Chapter 1
Histamine in Fish and Fishery Products

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Abstract The consumption of certain fish products containing high levels of histamine (and other biogenic amines) can result in an acute illness with allergy-like symptoms called scombroid syndrome. Fish accumulate toxic levels of histamine when their high level of histidine in muscle tissues is coupled with a proliferation of bacteria rich in the enzyme histidine decarboxylase. Other vasoactive amines—cadaverine, putrescine, etc.—may inhibit detoxification mechanisms that reduce the intestinal absorption of histamine. Moreover, histidine can be transformed by means of another metabolic pathway leading to accumulation in fish muscle of urocanic acid. Recently, interest has been extended to mesophilic and psychrotolerant bacteria. Histamine accumulation is traditionally correlated to microbially contaminated fish and poor storage conditions. In addition, the high thermal stability has to be considered. At present, different methods are available for the analytical determination of histamine ranging from the AOAC fluorometric method to HPLC, ELISA and rapid stick methods.

Keywords ELISA · Histamine · Histidine decarboxylase · HPLC · Mesophilic microorganism · Psychrotolerant bacterium · Refrigeration · Scombroid syndrome

Abbreviations

ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
HACCP	Hazard Analysis and Critical Control Points
HPLC	High-Performance Liquid Chromatography
WHO	World Health Organization

G. Caruso et al., *Microbial Toxins and Related Contamination in the Food Industry*, Chemistry of Foods, DOI 10.1007/978-3-319-20559-5_1

1.1 Histamine in Fish and Fishery Products: An Introduction

Scombroid fish poisoning, also named histamine poisoning, is one of the most challenging food safety problems in the seafood industry (Hungerford 2010). The consumption of mishandled fish belonging to the families of *Scombridae*, namely tuna and mackerel, *Clupeidae (*sardines and herrings) and *Engraulidae* (anchovies), may result in an acute illness with allergy-like or *Salmonella-like* infection symptoms (Lehane and Olley 2000). This 'scombroid syndrome' occurs if these foods contain high levels of histamine—2-(4-imidazolyl) ethylamine or 4-(2-aminoethyl) imidazole—and other vasoactive (biogenic) amines.

Normally, histamine can be important when related amounts are ≥50 mg/100 g of microbially contaminated fish. In detail, unsatisfactory raw fish usually show more than 50 ppm of histamine (toxic values should be between 100 and 500 ppm), while normal levels should not exceed 10–50 ppm (Chamberlain 2001). On the other side, urocanic acid—an imidazole compound derived from histidine in contaminated products—may be also considered when speaking of scombroid poisoning effects (Lehane and Olley 2000).

Basically, the accumulation of histamine and other decomposition compounds is observed in many fish types, including *Scombridae*. From the biochemical viewpoint, histamine is obtained in scombroid fish (*albacore, bonito*, skipjack, Spanish mackerel, saury, etc.) by means of the enzymatic conversion of free and abundant histidine in muscle tissues (Cattaneo 2011; Lehane and Olley 2000; Rawles et al. 1996; Ruiz-Capillas and Moral 2004; Taylor 1986; Tortorella et al. 2014). However, the abundant presence of free histidine is reported in many fish products, including also (Antoine et al. 1999; Chang et al. 2008; Hungerford 2010; Taylor 1986):

- Anchovies (Engraulis spp.)
- Herring (Clupea spp.)
- Pilchards (Sardina pilchardus)
- Mahi-mahi (Coryphaena spp.)
- Sardines (Sardinella spp.)
- Swordfish (Xiphias gladius).

Actually, histamine poisoning has been reported in relation to non-fish products such as Gouda, Swiss, Gruyere, Cheddar and Cheshire cheeses (Chambers and Staruszkiewicz 1978; Doeglas et al. 1967; Kahana and Todd 1981; Taylor 1986). However, these situations appear circumscribed to a few situations: apparently, cheeses might be considered as a potential problem when speaking of unusual ageing (Taylor 1986). Moreover, other fermented products—*Sauerkraut*, wines—or partially demolished foods (in relation to the protein fraction) such as Italian *pepperoni* and *salami* may contain occasionally high histamine levels (Dierick et al. 1974; Mayer and Pause 1972; Ough 1971; Taylor 1986; Taylor et al. 1978). Anyway, the most part of observed and reported histamine poisoning episodes is

correlated with the consumption of raw fish and finished seafood products (Hungerford 2010).

It has to be considered that the bacterial and enzymatic production of histamine from histidine is strictly correlated with storage temperatures: normally, thermal values should always remain below 4 °C (Hastein et al. 2006; Tsironi et al. 2008). On the other hand, this process may occur in all stages of the food chain (Cattaneo 2014; Kanki et al. 2004). In detail, the production of histamine can be easily be observed in skipjack and big-eye tuna fish at 22 °C after 24–48 h, while this phenomenon may be remarkably delayed at 10 and 4 °C. However, the amount of detectable histamine may be notable after 3 days under refrigerated storage (Silva et al. 1998).

The Food and Drug Administration (FDA) has published a detailed guideline in relation to retail food establishments (scombroid products). According to this document (FDA 2011), raw fish should have internal temperatures below or equal to 10 °C (if fish has been delivered 12 or more hours after death) or 4.4 °C (if fish has been delivered 24 or more hours after death). Anyway, temperatures should be evaluated after receipt (FDA 2011). Certainly, storage at 0 °C can determine the end of histamine production (Chamberlain 2001) but existing levels are not eliminated.

Recently, it has been recognised that the production of histamine from high levels-histamine fish can be assessed when temperatures are higher than 25 °C for 6 h or more (FAO/WHO 2012). On these bases, the recommended amount of histamine in fish products has been defined to be lower than 15 mg/kg on the condition that good hygienic practices and 'Hazard analysis and critical control points' (HACCP)—based strategies have been implemented. The Codex Alimentarius Commission has defined two different levels (100 and 200 mg/kg) in relation to the commercial acceptability and possible food safety problems of fish products respectively (Cattaneo 2014).

These values have a slightly different meaning in the European Union in relation to the Regulation (EC) No. 2073/2005 and subsequent amendments. In detail, nine samples have to be considered and four conditions are possible when speaking of normal fish products:

(a) All results have to be lower than 100 mg/kg. Fish products are fully acceptable
(b) One or two results only are found between 100 and 200 mg/kg while remaining samples are below 100 mg/kg. Fish products are fully acceptable
(c) One or more samples exceed 200 mg/kg. Fish products have to be recalled or withdrawn from the market
(d) More than two samples are found between 100 and 200 mg/kg. Fish products have to be recalled or withdrawn from the market.

These norms are valuable for unsalted fish products. Should salted fish be sampled (nine products), four conditions are possible when speaking of normal fish products (FAO/WHO 2012):

(e) All results have to be 200 mg/kg. Fish products are fully acceptable
(f) One or two results only are found between 200 and 400 mg/kg while remaining samples are below 200 mg/kg. Fish products are fully acceptable

(g) One or more samples exceed 400 mg/kg. Fish products have to be recalled or withdrawn from the market
(h) More than two samples are found between 200 and 400 mg/kg. Fish products have to be recalled or withdrawn from the market.

Interestingly, histamine levels in foods do not appear to be influenced by normal processing treatments such as cooking and smoking. Actually, these processes kill histamine producers but the existing amounts of histamine remain unchanged (Cattaneo 2014). In addition, cold storage cannot reduce the real incidence or diminish possible poisoning episodes when raw fish is partially compromised (FDA 2011).

1.2 Chemistry and Production of Histamine: Importance of Other Biogenic Amines

Actually, histamine is not produced in degraded fish only. In fact, a low amount of histamine is also naturally produced by human beings because of the decarboxylation of histidine (FAO/WHO 2012).

With exclusive reference to fish products, toxic levels of histamine are accumulated when their high level of histidine in muscle tissues is coupled with a proliferation of bacteria rich in the enzyme histidine decarboxylase (Alini et al. 2006). Histamine exerts its negative effects on specific receptors known as H_1 and H_2 receptors located on human cell membranes. H_1 receptors are implicated in allergic reactions with dilation of peripheral blood vessels (rash, namely of the lips and surrounding area, urticaria, headache), while H_2 receptors are responsible for gut motility (diarrhoea, cramps, vomiting). The onset of symptoms either without or with small amounts of histamine implicates other causes or contributory causes of intoxication.

Other vasoactive amines such as cadaverine and putrescine may inhibit detoxification mechanisms that reduce intestinal absorption of histamine by means of catabolic enzymes like histaminase (FAO/WHO 2012). These biogenic amines are produced by means of microbial spoilage and fermentation from amino acids; the precursor is ornithine (FAO/WHO 2012). The role of these molecules is not clear at present: potentially, putrescine and cadaverine might be considered as histamine potentiators (Taylor and Lieber 1979). On the other hand, the real role of these biogenic amines in scombroid poisoning episodes is not clear (FAO/WHO 2012). The same thing can be affirmed when speaking of tyramine, a monoamine molecule formed from tyrosine (Leuschner and Hammes 1999; Prester 2011; Taylor and Lieber 1979). Other notable biogenic amines with some food safety importance are tryptamine, spermine, spermidine and β-phenylethylamine (Shalaby 1996). In relation to the present section, the importance of these amines is reduced because they can be found in many foods including also dairy and meat products, nuts, chocolate, etc. (Emborg 2007).

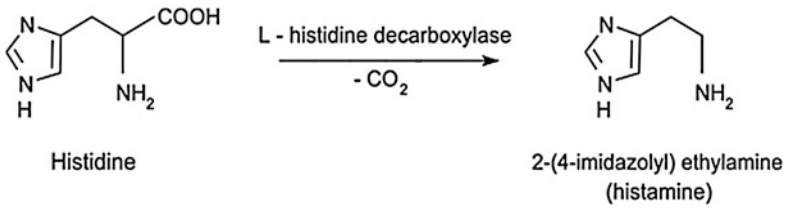

Fig. 1.1 Production of histamine by enzymatic decarboxylation of histidine. BKchem version 0.13.0, 2009 (http://bkchem.zirael.org/index.html) has been used for drawing this structure

It has been reported that the presence of histamine may not cause toxic effects at low levels. On the other hand, the contemporary presence of molecules, such as cadaverine and putrescine, can enhance histamine-related toxic effects when the ratio between histamine and remaining biogenic amines is 1:5 (Emborg and Dalgaard 2006; Naila et al. 2010).

After decarboxylation of histidine (Fig. 1.1), the produced histamine can be subsequently converted (Alini et al. 2006) into:

(1) Imidazole acetaldehyde and imidazole acetic acid by the diamine oxidase enzyme, or
(2) Methyl histamine by the methyl transferase enzyme.

In addition to its decarboxylation mechanism, histidine can be transformed by another metabolic pathway leading to accumulation in fish muscle of urocanic acid (Alini et al. 2006), caused by histidine lyase (histidase). The above-mentioned urocanic acid is an imidazole compound whose effects include activation and degranulation of connective tissue mast cells in the human body, releasing histamine from their metachromatic granules. The production of large amounts of histamine in fish is largely due to Gram-negative bacteria, whereas scombroid syndrome is seldom associated with Gram-positive bacteria (FAO/WHO 2012). For a long time, the production of toxic quantities of histamine has been correlated with the excessive growth of mesophilic bacteria belonging mainly to *Morganella morganii*, *Hafnia* and *Raoultella* (*planticola*) species under conditions of thermal abuse at temperatures between 20 and 40 °C. More recently, the interest has concerned also psychrotolerant bacteria belonging to *Morganella psychrotolerans* and *Phosphobacterium phosphoreum* species because they are able to grow below 0 °C (Emborg 2007). On the one side, mesophilic bacteria cause problems when produce suffers even short-term temperature abuse; as a result, bacterial growth is readily controlled by adopting low storage temperatures. On the other hand, psychrotolerant bacteria require short storage times even under low temperature conditions. Histamine accumulation occurs normally in the early stages of the fish supply chain (fishing procedures, storage conditions on board fishing vessels, distribution procedures) and this is certainly the case for fresh fish (FDA 2011). Histamine is a highly heat-stable compound so that once it has formed the produce cannot be

detoxified either by domestic cooking or by transformation treatments including autoclave stabilisation in the canning process. In addition, newly formed histidine may be found in canned fish when production problems expose the produce to unsuitable storage/temperature conditions. This produce is intended to be cooked before sterilisation or already cooked and ready for autoclave. As a consequence, possible post-contamination episodes can occur before the autoclave step or when the canned produce is used for multiple purposes—dressings, sandwiches, pizzas—without adequate hygiene measures (clean utensils, suitable storage temperature between subsequent uses).

Generally, the production of histamine is negligible in certain fish species in the early days after capture on condition that storage temperatures are below 4 °C (Taylor 1986). Substantially, psychrotrophs can prevail in refrigerated tuna and determine the rise of histamine amounts at 10 °C. In addition, the histidine decarboxylase activity is apparently detectable and remarkable at 4 °C (Silva et al. 1998). On the other hand, raw tuna and other fish species such as anchovies can reach notable and dangerous histamine amounts after 24 h only when stored at 22 °C (Behling and Taylor 1982; FDA 1982, 2005; Guizani et al. 2005; Rossano et al. 2006; Silva et al. 1998).

Another important reflection has to be considered when speaking of halotolerant and halophilic histamine-producing bacteria (Hernández-Herrero et al. 1999). Substantially, the production of histamine in fish has also been reported in salted products because of the remarkable activity of halotolerant microorganisms such as *Staphylococcus aureus*. Anyway, *Staphylococcus* spp. is considered the most reported histamine producer species when speaking of fermented salted fish such as anchovies. Other important histamine formers belong to *Vibrio* and *Pseudomonas* species (Hernández-Herrero et al. 1999). The selection of similar halotolerant microorganisms is caused by peculiar pH, water activity and sodium chloride values in salted anchovies.

Interestingly, the presence of these microorganisms has been considered (Hernández-Herrero et al. 1999) in relation to the contamination of fish by human activities (capture and unhygienic handling). When speaking of salted fish products, the production of histamine can only be contrasted (Hernández-Herrero et al. 1999) with adequate refrigeration temperatures and high sodium chloride levels (ideally >20 %).

1.3 Analytical Detection of Histamine

At present, different methods are available for the analytical determination of histamine ranging from the AOAC fluorometric procedure (the most important standardised analysis in the United States of America) to high-performance liquid chromatography (HPLC), considered the ‘gold’ reference test in the European Union (EU). Other remarkable methods include enzyme-linked immunosorbent assay (ELISA) tests—with the status of ‘AOAC Research Institute Performance

Tested Methods (SM) procedure'—and the latest rapid stick methods (lateral flow assay and similar tests) for the semi-quantitative determination of histamine in a matter of minutes.

In detail, the following list shows most used testing methods for the determination of histamine (and other biogenic amines, where possible) in seafood (Emborg 2007; FAO/WHO 2012):

(a) Sensorial evaluation. In other words, several controls may be preliminarily carried out by experienced panelists on seafood products. Naturally, this type of control is based on the perception of degradation and quality losses of sampled fish such as canned tuna. It has to be considered that inexperienced consumers may easily be unable to detect even high concentrations in histamine seafood if sensorial evaluation is preferred (Du et al. 2002; Őzogul et al. 2002)
(b) HPLC procedures (Duflos et al. 1999; Malle et al. 1996). These analytical methods (needed time: 1–2 h; limit of quantification: 1.5–5 ppb) are very useful for confirmation and quantification of histamine, in accordance with Reg. (EC) No. 2073/2005 and subsequent amendments (Emborg 2007; FAO/WHO 2012; Onal 2007). In detail, the European reference method is designed for the detection of histamine, putrescine, cadaverine, spermine and spermidine in all fish products. Detectable amines are extracted with perchloric acid and separated by HPLC after reaction with dansyl chloride (Emborg 2007). On the other side of the Atlantic Ocean, the official AOAC 977.13 fluorometric method recommends the extraction of histamine with methanol (Emborg 2007). Extracted substances have to be eluted through an ion exchange column with hydrochloric acid. Finally, a fluorometric detection is carried out with the addition of *o*-phthalaldehyde to eluted solutions
(c) Spectrofluorometric methods (FAO/WHO 2012). These protocols (needed time: 1 h) can reach 1.5 ppb as the limit of quantification, while analytical ranges are between 1.5 ppb and 100 ppm. Probably, the best advantage of spectrofluorometric methods is strictly correlated with low analytical costs
(d) ELISA systems. These methods are carried out with spectrophotometers. The protocol is fast, simple and is designed to manage multiple samples at the same time
(e) Colorimetry. Similarly to ELISA methods, they are carried out with spectrophotometers. The protocol is fast, simple and is designed to manage multiple samples at the same time. Colorimetric systems offer the same advantages of ELISA testing methods. In addition, a sort of semi-quantitative evaluation can be obtained by means of visual colorimetric protocols.

Other controls may be naturally made on fish and seafood products; however, the evaluation of microbial spoilage and the determination of bacterial species are not the aims of this book. However, it can be highlighted that new strategies also comprehend mathematical modelling programs when speaking of microbial spoilage. In particular, histamine production can be correlated with the growth of microorganisms such as *M. morganii* and *M. psychrotolerans* in certain fish and

fishery products. As a result, the prediction of histamine production may be carried out by means of predictive modelling systems such as the Seafood Spoilage and Safety Predictor (Dalgaard 2009; FAO/WHO 2012; Naila et al. 2010). From the practical viewpoint, several molecular methods for the detection of histamine-producing bacteria are essentially based on the detection of the gene encoding histidine decarboxylase. However, these testing methods need to be improved at present (Emborg 2007).

1.4 New Possible Strategies Against Histamine in Fish Products

Finally, the control of biogenic amines and histamine in the food industry (and correlated sectors) can be briefly described. At present, main strategies are (Brunazzi et al. 2014; Emborg 2007; López-Sabater et al. 1994; Naila et al. 2010; FAO/WHO 2012; Parisi 2009):

(a) The addition of starter cultures with the aim of degrading produced histamine
(b) The application of hydrostatic pressures
(c) Irradiation methods
(d) The addition of chemicals (citric acid, D-sorbitol, sodium and potassium sorbate, etc.) with different functions, including preservatives
(e) The modification of the microbial ecology in certain products (in accordance with mathematical predictive models)
(f) The use of innovative packaging solutions: modified atmosphere packaging techniques and active (smart) packages.

Many of these procedures can be surely defined 'risk management options' when speaking of safety and risk assessment (FAO/WHO 2012). The remaining part of possible strategies can be briefly listed as follows:

- Chilling
- Freezing
- Gutting and gilling of highly-perishable fish products
- Heating processes.

Naturally, the use of one or more of these strategies has to be considered in the ambit of HACCP plans. The use of a reasonable risk management approach can be very useful with reference to food safety and the reduction of rejection costs, although the decrease of histamine levels cannot be always correlated with economic advantages in specific business areas (FAO/WHO 2012).

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