HISTAMINE IN FISH

Alina PŞIBILSCHI*, Eufrozina NIGA*

* University "Stefan cel Mare" of Suceava , Food Engineering Faculty, 13 Universitatii St., cod 720246 Suceva, Romania

Abstract: *Most of the fish naturally contain relatively large amounts of histidine (an amino acid) in their muscle. After the fish dies, the variety of live bacteria on the fish continue to grow, and in the process, can change histidine to histamine, especially at high temperatures. The level of histamine in fish, frozen or fresh is affected by the presence of certain proteins which are converted to allergenic products by bacteria present. Correct temperature control can limit the production of histamines.*

Keywords*: histidine, histamine, fish, temperature.*

Introduction

Histamine was discovered in 1910 by Dale and Laidlaw, and it was identified as a mediator of anaphylactic reactions in 1932. Histamine belongs to the biogenic amines and is synthesized by the pyridoxal phosphate (vitamin B_6) – containing L-histidine decarboxylase (HDC) from the amino acid histidine. It is a hydrophilic vasoactive amine.

Once formed, histamine is either stored or rapidly inactivated. Histamine released into the synapses is broken down by acetaldehyde dehydrogenase. It is the deficiency of this enzyme that triggers an allergic reaction as histamines pool in the synapses. Histamine is broken down by histamine-N-methyltransferase and diamine oxidase. Some forms of foodborne disease, so-called "food poisonings," are due to conversion of histidine into histamine in spoiled food, such as fish.

Histamine is a potent mediator of numerous biologic reactions. Histamine exerts its effects by binding to its 4 receptors [histamine1 receptor (H_1R), H_2R, H_3R, and and H_4R] on target cells in various tissues (Table 1).

Table 1: Mechanism of action (Ababouch L., 1990)

Type	Location	Function
H_1 histamine receptor	Found on smooth muscle, endothelium, and central nervous system tissue	Causes vasodilation, bronchoconstriction, smooth muscle activation, separation of endothelial cells (responsible for hives), and pain and itching due to insect stings; the primary receptors involved in allergic rhinitis symptoms and motion sickness.
H_2 histamine receptor	Located on parietal cells	Primarily stimulate gastric acid secretion
H_3 histamine receptor	-	Decreased neurotransmitter release: histamine, acetylcholine, norepinephrine, serotonin
H_4 histamine receptor	Found primarily in the thymus, small intestine, spleen, and colon.	Unknown physiological role.

It causes smooth muscle cell contraction, vasodilatation, increased vascular permeability and mucus secretion, tachycardia, alterations of blood pressure, and ar-

rhythmias, and it stimulates gastric acid secretion and nociceptive nerve fibers. Histamine can be metabolized in 2 ways: by oxidative deamination by DAO (former name: histaminase) or by ring methylation by histamine-*N*-methyltransferase (HNMT) (Figure 1).

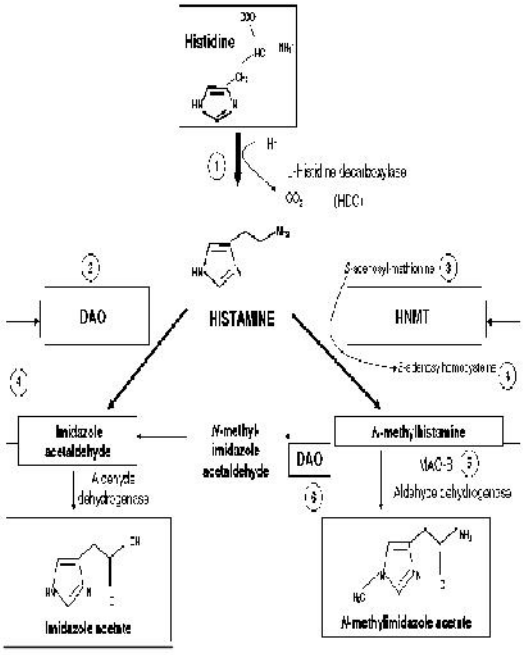

Figure 1. Summary of the histamine metabolism (Ababouch L., 1990)

The biogenic amine histamine is synthesized by decarboxylation of the amino acid histidine catalyzed by L-histidine decarboxylase (HDC) (1). Histamine can be metabolized by extracellular oxidative deamination of the primary amino group by diamine oxidase (DAO) (2) or intracellular methylation of the imidazole ring by histamine-*N*-methyltransferase (HNMT) (3). Therefore, insufficient enzyme activity caused by enzyme deficiency or inhibition may lead to accumulation of histamine. Both enzymes can be inhibited by their respective reaction products in a negative feedbackloop (4). *N*-Methylhistamine is oxidatively deaminated to *N*-methyl-imidazole acetaldehyde by monoamine oxidase B (MAO-B) (5) or by DAO (6). Because the methylation pathway takes place in the cytosolic compartment of cells, MAO B (5) has been suggested to catalyze this reaction in vivo (Ababouch L., 1990).

Biogenic amine production and its control

Almost all foods has bacteria on it right from the start. This is because most food comes from the natural environment where bacteria are always present. When meat is stored in the temperature danger zone of between 5˚C and 60˚C, even for short periods, the number of bacteria present can multiply very quickly. Fish are a particular concern when it comes to maintaining temperatures of less than 50˚C.This is because if the fish contain an amino acid called histidine, the bacteria can turn this into histamine which is a physiological amine responsible for many allergic reactions. Once histamine is formed it is not destroyed by freezing, smoking, cooking.

Many types of bacteria are capable of producing the biogenic amines regarded as causing histamine poisoning. Also, the enzymes responsible (decarboxylases), once produced, may still be active even if the bacteria that produced them are subsequently controlled. Control of biogenic amine production is best summarised by food type. Histamine food poisoning is most often associated with a particular group of fish, including the scombroid fish, that have high free levels of the amino acid histidine in their flesh. Fresh scombroid fishes do not contain free histamine, and amines are only produced during temperature abuse and spoilage. For example storage of mackerel for 18 days at 0˚C resulted in little histamine formation, but high levels were found after only 5 days storage at 10˚C levels. Scombroid fish can have a 14 day safe shelf life at 0˚C if chilled quickly (meaning reducing the internal temperature to 10˚C or less in 6 hours), but this reduces

to only 7 days at 4.4˚C (these times include time on the boat). The fish should not be exposed to temperatures > 4.4˚C for more than 4 hours after the initial chilling. Vacuum packaging is not an effective means of retarding the production of amines. Salting may result in the selection of salt tolerant bacteria that may also produce amines. Preservatives, or other interventions that inhibit the growth of bacteria, will also inhibit the production of amines. Fish will store indefinitely if frozen (Silva C.C.G. Da Ponte, 1998).

The Illness

Incubation: Ranges from several minutes to several hours. Mean incubation period around 1 hour (Mitchell J., 1993).

Duration: Normally lasts for a few hours but can last for days.

Symptoms: Histamine. May include rash, localised skin inflammation, nausea, vomiting, diarrhoea, abdominal cramps, low blood pressure, headache, tingling, flushing and severe respiratory distress. The most consistent sign is a flushing of the face and neck causing heat and discomfort, which can appear similar to sunburn. This acts indirectly to increase blood pressure by narrowing peripheral blood vessels and increasing the output from the heart. Other symptoms include dilation of the pupils, swelling of the eyes and tear production, salivation, increased respiration and blood sugar concentration.

Toxins: The actual nature of the toxin is the subject of much debate, at least in fish. The biological effects of histamine are reported to be increased in the presence of other spoilage products, as fish containing a level of histamine seem to be more toxic than the same amount of histamine administered orally by itself. Another theory suggests that an unknown toxin from spoiled fish actually mediates the release of histamine from the body's cells. Whatever the actual toxin(s) involved, biogenic amines in food are at least indicators of the presence of these toxin(s).

At Risk Groups: All consumers are at risk, although there may be sub-groups of different susceptibilities. The enzymes that these drugs inhibit are those that remove these toxins in healthy individuals.

Long Term Effects: Rarely, cardiac and respiratory complications occur.

Dose: 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.

Treatment: Administration of antihistamines for histamine poisoning.

Fish are the most likely to be involved with histamine poisoning. These include mackerel, tuna, bonito, and butterfly kingfish. Other fish species also implicated include sardines, pilchards, anchovies, herring and marlin (Lehane L., 2000).

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 contained 100 mg/100g or more. Fermented fish products may contain appreciable levels of histamine, but there is no association between consuming this sort of food and disease.

In 1995, a study effectuated by the FSV, of 55 samples purchased from supermarkets, 8(15) exceeded the then-regulated level (100mg/kg) of histamine (table 2).

Table 2: Average levels of biogenic amines, mg/kg (Bartholomev B.A., 1997)

Food/Amine mg/kg	Crab, Tuna	Sardines, Anchovies	Dried Mackerel	Squid Prawns
Histamine	10	30	35	40
Putrescine	2	25	25	540
Cadaverine	20	50	65	540

Outbreaks and incidents

New Zealand - *Kahawai*: 3 cases (histamine content 200 mg/100g).

Overseas: - *Blue Marlin*: 28 cases among 57 exposed, 13 hospitalised. Control measure failure: temperature abuse.

Tuna: 7 cases, three hospitalisations (histamine content 2000mg/100g). Control measure failure: likely temperature abuse.

Western Australian *Salmon*: 7 cases, one hospitalisation (histamine content of two samples tested 60 and 245 mg/100g).

Fried White Tipped *Mackerel*: 41 cases, all hospitalised. Control point failure: temperature abuse.

Kingfish: 2 cases: (histamine content 600 mg/100g).

Smoked Mackerel: 4 cases (histamine content 300 mg/100g). Control point failure: temperature abuse.

Tuna Burgers and fillets: 5 outbreaks involving 20 cases (histamine content up to 325 mg/100g in burgers). Control point failure: inadequate refrigeration, failure to clean and sanitise grinder between preparation of burgers (Bartholomev B.A., 1997).

Materials and methods

Samples of the three fish were selected for replicate determination and subsamples from each fish were selected for duplicate determination.

The tuna fillets were cut into single samples that were smaller, more manageable portions, and stored in individual sterile plastic jars.

Samples were stored at ambient temperature (20°C), at 4°C and at 0°C. For storage at ambient temperature, samples were placed in a laboratory in thermostat and left at 20°C for the period of the experiment. For storage at 4°C, the samples were stored in the laboratory's cool room (temperature range 4-6°C). For storage at 0°C the samples were packed in ice and again stored in the laboratory's cool room.

Biogenic amines are extracted from the finely minced fish flesh using 75% methanol in water (AOAC, 16th Edition). The extract is passed through an anion exchange resin to remove potential interfering compounds. The biogenic amines pass through the column. The solvent is removed under vacuum and the residue treated with dansyl chloride. The residue is then mixed with water and the dansylasted biogenic amines extracted with diethyl ether. The diethyl ether is removed under vacuum and the residue dissolved in acetonitrile. The individual amines are then determined by HPLC using a C_{18} reverse phase column with UV detection at 254 nm (Eerola S. et al., 1993).

Results and discussion

Storage at ambient temperature (20°C) - The biogenic amine data for the trial at ambient temperature indicate histamine is produced in significantly large quantities as the fish degrades during the life of the experiment (Table 3). At ambient temperature (20°C), histamine production commenced after approximately 40 hours and increased to the order of 100 mg/kg after 50 hours. After 90 hours the level of histamine for sample 1 was 1200.2 mg/kg, sample 2 reached a maximum histamine level of 1200.2 mg/kg after 70 hours and sample 3 reached a maximum histamine level of 1200.2 mg/kg after 90 hours.

Table 3: Storage at ambient temperature (20°C)

Time, h	Histamine, mg/kg			
	Sample 1	Sample 2	Sample 3	Average
10	0	0	0	0
20	16.3	0	7.2	7.83
30	17.9	2.8	8.7	9.8
50	71.8	150.4	90.2	104.13
70	730.1	1200.2	1000.1	976.8
90	1200.2	640.5	1200.2	1013.6

Storage at 4°C - After day 17, histamine production has reached its maximum in all the fish (table 4).

Table 4: Storage at 4°C

Time, days	Histamine, mg/kg			
	Sample 1	Sample 2	Sample 3	Average
0	1	1	1.2	1.06
5	42	2	20.8	21.6
10	53.2	73	57	61.06
14	17	130.1	100.8	82.63
17	61.1	150.3	136.7	116.03
20	32	18.7	25	25.23

Storage at 0°C - At 0°C the production of histamine does not begin until after day 4 (Table 5). The maximum histamine levels produced are 46, 77 and 65.2 mg/kg for sample 1, sample 2 and sample 3.

Table 5: Storage at 0°C

Time, days	Histamine, mg/kg			
	Sample 1	Sample 2	Sample 3	Average
0	1	1	1	1
5	1	2.3	1.7	1.56
10	25.3	13.5	18.9	19.06
14	46	46	45.4	45.8
17	26.8	77	65.2	56.3
20	15.02	32.4	29.1	25.5

The averaged levels of histamine in sample 1, sample 2 and sample 3 at 0°C, 4°C are summarised in fig. 2.

Conclusions

Histamine is not destroyed by cooking. Therefore, the best way to keep histamine at a minimum is to ensure proper temperature control .

In some cases, low levels of histamine may already be present in the fish when you receive it.

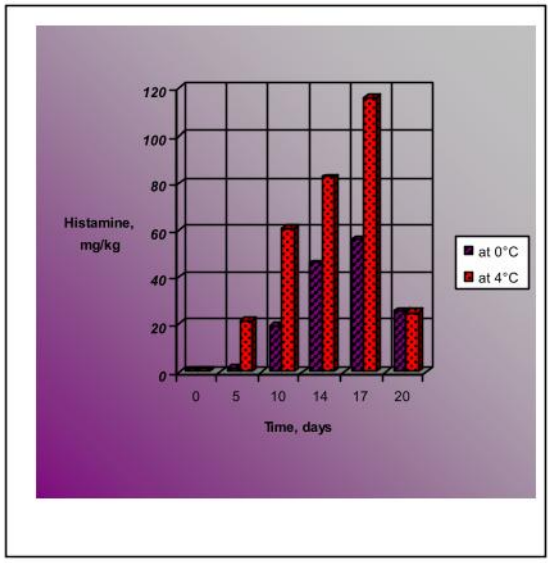

Figure 2: Storage at 0°C and 4°C

To stop it increasing to levels of concern, you should always:

- before leaving the dock, pack cooler have to be large enough for the fish you hope to catch, full with bags of ice;
- on the water, before casting, prepare the coolers by opening a couple bags of ice and forming a layer of ice on each cooler bottom;
- as soon as the fish is brought on board, stun it by clubbing it with a blunt instrument, aiming for the soft part of the head between the eyes;
- purchase from reputable suppliers who store the fish on ice or under refrigeration;
- receive product at refrigerated temperatures (<5°C);
- place the fish under refrigeration as soon as it is received;
- keep the fish at refrigerated temperatures when not being used;
- if the fish is frozen, thaw the fish under refrigeration.

Adequate processing guidelines for fish have been derived from published information. To prevent the formation of his-

tamine we have to hold fish at the temperature recommended in the table 6 (Silva C.C.G. Da Ponte, 1998).

Table 6: Adequate processing guidelines (Silva C.C.G. Da Ponte, 1998).

Temperature	Time
-18˚C	no limit
0˚C	up to 14 days
4.4˚C	up to 7 days
10˚C	up to3 days
21˚C	0 days

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