NSW Food Authority

safer food, clearer choices

Presence of histamine in anchovies

NSW/FA/FI079/1007

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Executive summary

In 2009 a case of scombroid poisoning occurred due to high levels of histamine in anchovies. In response, the Authority conducted a survey on histamine levels in anchovies in oil and dried anchovies. Between May 2009 and August 2009, 45 samples of anchovies in oil and 34 samples of dried anchovies were purchased from retail outlets across Sydney and tested for histamine. Standard 2.2.3 in the Australia New Zealand Food Standards Code (the Code) states that the level of histamine in fish and fish products must not exceed 200 mg/kg.

- 97.8% of anchovies in oil contained less than 200 mg/kg of histamine and were compliant with the Code.
- 97.1% of dried anchovies contained less than 200 mg/kg of histamine and were compliant with the Code.

Appropriate enforcement action was taken on samples with levels greater than 200 mg/kg.

The average level of histamine in anchovies in oil was 33 mg/kg and ranged from not detected to 518 mg/kg. The average level of histamine in dried anchovies was 35 mg/kg and ranged from not detected to 483 mg/kg.

One sample of anchovies in oil was tested after storage at room temperature for several months (the manufacturer's storage instructions were store at refrigerated temperatures). The level of histamine in this sample was 2116 mg/kg. Subsequent samples of this batch were sourced from the wholesaler (these had been correctly stored) and no detectable levels of histamine were found. This result highlights the importance of correct storage. It was re-iterated to the wholesaler to ensure his stockists were storing this product correctly.

Introduction

Anchovies (*Engraulis* spp) are small, common salt water fish. They are found in temperate waters such as the Mediterranean. Popular in Australia, 967 tonnes of canned anchovies worth $9,247,000 were imported into Australia during 2007-2008 (ABARE, 2009).

Canned anchovies or anchovies in oil are prepared by gutting and placing them in salt brine for 6-10 months for curing. During processing the salt levels are critical as too much salt will rupture the fish and the anchovies will become unpalatable and too little salt will allow the growth of spoilage organisms. After curing, the anchovies may be washed, skinned, filleted, scaled, trimmed and packed in either salt or oil (Napoleon Co Anchovies, n.d.). Alternatively anchovies can be dried naturally or artificially and sold as a dried product.

In 2009, a case of scombroid poisoning occurred in NSW that was linked to canned anchovies, The anchovies implicated were tested and found to contain 365 mg/kg of histamine.

Histamine

Histamine is a biogenic amine that can be found in decaying fish and is a cause of food borne illness; specifically scombroid poisoning.

Fresh caught fish does not contain histamine (ESR, 2001). However, once fish starts to decay or is subjected to temperature abuse histamine can start to accumulate. Histamine is formed when histidine, a naturally occurring compound in fish, is converted into histamine by certain bacteria. Histamine formation in fish is controlled by three main factors:

1. Levels of free histidine

   Histidine is naturally found in high levels in the muscular flesh of dark fleshed fish such as mackerel, tuna, sardines and anchovies. These are often the main fish implicated in scombroid poisoning (Lehane and Olley, 1999).

2. Contamination levels of histidine decarboxylating bacteria

   It is believed that the bacteria responsible for histidine conversion into histamine (mainly from the *Enterobacteriaceae* family such as *Klebsiella*, *Morganella* and *Hafnia*) are either present in the gills or gut cavity, or introduced during poor handling and processing (as discussed in Lehane and Olley, 1999). High levels of histamine have been found in fish with high initial bacterial loads (Pons-Sanchez-Cascado, Veciana-Nogues, Bover-Cid, Marine-Font, Vidal-Carou, 2005; Veciana-Nogues, Albala-Hurtado, Marine-Font, Vidal-Carou, 1996).

3. Temperature abuse

   During temperature abuse bacterial growth to the point of histamine production can occur in as little as 3-4 hours (Ross and Sanderson, 2000). This can result in histamine reaching toxic levels before organoleptic changes take place. Once the levels of bacteria have increased and started to produce histamine, residual enzyme activity will still occur at refrigerated temperatures (although bacterial growth ceases) (Ross and Sanderson, 2000). Previous studies have shown that histamine can form during processing and storage (Pons-Sanchez-Cascado et al, 2005; Veciana-Nogues et al, 1996). Cooking does not destroy histamine.

A low level of histamine in food is not necessarily hazardous. Many foods contain small amounts of histamine that are easily detoxified by enzymes in the intestinal tract (Shalaby, 1996). It is believed that the ingestion of other amines, such as cadaverine and putrescine

(compounds produced in decaying foods), prevent the breakdown of histamine in the intestinal tract, which enhances the effect of histamine. Research has indicated that histamine in food appears to be more toxic than the equivalent amount taken orally (as discussed in Lehane and Olley, 1999).

There are still many unknowns regarding histamine and its toxicity.

Regulatory limits

Prescribed limits for histamine in fish have been set by various regulatory bodies around the world:

- The Australia New Zealand Food Standards Code (the Code) prescribes the limit for histamine in fish at 200mg/kg.
- US Food and Drugs Administration sets 50mg/kg as an indicator of decomposition and 200mg/kg as hazardous (FDA, 2001).
- Codex sets 10mg/100g (100mg/kg) as an indicator of decomposition and 20mg/100g (200mg/kg) as an indicator for poor handling and hygiene. (Codex Alimentarius, 2003; Codex Alimentarius, 2007)
- The European Communities microbiological criteria for foodstuffs states that nine samples must be tested in a batch with the mean less than 200 mg/kg with no sample greater than 400 mg/kg and no more than two samples greater than 200 mg/kg (European Commission, 2006).

Other proposed guidelines

Shalaby (1996), proposed the following guidelines for histamine in fish

- < 50mg/kg safe for consumption
- 50 - 200mg/kg possibly toxic
- 200-1000mg/kg probably toxic
- >1000mg/kg toxic and unsafe for consumption

Scombroid poisoning

Symptoms of scombroid poisoning include a tingling or burning sensation in the mouth, a rash on the upper body and a drop in blood pressure. Nausea, vomiting, and diarrhoea may also occur. Onset of intoxication is very quick, from immediate to 30 minutes, usually lasting around 3 hours; however in some severe cases symptoms can last days. It is thought that Scombroid poisoning often goes unreported as the symptoms can be attributed to an allergic reaction and are often of short duration (Lehane and Olley, 1999; Attaran and Probst, 2002).

The threshold level of histamine for effect is unknown and is thought to be influenced by an individual's susceptibility to histamine and other biogenic amines such as cadaverine and putrescine (other compounds produced in decaying foods) present in the fish (Lehane and Olley, 1999). Most poisonings occur at levels greater than 200mg/kg however symptoms have been seen from consuming foods containing as little as 50mg/kg (ESR, 2001) and do not result in a noticeable organoleptic change in the food to warn the consumer.

Outbreaks

Scombroid poisoning from fish has been well documented. OzFoodNet reported between 1995 and 2008 there were 32 foodborne illness outbreaks linked to histamine totalling 126 cases and 17 hospitalisations (although as noted previously, that it is believed scombroid poisoning is commonly mistaken for an allergic reaction and thus underreported).

Diagnosis is often based on clinical presentation and food consumption history. Sobel and Painter (2005) reported that between 1998 and 2002 there were 167 histamine poisoning outbreaks in America that affected 703 persons with 38 hospitalisations.

Survey objective

To determine the level of histamine in anchovies sold in NSW.

Materials and method

Forty five anchovies in oil products and 34 dried anchovy products were purchased from retail outlets across metropolitan Sydney between May 2009 and August 2009.

Anchovies in oil samples purchased were predominantly imported from Italy, Morocco and Greece. Dried anchovy samples purchased were predominantly imported from China, Thailand and Vietnam. Samples were stored as purchased until transported to the laboratory for testing (eg if purchased at room temperature the product was stored at room temperature). Samples were submitted to the Division of Analytical Laboratories at Lidcombe for testing. Samples were analysed for histamine, sodium content, pH value and water activity.

pH value

Where possible the pH was measured directly from the sample. If not possible, any oil was drained from the sample and the sample was then blended to a paste with the addition of 10 to 20 ml of distilled water where necessary.

Salt content

The sample was digested with nitric acid then diluted with distilled water. The solution was then analysed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) for sodium content.

Water activity

A few drops of a hydroscopic detector liquid was added to the headspace of a sealed container containing sufficient sample. Over time, the water activity of the sample and the detector liquid become equal. Water activity was then determined by measuring the refractive index of the detector liquid. This method is an in-house method based on a CSIRO, published paper and AOAC 978.18 method.

Histamine

The sample was first homogenised and then a maximum of 4g of the homogenised sample was made alkaline by the addition of sodium hydroxide and extracted through chloroform. Histamine was then transferred from the chloroform extract into a dilute hydrochloric acid solution; an aliquot is derivatised with orthophthaldehyde (OPA) for HPLC analysis using fluorescence detection.

Results and discussion

In total, 79 anchovy products were tested for the histamine content. The results are presented in Table 1. Distribution of the different levels of histamine observed in each category is outlined in Figure 1. Raw data is presented in Appendix 1.

- 97.8% of anchovies in oil contained less than 200 mg/kg of histamine and were therefore compliant with the Code.
- 97.1% of dried anchovies contained less than 200 mg/kg of histamine and were therefore compliant with the Code.

Appropriate enforcement action was taken on products where the level of histamine was greater than 200 mg/kg.

Water activity, pH and sodium levels are presented in Table 2.

Analysis of variance showed no correlation between levels of histamine, pH, water activity or sodium content.

Table 1: Histamine results

Product type	Histamine level		
	Acceptable		Unacceptable
	not detected	<200 mg/kg	>200 mg/kg
Anchovies in oil (n=45)	28.9% (13)	68.9% (31)	2.2% (1)
Dried anchovies (n=34)	55.9% (19)	41.2% (14)	2.9% (1)

Table 2: Water activity, pH and sodium results

Product type	aw		pH		sodium (mg/kg)	
	average	range	average	range	average	range
Anchovies in oil (n=45)	0.78	0.97-0.73	5.6	6.2-4.0	5062	7117-765
Dried anchovies (n=34)	0.57	0.88-0.22	6.9	9.5-5.8	3140	19736-105

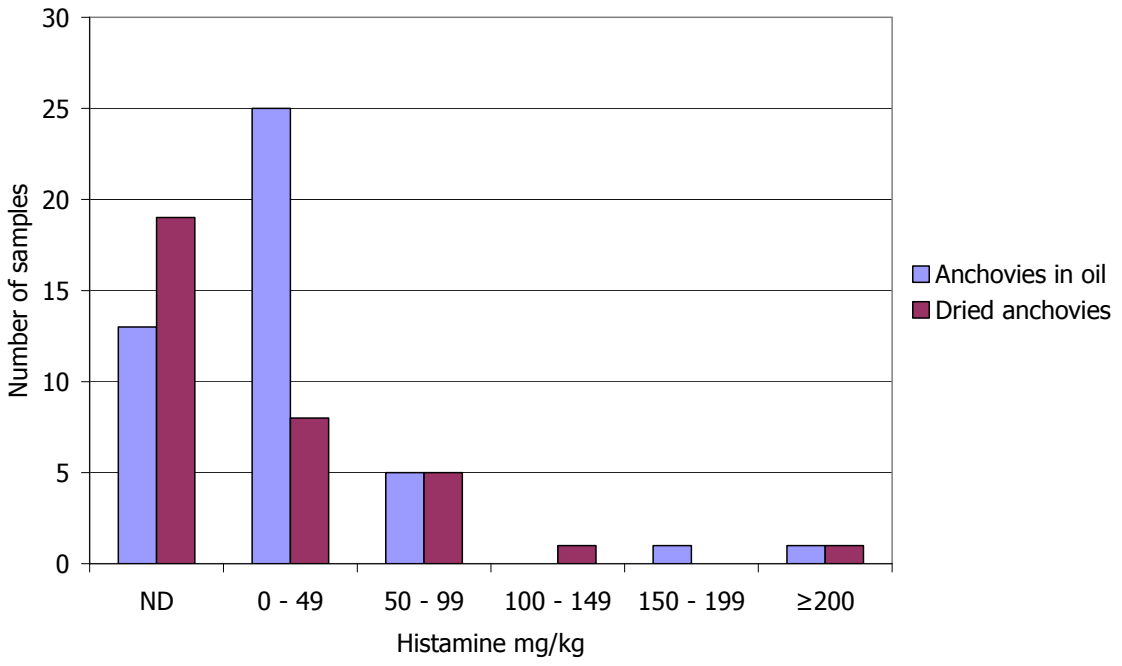

Figure 1: Distribution of histamine in samples tested

Anchovies in oil

A total of 45 batches (totalling 30 brands) of anchovies in oil were analysed for histamine. This included:

- 29 products sampled singularly
- 12 products sampled in duplicate
- 2 products sampled in triplicate
- 2 products sampled in quadruplet

It is not unexpected to detect low levels of histamine in anchovies in oil. The average histamine level of all products analysed (using the average of replicates tested) was 33 mg/kg and ranged from not detected to 518 mg/kg. This level is comparable to a previous study which found the average histamine level of anchovies in oil was 35 mg/kg (Veciana-Nogues, Vidal-Carou & Marine-Font 1989).

One product, tested in duplicate, had levels of 224 mg/kg and 86 mg/kg, giving the product an average histamine content of 155 mg/kg. No action was taken on this product as the average level was lower than the regulatory limit of 200 mg/kg.

Histamine formation does not occur homogenously in fish so variation of histamine levels within a product batch can occur. This was observed by Lee et al (2005) who analysed eighteen cans of a batch of anchovies in oil and fourteen cans of a second batch for histamine. The levels of histamine in these batches ranged from 14.2 mg/kg to 1427.9 mg/kg and 2.4 mg/kg to 30.2 mg/kg respectively.

An excessively high level of histamine was detected in one product batch in this survey. An imported product from Greece contained 2116 mg/kg of histamine. An initial sample of this batch was tested 4 months prior to this sample and contained 5 mg/kg of histamine. Both these samples were purchased at the same time from the same retailer who was storing them at room temperature. The sample which contained 2116 mg/kg of histamine was tested after being stored at room temperature for four months after purchase.

This product provided storage instructions of between 0 & 5°C ; however both samples tested were purchased from a retail outlet storing the product at room temperature. Two additional samples of the same batch, which had been stored under refrigeration, were sourced from the wholesaler and tested. These samples had no detectable histamine. This high result gives emphasis to the importance of correct storage. The two initial samples were not included in the overall analysis of results as they were improperly stored prior to testing. However the additional samples of this batch sourced from the wholesaler were included in analysis (as they were stored according to manufacturer's instructions). It was reiterated to the wholesaler to ensure that stockists were storing the product correctly. Temperature abuse is a known contribution factor of elevated histamine levels (Rodriguez-Jerez, Lopez-Sabater, Hernandez-Herrero, Mora-Ventura 1994; Lee et al, 2003).

Table 3 outlines the pH, water activity and sodium levels of these four samples.

Table 3: Histamine, aw, pH and sodium differences between temperature abused samples and non temperature abused samples of the same batch

	histamine (mg/kg)	aw	pH	sodium (mg/kg)
sample 1	5	0.79	5.6	5787
sample 2	2116	0.80	6.1	5183
sample 3	ND	0.79	5.4	6232
sample 4	ND	0.79	5.4	6083

ND – not detected

The pH of sample 2 indicates that bacterial activity has occurred after production.

Rodriguez-Jerez et al, 1994 observed that samples of semi preserved anchovies in oil that had a higher pH contained higher microbial counts of decarboxylating bacteria than those with a lower pH.

Products re-tested after opening

After initial testing four products were stored under refrigeration for several months then re-tested (Table 2). The average change in histamine level was almost negligible at 3 mg/kg ranging from -5 mg/kg to 11 mg/kg. The pH of these samples ranged from 5.3 to 5.7 and the water activity ranged from 0.74 to 0.77.

Similar results have been seen with canned tuna (Kerr, Lawicki, Aguirra, Rayner, 2002).

Table 4: Results for products re-tested after initial analysis

Sample No.	Initial histamine level mg/kg	Histamine level after re-testing mg/kg	difference in histamine levels	Length stored prior to retesting
5	28	39	11	4 months
12	29	24	-5	4 months
21	52	59	7	3 months
25	ND	ND	0	3 months

Dried anchovies

A total of 34 batches (totalling 24 brands) of dried anchovies were analysed for histamine. This included:

- 30 products sampled singularly
- 3 products sampled in duplicate
- 1 product sampled in triplicate

The average histamine level of all products analysed (using the average if replicates tested) was 35 mg/kg ranging from not detected to 483 mg/kg.

One product had an average histamine level above 200 mg/kg (Table 1). High levels of histamine have previously been detected in dried fish imported from Asia (Rigg, 1997).

The product with a histamine level greater than 200 mg/kg was sampled in triplicate. The three sub- samples contained 267 mg/kg, 497 mg/kg and 685 mg/kg of histamine resulting in an average of 483 mg/kg. This product was recalled from the market. This again demonstrates how the levels of histamine in one batch can vary significantly. Lee et al, 2005 and 2003 concluded that histamine formation in fish was an 'individual event' not a 'group event' and that a large number of subsamples were required to get a representative histamine value. The pH and water activity of this product ranged from 6.0 to 6.1 and 0.65 to 0.74 respectively. Given these product values, histamine formation is likely to have occurred during the manufacturing process.

Conclusion

The average level of histamine in dried anchovies and anchovies in oil tested was well below the regulatory limit of 200 mg/kg, at 33 mg/kg and 35 mg/kg respectively.

This survey illustrates that the majority of anchovies in oil and dried anchovies contain less than 200 mg/kg of histamine and are compliant to the Code but also highlights the importance of correct storage for these products both prior to, and after opening.

Salt is used for product stability and safety. As a consequence, anchovies contain high levels of sodium however anchovies are only eaten in small portions infrequently by the general population.

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Appendix 1

Anchovies in oil raw data

Sample number	Number of replicates analysed	Country of origin	Average histamine level (mg/kg)	pH	aw	Sodium (mg/kg)
1	1	China	9	6.0	0.86	2130
2	2	Italy	ND	6.1	0.75	5638
3	2	Italy	4	5.5	0.75	5830
4	1	Italy	47	5.8	0.81	6319
5	1	Spain	14	5.8	0.78	5607
6	1	Italy	518	6.2	0.74	5412
7	2	Italy	17	5.7	0.78	4883
8	1	China	28	6.0	0.89	1452
9	1	Italy	13	5.7	0.78	5462
10	1	Italy	ND	-	-	-
11	1	Albania	ND	5.7	-	5856
12	1	Italy	ND	5.8	0.74	6816
13	1	Italy	13	5.7	0.74	7117
14	2	Italy	7	5.8	0.74	5715
15	1	Italy	ND	6.1	0.74	5633
16	1	Albania	5	5.7	0.74	5531
17	1	Italy	8	5.4	0.77	5951
18	3	Italy	5	5.6	0.75	5978
19	1	Italy	96	5.4	0.75	5842
20	1	Morocco	ND	5.4	0.73	5255
21	4	Greece	2	5.6	0.79	582
22	2	Italy	ND	5.6	0.78	5380
23	1	Italy	29	5.3	0.75	5879
24	2	Italy	21	5.2	0.75	5951
25	2	Italy	ND	5.4	0.75	5150
26	3	Italy	39	4.8	0.88	2780
27	2	Italy	19	5.5	0.76	5570
28	1	Italy	23	6.0	0.74	5767
29	1	Taiwan	76	5.4	0.87	2851
30	1	Italy	28	5.6	0.77	5405
31	2	Italy	96	5.6	0.77	5937
32	1	Italy	ND	5.9	0.77	6373

Sample number	Number of replicates analysed	Country of origin	Average histamine level (mg/kg)	pH	aw	Sodium (mg/kg)
33	1	Italy	ND	5.7	0.74	6273
34	2	China	21	6.1	0.81	2891
35	1	unknown	ND	4.3	0.92	765
36	1	Italy	ND	5.7	0.75	5152
37	1	Morocco	52	5.5	0.74	5752
38	1	Morocco	21	4.0	0.93	934
39	4	Morocco	3	5.6	0.75	5341
40	1	Italy	68	5.5	0.75	4935
41	2	Italy	ND	5.3	0.75	5813
42	2	Chile	155	5.9	0.74	6116
43	1	Taiwan	11	5.2	0.90	2360
44	1	Taiwan	11	5.5	0.84	2206
45	1	unknown	31	5.5	0.77	6610

- not tested
ND not detected

Dried anchovy raw data

Sample number	Number of replicates analysed	Country of origin	Average histamine level (mg/kg)	pH	aw	Sodium (mg/kg)
1	1	Thailand	ND	9.1	0.37	1421
2	1	Korea	ND	5.8	0.22	19736
3	1	China	65	6.3	0.69	3772
4	1	Thailand	ND	6.3	0.51	966
5	1	Thailand	ND	9.4	0.40	2085
6	1	Thailand	ND	8.8	0.34	1386
7	2	Thailand	ND	8.5	0.27	1552
8	1	Vietnam	16	7.2	0.81	1641
9	1	Vietnam	38	6.2	0.66	4697
10	1	Vietnam	41	7.9	0.72	1663
11	1	unknown	ND	6.4	0.68	3892
12	1	Indonesia	21	6.3	0.80	3700
13	1	China	ND	6.4	0.73	4077
14	2	China	38	6.1	0.64	4611
15	2	China	ND	6.5	0.70	4308

Sample number	Number of replicates analysed	Country of origin	Average histamine level (mg/kg)	pH	aw	Sodium (mg/kg)
16	1	Taiwan	ND	-	0.34	3170
17	1	Thailand	ND	-	0.34	3170
18	3	Vietnam	483	6.1	0.68	3187
19	1	Thailand	ND	9.1	0.37	1361
20	1	Thailand	ND	9.5	0.44	1569
21	1	China	10	7.4	0.64	3258
22	1	Malaysia	ND	6.7	0.73	3849
23	1	Vietnam	119	6.5	0.88	2807
24	1	China	86	6.3	0.43	1094
25	1	China	ND	6.5	0.43	105
26	1	China	67	6.1	0.72	3981
27	1	China	46	6.1	0.55	718
28	1	Malaysia	ND	6.5	0.65	3440
29	1	China	ND	6.4	0.54	202
30	1	Vietnam	51	6.1	0.65	4568
31	1	China	ND	6.7	0.26	161
32	1	Vietnam	45	6.9	0.80	1668
33	1	China	58	6.1	0.74	4682
34	1	Japan	ND	7.9	0.91	371

- not tested
ND not detected

NSW Food Authority
6 Avenue of the Americas
Newington NSW 2127
PO Box 6682 Silverwater NSW 1811
Phone 1300 552 406
Fax 02 9647 0026
www.foodauthority.nsw.gov.au