Histamine Formation in Fermented Seafood Products

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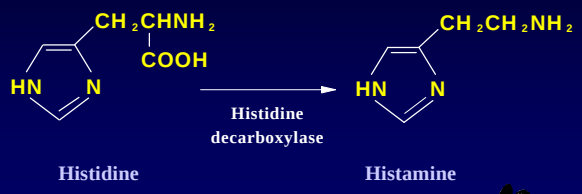
Scombroid Poisoning

- Causative agent: Histamine
- Caused by ingestion of fish containing >50 mg/100 g fish (500 ppm)
- U.S. FDA guideline: 50 ppm
- Stable to heat and freeze
- No color or odor to indicate its presence
- Symptoms
  - Rash, edema, headache, flushing, tingling, cramps, diarrhea, nausea, and dizziness

Anchovies are a semipreserved fish product obtained without heating, and therefore histamine contents are subject to change during its storage. Anchovies used as the starting material contain high levels of free histidine in the muscle, which is the substrate of histidine decarboxylase. In addition, canned anchovies are produced by natural fermentation process at ambient temperature without temperature control. The only process barrier for the formation of histamine is the high concentration of salt used in the process, often exceeding 15%. However with the health consciousness of the public, the salt concentration has been lowered to satisfy the consumer's demand. Canned anchovy products usually sold in groceries without refrigeration, which can lead to continuous formation of histamine until consumed.

Histamine formation



Chemical mechanisms of histamine formation is decarboxylation of free histidine by the bacterial enzyme, histidine decarbxylase. The decarboxylation of histidine is histamine, one of the active biogenic amines involved in neurotransmission and other important biological functions in human. It is often recognized that histamine may not be the sole etiological agent for scombroid poisoning. It has been consistently shown that other biogenic amines formed together with histamine enhances the toxicity of histamine both directly and indirectly affecting health.

Species Susceptible to Histamine

Yellowfin

Mahimahi

Skipjack

Sardine

Bluefish

Several fish species are susceptible to histamine. They may or may not belong to Scombridae. What is important is the content of free histidine in muscle. The term "free histidine" is used to describe the histidine in the free amino acid pool in the muscle in comparison to that incorporated in peptide as in skeletal muscle. The Scombrid fish has been shown to contain extremely high levels of histamine, sometimes exceeding 1% by wet weight.

Prerequisites for Formation of Histamine in Fresh Fish

- **Histidine**
- **Bacteria**
- **Histidine decarboxylase**
- **Conditions to allow bacterial growth and enzyme activity**
  - **Time**
  - **Temperature**

Histamine formation often requires the combination of right conditions which allows its formation. Histamine is formed by the bacterial enzyme, histidine decarboxylase. The enzyme decarboxylates "free" histidine in fish and forms histamine. Often the enzyme activity curve follows the bacterial growth curve, and its activity is highly visible following bacterial exponential growth. Therefore, the histamine formation is most often observed in fish containing high levels of histidine exposed to high temperature or not chilled properly for a lengthy period of time.

Challenges for canned anchovies

- Imported
- Produced by fermenting outdoors at ambient temperature
- Lower salt concentration
- Stored at room temperature up to 2 years or more until sold
- In the can but not heat-treated!
- Consumed in small quantities
- Other similar products: fish sauce

Anchovies are a semipreserved fish product obtained without heating, and therefore histamine contents are subject to change during its storage. Anchovies used as the starting material contain high levels of free histidine in the muscle, which is the substrate of histidine decarboxylase. In addition, canned anchovies are produced by natural fermentation process at ambient temperature without temperature control. The only process barrier for the formation of histamine is the high concentration of salt used in the process, often exceeding 15%. However with the health consciousness of the public, the salt concentration has been lowered to satisfy the consumer's demand. Canned anchovy products usually sold in groceries without refrigeration, which can lead to continuous formation of histamine until consumed.

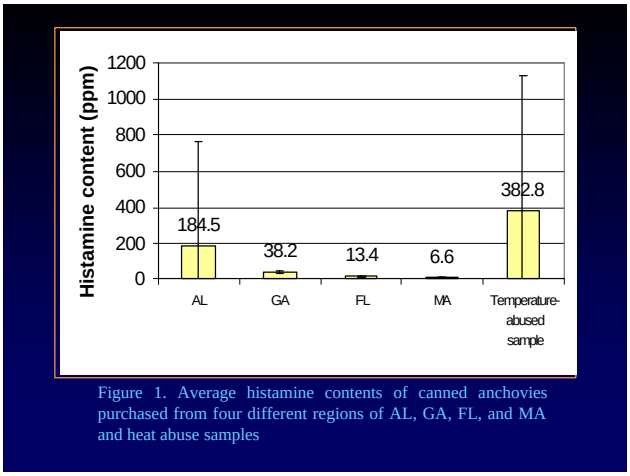


Figure 1. Average histamine contents of canned anchovies purchased from four different regions of AL, GA, FL, and MA and heat abuse samples

Figure 1. The canned anchovies obtained from four different regions in the Southeast and Northeast showed a wide range of histamine contents. The products obtained from the Massachusetts with the coldest climate among the regions tested showed the least amount of histamine with the average of 6.6 mg histamine/100 g fish. The products obtained from the Southern areas of Florida or Georgia showed the slightly increased levels of histamine, but still below the FDA guideline, 5 mg/100 g fish. However, the samples obtained from the Alabama region showed almost four times the FDA guideline, averaging at 184.5 ppm histamine/100g fish. Although the average was high, most samples contained acceptable levels of histamine. Of the twelve samples analyzed, ten contained less than 17 mg histamine/100 g of fish. The two products containing high levels of histamine resulted in the overall high histamine content. This data brought our awareness that histamine formation is an individual event rather than the group event, therefore more focus should be given to individual products during monitoring. Similar result was obtained with temperature-abused sample which were stored at room temperature over a year period of time after the purchase at the local grocery stores. The long-term storage increase the total average histamine content of the products. However, similar to what was observed with the products obtained from the Alabama region, we also observed the small number of products with extremely high content of histamine which drove the average content of histamine high, although the majority of the products were safe with the histamine levels below the FDA guideline.

Table 1. Histamine contents in canned anchovy obtained from four different regions of AL, MA, GA, and FL

Regions	Histamine[a]	Regions	Histamine
AL	2015 ± 13.1	MA	7.29 ± 1.23
	90.7 ± 0.10		6.53 ± 0.13
	13.4 ± 0.04		5.99 ± 0.01
	11.3 ± 0.22	GA	44.8 ± 1.10
	6.94 ± 0.37		35.4 ± 0.12
	5.33 ± 0.10		34.4 ± 0.26
	13.8 ± 0.09	FL	8.93 ± 0.51
	8.80 ± 0.14		15.3 ± 0.10
	8.18 ± 0.15		10.1 ± 1.35
	7.61 ± 0.24		10.7 ± 1.18
	14.9 ± 0.43		17.0 ± 2.01
	18.0 ± 0.13		18.3 ± 1.09

[a] Histamine contents (ppm) in sample were determined by AOAC method. Average ± standard deviation (n=2).

Table 1 is shown to support the data shown in Figure 1.

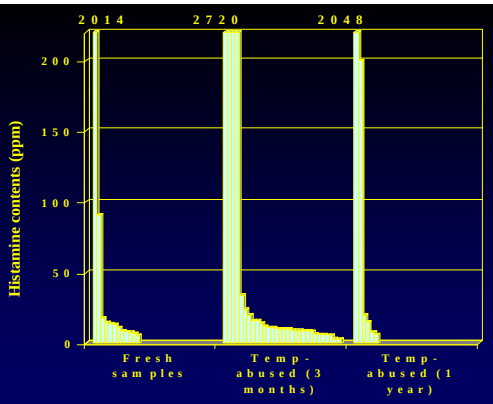


Figure 2. Histamine contents of the fresh and temperature-abused samples for 3 months to 1 year. All samples were obtained from local groceries in AL.

Figure 2. From the previous observation that the histamine formation is an individual event, we conducted the storage study for three months and one year. As observed previously, most of the canned anchovy products contained histamine levels below the FDA guideline. What made the difference was the frequencies of the incidents that the canned products contained elevated levels of histamine. As the storage period increased, the higher ratio of cans were detected to contain histamine over 200 mg/100 g of fish (or 2000 ppm). The average frequency of the canned anchovies containing over 200 mg/100 g of fish was approximately 10%, which increased to over 15% with the sample storage at room temperature for 1 year.

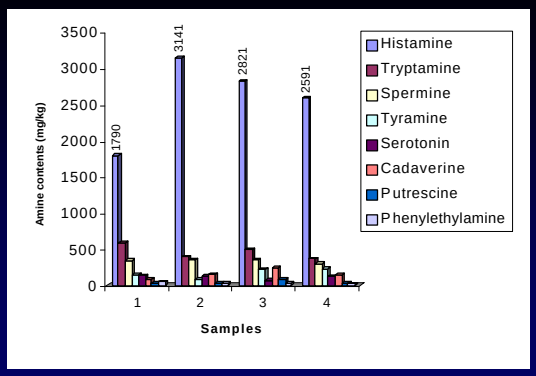


Figure 3. Biogenic amine contents in the canned anchovies containing over 1000 ppm

Figure 3. When biogenic amine content were analyzed for a few samples selected from those containing over 1000 ppm histamine (or 100 mg histamine/100 g of fish), it was shown that histamine was the amine with the highest level among all the biogenic amines. The second most prevalent biogenic amine was tryptamine followed by spermine, tyramine and cadaverine. This result provided an evidence that histamine is a good indicator for scombroid poisoning due to its prevalence and high concentration in seafoods compared with other biogenic amines.

Table 2. Aerobic and anaerobic plate counts of commercial canned anchovy containing over 50 ppm histamine

Brand	Sample code	Histamine contents[a]	APC[b] 0.5%[d]	APC[b] 5%	ANPC[c] 0.5%	ANPC[c] 5%
	A	1976 ± 21.9	N.D.[e]	N.D.	N.D.	N.D.
		2666 ± 103	N.D.	N.D.	N.D.	N.D.
		2639 ± 21.9	N.D.	N.D.	N.D.	N.D.
		2720 ± 123	N.D.	N.D.	N.D.	N.D.
	B	6.69 ± 0.03	N.D.	N.D.	N.D.	N.D.
		12.1 ± 0.30	N.D.	N.D.	N.D.	N.D.
		24.0 ± 0.45	N.D.	N.D.	N.D.	N.D.
		34.2 ± 3.01	N.D.	N.D.	N.D.	N.D.
		16.0 ± 4.50	N.D.	N.D.	N.D.	N.D.
		10.5 ± 1.07	N.D.	N.D.	N.D.	N.D.

[a] Histamine was determined by AOAC method. Average ± standard deviation.
[b] Aerobic plate count (CFU/g).
[c] Anaerobic plate count (CFU/g).
[e] Not detected (<10^2 cfu/g).

Table 2. To identify the prevalent histamine-forming bacteria, we grouped the canned anchovies into the low and high level containing histamines. The low level group contained less than 50 ppm histamine, while the high level group contained higher than 2000 ppm histamine. Regardless of the histamine contents, no viable bacteria were detected for both aerobic and anaerobic counts supplemented with 0.5% to 5% salt. This data indicated that the common bacteria flora are not histamine formers. Based on the previous data that the canned anchovies continued to form histamine, although in small numbers of the cans, it is speculated that the histamine formers are small in numbers and may not be detected by the conventional microbiological method.

Table 3. Aerobic and anaerobic plate counts in commercial canned anchovy showing the positive results on aerobic plate counts

Brand	Sample code	Histamine contents[a]	APC[b] 0.5%[d]	APC[b] 5%	ANPC[c] 0.5%	ANPC[c] 5%
	C*	16.1 ± 0.05	4.3×10^2	3.5×10^2	N.D.[e]	N.D.
		8.74 ± 2.38	4.7×10^2	4.2×10^2	N.D.	N.D.
		8.82 ± 0.20	4.1×10^2	2.9×10^2	N.D.	N.D.
		8.53 ± 0.10	3.3×10^2	2.6×10^2	N.D.	N.D.
		9.24 ± 0.03	1.7×10^2	1.2×10^2	N.D.	N.D.
		3.38 ± 0.49	2.9×10^2	1.7×10^2	N.D.	N.D.
		3.04 ± 0.69	3.1×10^2	3.3×10^2	N.D.	N.D.
		14.3 ± 0.25	2.0×10^2	N.D.	N.D.	N.D.

[a] Histamine content (ppm) in sample were determined by AOAC method. Average ± standard deviation.
[b] Aerobic plate count (CFU/g).
[c] Anaerobic plate count (CFU/g). All samples showed negative result in anaerobic plate count.
[e] Not detected (<10^2 cfu/g).

Table 3. To confirm the previous data, the histamine contents were measured for the cans containing high numbers of aerobic plate counts (over 100 cfu/g). It was again shown that cans with bacterial counts did not correlate with the histamine contents, as none of them had histamine levels over 20 ppm, well below the FDA guideline.

Table 4. Identification of bacterial isolates from commercial canned anchovy without enrichment

Strain[a]	Histamine[b]	NaCl
Bacillus licheniformis	0.67	0.5%
Bacillus licheniformis	1.35	
Bacillus licheniformis	0.73	
Bacillus subtilis	1.98	
Bacillus subtilis	6.96	
Bacillus subtilis	4.05	
Bacillus licheniformis	0.98	5%
Bacillus megaterium	1.08	
Bacillus licheniformis	1.75	
Bacillus licheniformis	2.88	
Bacillus subtilis	0.33	
Bacillus licheniformis	2.09	

[a] Strains were identified by Microbial Identification System (MIS)
[b] Histamine production by isolate was determined by AOAC method.

Table 4. When the bacteria isolated from the canned anchovies from the the aerobic count plates supplemented with 0.5% to 5%, all the species identified belonged to Bacillus. Each isolate shown in cultures showed that they were poor histamine formers with all showing the histamine content in culture below 10 ppm. Behling and Taylor have shown that high histamine formers were capable of forming histamine higher than 1000 ppm in culture. Therefore, it was concluded that most bacteria present in canned anochovies were not histamine formers.

Table 5. Histamine production by isolates from canned anchovy with enrichment

	Ranges of histamine produced (No. isolates tested[b])			
Brand	0.5%[c]	5%	10%	15%
	0.00-4.10 (6)	0.14-1.60 (5)	N.D. [d]	N.D.
	0.11-1.55 (16)	0.00-0.82 (15)	N.D.	N.D.
	1.83-5.09 (21)	0.09-5.62 (9)	1.39-2.84 (4)	N.D.
	0.00-2.17 (8)	0.24-3.90 (9)	N.D.	N.D.
	1.02-4.04 (14)	1.08-4.96 (9)	N.D.	N.D.
	0.38-3.98 (7)	0.35-1.19 (11)	1.75-3.63 (19)	N.D.
	0.68-3.66 (27)	0.64-1.45 (11)	N.D.	N.D.
	0.63-5.77 (29)	2.64-4.19 (10)	0.66-3.80 (7)	N.D.
	0.38-4.82 (10)	0.70-3.03 (10)	0.82-3.40 (6)	N.D.
	0.90-2.87 (7)	0.70-5.36 (12)	1.44-3.85 (7)	N.D.
	0.11-3.89 (8)	0.34-1.02 (5)	1.10 (1)	N.D.
	0.00-1.85 (33)	0.21-1.81 (12)	N.D.	N.D.
	0.56-4.36 (9)	1.33-2.01 (3)	N.D.	N.D.
	0.42-1.25 (11)	0.78-3.51 (12)	N.D.	N.D.
	0.54-2.18 (8)	1.08-4.05 (9)	N.D.	N.D.

[a] Histamine production by isolate was determined by AOAC method.
[b] Colonies were selected from TSA (Tryptic Soy Agar) after enrichment by using TSB (Tryptic Soy Broth) supplemented with NaCl
[c] NaCl concentration in TSA.
[d] Not detected.

Table 5. To help isolate potential histamine forming bacteria, enrichment method was employed prior to plating. It was shown that most numbers of bacteria were isolated by the supplementation of salt at 0.5%. With the supplementation of 15%, which is the concentration of salt used in canned anchovies, no bacteria were isolated. Despite the levels of salt supplemented, no isolates were detected which were capable of forming high levels of histamine. Most isolates only produced negligible levels of histamine, below 10 ppm.

Table 6. Identification of bacteria from commercial canned anchovy with enrichment

Strain[a]	Histamine[b]	NaCl conc.[c]
Bacillus subtilis	0.65	5%
Bacillus subtilis	1.30	
Bacillus subtilis	0.58	
Bacillus subtilis	1.02	
Bacillus cereus	1.19	
Bacillus cereus	1.02	
Bacillus cereus	0.74	
Bacillus cereus	0.93	
Bacillus amyloliquefaciens	1.10	10%
Bacillus licheniformis	3.18	
Bacillus licheniformis	2.74	
Bacillus licheniformis	2.76	
Bacillus licheniformis	3.63	
Bacillus licheniformis	3.14	

[a] Strains were identified by Microbial Identification System (MIS)
[b] Histamine production by isolate was determined by AOAC method.
[c] NaCl concentraion in TSA.

Table 6. As seen with Table 4, all the bacteria isolated with enrichment were also identified as Bacillus.

Conclusions

- Great individual variations
- High histamine found randomly
- Starting materials important
- Histamine increases during storage
- Control by cold storage?

In conclusion, we found that there were great variations in individual cans. This fact may necessitate the need to analyze a large numbers of cans in the product lot to ensure detection of the cans containing high levels of histamine. Our study showed that even within a lot with high average contents of histamine, only a small numbers of cans contained histamine levels over the FDA guideline, 50 ppm, and the majority of the cans contained histamine below the guideline. Our result showed that the starting material quality of fish may be very important as once histamine forming bacteria are present, it is difficult to remove or destroy them. The main bacterial flora present in the products were identified as Bacillus. Although it is difficult to isolate the histamine formers by the conventional methods, our result showed that they are present in the canned anchovies as evidenced by the detection of more ratio of cans containing high levels of histamine during storage without temperature control. Therefore it has been suggested to store canned anchovies in cold storage to minimize the formation of histamine during storage and marketing.

Wholesome Safe Seafood
Anytime Anywhere!

Seafood safety is a necessity not a choice!