for their production. In the present study, histamine was found in the brined and final marinated fish products but not in the respective raw materials.

Storage temperature is one of the most important factors contributing to histamine formation in fish (5, 9, 28). Although a temperature in the range of 25–30°C is conducive to the formation of histamine, high concentrations of this amine have also been reported in chilled foods (4–10°C) (18, 21). Some studies showed that histamine is usually not detected or identified only at low levels in fresh fish and fish products (24, 29). Nevertheless, the inferior hygiene as raw fish is handled and/or hygienically insufficient processing of ready-to-eat fish products can result in a high histamine content (1, 25). It was identified in Spain where 657 mg/kg of histamine was detected in canned fish (11). Other elements, such as mechanised conservation processes (pasteurisation, high pressures and irradiation) and packaging (under vacuum and/or in modified atmospheres), have been widely studied as possible potentiators of the activity of microorganisms responsible for the formation of histamine (3, 17). It has been suggested that smoking fish delays the histamine formation process by activity which is bacteriostatic to some bacteria and by the preservative effect of smoke compounds (15). On the other hand, there are results showing that processing operations, including smoking, may contribute to histamine production (2, 7, 14). In the present study, the histamine content in smoked fish was much lower than in raw material. The obtained results may suggest that the smoking process reduces histamine formation in fish products.

The obtained results support the assumption that the analysed fish products were generally safe in terms of histamine content. To protect fish from contamination with histamine-producing bacteria and consumers from scombroid fish poisoning, factors that enhance this amine's formation should be minimised during processing.

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