

Effect of Storage Conditions on Histamine Formation in Fresh and Canned Tuna

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INTRODUCTION

In Victoria, in the last few years, there have been a number of outbreaks of histamine poisoning that have been attributed to the consumption of fish products, in particular, canned tuna from take away sandwich bars or tuna fillets from unprocessed fresh tuna (personal communication, Infectious Diseases Unit, Department of Human Services). Tuna, and other scombroid fish, have also been implicated in outbreaks of histamine poisoning overseas (Laurent et al 1995, Kim et al 1999, Ababouch 1990, Lonberg 1984, Murray et al 1989, and Maher et al 2000).

Histamine is a member of a group of compounds known as biogenic amines. Putrescine and cadaverine are also classed as biogenic amines. Biogenic amines are biologically active compounds normally produced by decarboxylation of free amino acids and are present in a variety of foods, eg fish, fish products, meat wine, cheese and fermented foods. The presence of biogenic amines in these foods is an indicator of food spoilage (Veciana-Nogues et al 1990). Recent storage trials on the generation of biogenic amines in vacuum-packed beef (Kaniou et al 2001) and in cheese (Pinho et al 2001), highlight the continued worldwide concern into food storage and food safety issues. The formation of biogenic amines is dependent upon the availability of free amino acids, the presence of decarboxylase containing microorganisms (bacteria with enzymes that can

decarboxylate the free amino acids) and conditions that favour bacterial growth and enzymic processes (Halasz et al, 1994).

Histamine is produced in fish tissue by the decarboxylation of free histidine by bacteria containing the enzyme histidine decarboxylase with the optimum temperature for histamine production being 25°C (Kim et al 1999). The content of free histidine in tuna fish muscle is typically quite high compared to other fish species (Ababouch et al 1985). Therefore, in tuna fish, there is an increased risk that toxic levels of histamine can be formed, especially under unfavourable storage conditions (ie ambient temperature) and mishandling during processing (Ababouch et al 1985, Silva et al 1998).

Ingestion of food containing small amounts of

histamine has little effect on humans, but in large amounts histamine can be toxic. The intestinal tract of humans contains the enzymes diamine oxidase (DAO) and histamine-N-methyl transferase (HMT), which convert histamine to harmless degradation products. However, for large doses of histamine, the capacity of DAO and HMT to detoxify histamine is limited, resulting in toxic effects as histamine enters the bloodstream (Taylor, 1986). Symptoms of toxicity include vomiting, diarrhoea, abdominal cramps, perspiration, flushing, headaches and burning sensations in the mouth (Taylor and Bush, 1988). Putrescine and cadaverine can inhibit these enzymic reactions and therefore potentiate the toxicity of histamine (Eitenmiller et al, 1980).

Storage of fish under refrigerated conditions from the time it is caught until when it is consumed has been found

to be very important in reducing outbreaks of histamine poisoning. Low temperatures control bacterial histamine formation during fish processing. In the retail market, tuna fish are usually covered with ice or simply placed on ice to prevent spoilage. However, storage under these conditions for long periods of time can result in microbial growth and conversion of histidine to histamine. A better understanding of the lower temperature limit for bacterial growth and histamine production is therefore important to control fish quality and safety (Chen et al 1988). For this reason, Food Safety Victoria initiated a project to investigate the effect of storage conditions on the concentration of histamine in fish tissue. The levels of the other common biogenic amines, (B-phenethylamine, putrescine, cadaverine, spermidine and spermine) in the samples were also monitored in the study. The levels of total volatile nitrogen (TVN) in the samples, which is commonly used as a spoilage indicator, were also determined to investigate the potential correlation between TVN levels and histamine formation.

In take-away food outlets, histamine poisoning incidents can arise from two potential sources. The canned tuna used may contain high levels of histamine as a result of poor handling between the time the fish is caught and the time it is canned. Secondly, the canned tuna becomes contaminated with bacteria that produce biogenic amines. The source of the bacteria may simply be as a consequence of poor hygiene or practices, or it may be present in other foodstuffs, eg in sandwich dressings such as mayonnaise where the bacteria is benign without the precursor amino acids to form amines (Leuschner and Hammes 1999). The presence of bacteria could result in elevated histamine levels in the food and hence result in cases of food poisoning. Therefore a small survey of tuna sandwiches from take away food outlets was also undertaken to investigate whether significant levels of bacteria, which could potentially give rise to high levels of histamine, were present in the products.

Recommendations for appropriate storage and handling practices for fresh and canned tuna will result from the investigations. Areas for future research will also be identified.

SAMPLING AND SAMPLE PRESENTATION

Stability Trial

Two large fillets of “Yellow Fin” Tuna were purchased from two different outlets at the Melbourne Wholesale Fish Market, Footscray Road, Footscray, on Tuesday the 5th September 2000. The suppliers’ names and addresses are listed in Appendix 1. Samples of the two fish were selected for replicate determination and sub-samples 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. Care was taken to minimise bacterial contamination from benches and equipment used in the preparation. To this end, knives and chopping boards were sterilised in an autoclave prior to use. Prior to, and during sample preparation, a solution of 85% methanol was used to clean equipment and surfaces. Gloves were worn at all times.

Samples were stored at ambient temperature (17°C), at 4°C and at 0°C. For storage at ambient temperature, samples were placed in a laboratory fume cupboard and left for the period of the experiment. The temperature

in the fume cupboard was monitored using a data logger. 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. Samples were taken at the appropriate times (Sampling Protocol, Appendix 2) and stored at -70°C to minimise bacterial activity that could influence the results prior to analysis. All samples were assayed collectively to optimise efficiency and to limit possible day-to-day variability of analyses.

Bacterial content of tuna sandwiches

The survey of take away food outlets involved the collection of tuna sandwiches from randomly selected outlets in the Western metropolitan area of Melbourne. The dates of sampling, addresses of take away food outlets and brief descriptions of the condition of the tuna used for the sandwich preparation are listed in Appendix 1. As soon as possible after collection, the sandwiches were delivered to the Melbourne Diagnostic Unit (MDU) for the determination of standard bacterial plate count.

METHODS

Biogenic Amines

Biogenic amines were determined using a method based on an extraction procedure described in the AOAC Official Methods of Analysis (1990 and 1997) and the derivatisation and HPLC procedures described by Moret et al (1992) and Eerola et al (1993).

Biogenic amines are extracted from the finely minced fish flesh using 75% methanol in water. 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 dansylated 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 C18 reverse phase column with UV detection at 254 nm.

Total Volatile Nitrogen (TVN)

The TVN levels in the samples were determined by the method outlined in Pearson (1970). The minced sample was mixed with magnesium oxide in cold water and homogenised with a Waring blender. The mixture was then steam distilled, the volatile components collected and the level determined by titrimetry.

Standard Plate Count

A method based on the Australian Standard Method AS1766.1.3 (1991) was used to determine the Standard Plate Count. This was carried out at the Melbourne Diagnostic Unit.

A 10g sample of the previously homogenised sandwich was mixed with a diluent and homogenised in a stomacher. Serial dilutions of the mixture were made and the diluted samples added to plate count agar. Samples were then incubated at 30°C for 72 hours. Colonies were counted and the levels of bacteria in the original sample determined. Each sample was analysed in duplicate.

RESULTS AND DISCUSSION

Stability trial

The following biogenic amines were determined: b-phenethylamine (b-phen), putrescine (Put), cadaverine (Cad), histamine (Hist), spermidine (Spd) and spermidine (Spm). The amine levels of the tuna fish samples stored at ambient temperature (17°C), 4°C and 0°C are tabulated in Tables 1, 2 and 3 respectively. The biogenic amine data from Tables 1, 2 and 3 are presented graphically in Appendix 3 - Supplementary Graphs.

Storage at ambient temperature (17°C)

The biogenic amine data for the trial at ambient temperature indicate that only histamine (in particular) and cadaverine are produced in significantly large quantities as the fish degrades during the life of the experiment (Table 1). At ambient temperature (17°C), histamine production commenced after approximately 40 hours and increased to the order of 100 mg/Kg after 48 hours. After 96 hrs the level of histamine for fish 1 was 1200 mg/kg. Fish 2 reached a maximum histamine level of 1200 mg/Kg after 72 hours.

Table 1

Storage at Ambient temperature

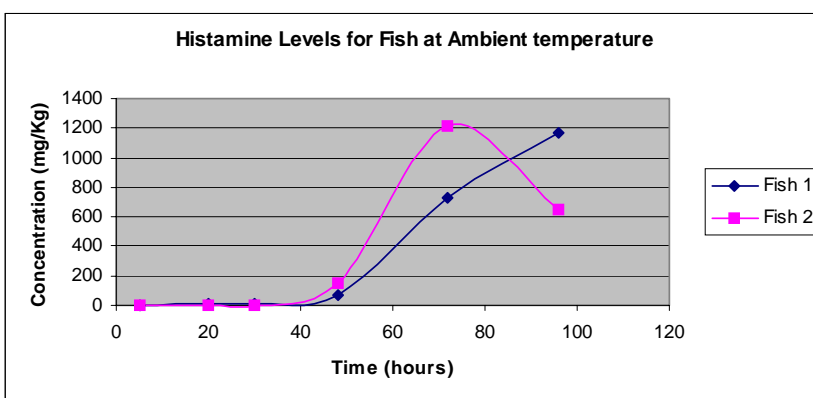
Fish 1						
Hours	β-phen	Put	Cad	Hist	Spd	Spm
5	0	10	10	0	0	0
20	0	8	37	16	7	4
30	0	4	37	17	31	19
48	0	7	22	71	0	0
72	0	25	88	730	3	14
96	38	35	120	1200	3	35

Fish 2						
Hours	β-phen	Put	Cad	Hist	Spd	Spm
5	0	10	0	0	0	0
20	0	2	3	0	0	0
30	0	4	38	2	0	0
48	0	13	120	150	5	13
72	8	39	350	1200	13	16
96	0	35	250	640	0	17

Note: amine levels are in mg/Kg

The histamine levels of Fish 1 and Fish 2 over 96 hours of the storage trial are compared in Graph 1.

Graph 1



Storage at 4°C

Cadaverine and histamine are the predominant amines produced at this temperature (Table 2). Histamine was produced at higher levels than cadaverine at ambient temperature, however at 4°C, the reverse occurs. At 4°C, amine production begins immediately. Initially, fish 1 has increasing levels of both histamine and cadaverine, whilst Fish 2 has increasing levels of cadaverine only. Histamine production in Fish 2 does not begin until after day 6. After day 15, histamine production has reached its maximum in both Fish 1 (60 mg/kg) and Fish 2 (150 mg/kg).

Storage at 0°C

At 0°C the production of both histamine and cadaverine does not begin until after day 4 (Table 3). The maximum histamine levels produced are 45 and 80 mg/Kg for Fish 1 and Fish 2 respectively, and therefore both are below the Australian Food Standards Code (2001) safe level of 200 mg/kg. As was observed for the fish at 4°C, Fish 2 was slower to produce histamine but again produced a higher level than Fish 1.

Table 2

Storage at 4°C						
Fish 1						
Days	β-phen	Put	Cad	Hist	Spd	Spm
0	4	8	8	1	1	3
6	3	16	96	42	6	0
10	6	10	92	53	6	0
13	0	2	37	17	3	0
15	3	13	96	61	3	0
17	0	4	20	25	2	3
20	0	10	29	32	1	0
Fish 2						
Days	β-phen	Put	Cad	Hist	Spd	Spm
0	2	17	21	1	0	2
6	3	12	66	2	30	0
10	2	11	130	73	3	1
13	0	14	210	130	0	0
15	2	16	100	150	1	0
17	4	27	110	140	2	2
20	0	8	4	18	4	5

Table 3 Storage at 0°C

Fish 1						
Days	β-phen	Put	Cad	Hist	Spd	Spm
0	4	8	8	1	1	3
4	4	9	17	1	2	5
8	4	6	100	25	2	1
13	5	15	95	46	0	2
17	1	9	56	26	6	1
20	0	7	34	15	10	3
Fish 2						
Days	β-phen	Put	Cad	Hist	Spd	Spm
0	2	17	21	1	0	2
4	4	3	3	2	1	3
8	5	12	84	13	1	0
13	4	9	85	46	2	2
17	24	14	130	77	2	0
20	8	23	82	32	6	0

Note: amine levels are in mg/Kg

Cadaverine production was reasonably similar for both fish at both temperatures. This may suggest that the bacteria responsible for cadaverine production are still active at 0°C. Cadaverine is produced by the decarboxylation of the amino acid lysine.

The averaged levels of histamine in Fish 1 and Fish 2 at 4°C and 0°C are summarised in Table 4 and Graph 2.

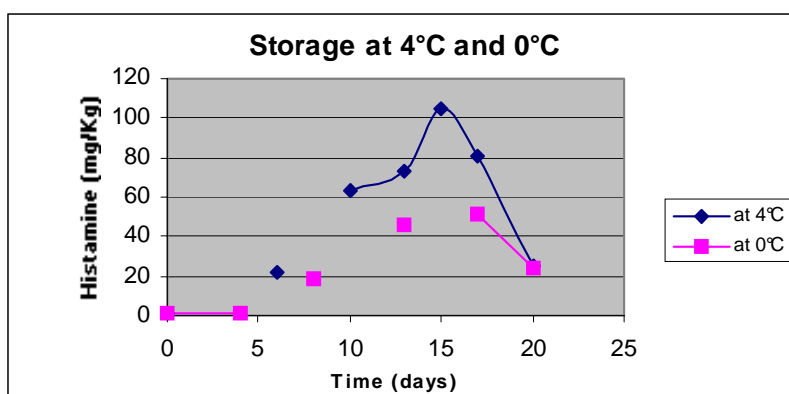
Histamine production is controlled when the temperature is maintained around 0°C or below. However our results show that even at 4°C problematic levels of histamine can be produced over time. Other workers have found similar results (Behling and Taylor 1982, Veciana-Nogues et al, 1997, Ben-Gigirey et al, 1998, Kim et al, 1998), which suggests that the fish contain bacteria capable of histamine production at low temperatures. Several investigators have shown that fish subjected to storage at 20°C for a short period of time will yield high levels of histamine even though the fish had initially been stored at low temperatures (Behling and Taylor 1982). Prior storage of the fish at high temperatures may influence the findings of cold storage trials.

Table 4

	Storage at 4°C	Storage at 0°C
Day	Histamine (mg/Kg)	Histamine (mg/Kg)
0	1	1
4	na	1
6	22	na
8	na	19
10	63	na
13	73	46
15	110	na
17	70	51
20	25	24

na = not assessed

Graph 2



Total volatile nitrogen

Total volatile nitrogen and histamine were analysed in each of the samples to establish if there was a link between the two sets of data. If a relationship did exist, then the TVN procedure, which is quite rapid, could be used as a simple means to assess possible histamine content. Data from

the determination of total volatile nitrogen from the tuna fish samples at the three storage temperatures are summarised in Table 5 and Graphs 3 and 4.

Table 5

Storage at Ambient				Storage at 4°C				Storage at 0°C			
Hours	Fish 1	Fish 2	Average Fish 1&2	Days	Fish 1	Fish 2	Average Fish 1&2	Days	Fish 1	Fish 2	Average Fish 1&2
0	410	390	400	0	410	390	400	0	410	390	400
5	260	220	240	2	Na	310	310	2	320	na	320
20	270	240	260	4	Na	260	260	4	720	810	770
30	360	300	330	6	320	240	280	6	300	Na	300
48	550	410	480	8	Na	240	240	8	470	780	630
72	710	670	690	10	390	450	420	10	440	Na	440
96	1,400	1,300	1,400	13	1,200	1,100	1,100	13	790	1,300	1,000
				15	1,100	1,500	1,300	15	620	Na	620
				17	1,700	1,200	1,500	17	740	700	720
				20	1,800	1,800	1,800	20	1,300	1,300	1,300

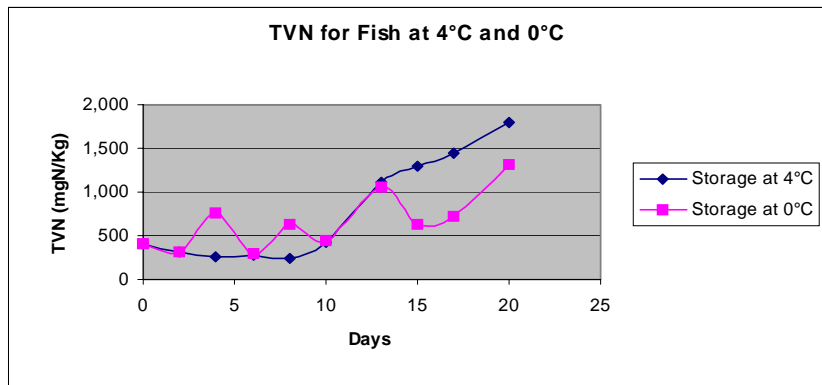
Note: units for the total volatile nitrogen are mg N/Kg.

na = not assessed

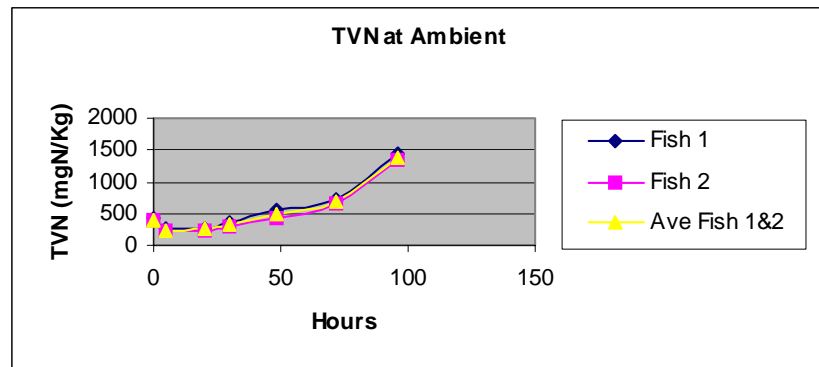
The data (Table 2) shows that levels of TVN rapidly increase when the fish is stored at room temperature (range 17°C + 4°C). The initial TVN levels obtained compare reasonably well with other published data on a variety of fish species (Mendes 1999 and Silva et al 1998), although no specific data for Yellow Fin tuna exists. Pearson (1970) suggests that for white-fleshed fish, TVN levels below 200 mgN/Kg indicate that the fish is fresh, whereas the fish would be rejected for human consumption when the TVN level exceeds approximately 500 mgN/Kg (Silva, 1998). For the ambient trial this level is reached after 2 days and for the trials at 4°C and at 0°C this level is reached after 10 days.

The results suggest that there is no relationship between TVN and histamine formation. At each temperature, TVN increased continuously with time. The

Graph 3



Graph 4



biogenic amines, eg histamine, typically show a period of time where histamine is not formed, production then commences and then declines with time. At 0°C, problematic levels of

histamine are not encountered, however, the corresponding TVN values indicate that the fish would not be fit for consumption.

BACTERIAL CONTENT OF TUNA SANDWICHES

Results for the determination of Standard Plate Count (SPC) and a description of the 12 samples of tuna sandwiches are tabulated in Table 6. The Standard Plate Count determination is used as a general-purpose analysis to cover a wide range of bacteria that may be present. It does not specifically indicate that the bacteria required for amine production were present (Ben-Gigirey et al 1999).

Levels below 10,000 Standard Plate Counts/g 30°C/3 days are not significant. Six of the 12 samples had SPCs that were above this level with four of this six being more than a factor of 10 above this level. High SPC levels are due to either an inadequate canning process (which should cook and sterilise the contents of the can) or the introduction of bacteria through cross contamination once the can has been opened. This could occur through the shared use of utensils to prepare sandwiches.

Samples 2a and 3a both gave SPC levels greater than 10,000 SPCs/g 30°C/3 days and both were canned tunas that had been mixed with other ingredients. Leuschner and Hammes (1999) carried out a study in which tuna was mixed

Table 6

Sample reference number	Description	Result (SPC/g 30°C/3 days)
0010057-01a	Shredded Tuna (chunky, nice looking texture). In serving container upon purchase.	<250
0010057-02a	Shredded to Pasty Tuna with spring onions (OK) In serving container upon purchase	380,000
0010057-03a	Shredded Tuna with spring onions & corn (chunky, nice looking texture). In serving container upon purchase.	74,000
0010057-04a	Finely shredded to pasty Tuna (average) Straight out of tin – Home Brand Sandwich Tuna	<250
0010057-05a	Shredded Tuna (chunky, nice looking texture). In serving container upon purchase.	140,000
0010057-06a	Flaked / Shredded Tuna (chunky, nice looking texture). Straight out of tin (Ocean Blue Tuna in brine).	<250
0010057-07a	Shredded Tuna (chunky, nice looking texture) In serving container upon purchase.	Approx 210,000
0010057-08a	Shredded Tuna (chunky, nice looking texture). In serving container upon purchase.	9,200
0010057-09a	Shredded Tuna (chunky, nice looking texture). In serving container upon purchase.	>30 x 10 ⁶
0010057-10a	Finely shredded to pasty Tuna (average). Straight out of tin.	13,000
0010057-11a	Finely shredded to pasty Tuna (average). Straight out of tin.	1,200
0010057-12a	Shredded Tuna (chunky, nice looking texture). In serving container upon purchase.	770

with mayonnaise and found high levels of amines after one day. Tavangaran (1984) found that the presence of soy protein increased the production of histamine in tuna inoculated with histamine producing bacteria. This suggests that bacterial loads can be increased by the

common practice of mixing tuna with other foods or dressings. However, there is not enough evidence to suggest that this is occurring in our study.

SUMMARY AND RECOMMENDATIONS

Stability trial

The results from the tuna stability trial show that high levels of histamine (and cadaverine) are produced within a short period of time at ambient temperature (17°C) and that the levels are much lower when the samples are stored at either 4°C or 0°C. Therefore, the fish should be stored at low temperatures (eg <4°C), to prevent the formation of histamine. Histamine formation occurs (slowly) with a relatively small increase in temperature from 0°C to 4°C. It would be useful to gather more information regarding the storage of fresh tuna and note practices that may contribute to amine production. One possible solution would be to freeze the tuna without compromising consumer appeal or introducing prohibitive costs.

Bacterial content of Tuna Sandwiches

The results from the survey of take-away food outlets indicate that there is a potential problem with the bacteria levels in sandwiches manufactured in these outlets. The high SPC levels from some food outlets suggests that poor food handling may be a problem. A larger survey could be undertaken to determine if this is an issue elsewhere. Identification of the bacteria would also indicate the type of bacteria present and the possible health implications.

The results of this survey suggest that it might be prudent to inform or remind take-away food outlets and fish retailers of the potential problems associated with inadequate storage of tuna.

Acknowledgments

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APPENDIX I

SCL Reference	Address	Sample Description	Date and Time Collected
Fish 1	Tasmanian Pacific Oyster Company Stall 24 Melbourne Wholesale Fish Market	Large fillet of fresh Yellow Fin Tuna	1pm 05/09/2000
Fish 2	Ash Bros Seafood's Pty Ltd Stall 24 Melbourne Wholesale Fish Market	Large fillet of fresh Yellow Fin Tuna	2pm 05/09/00
0010057-01a	The Country Loaf 51-53 Synnot Street, Werribee In serving container upon purchase	Shredded Tuna (chunky, nice looking texture)	11.15am 10/10/2000
0010057-02a	Ferguson Plarre Bakehouse P/L Werribee Plaza Shopping Centre Hoppers Crossing	Shredded to Pasty Tuna with spring onions (OK) In serving container upon purchase	11.25am 10/10/2000
0010057-03a	Central Square Coffee Shop Central Square Shopping Centre, Altona Meadows	Shredded Tuna with spring onions & corn (chunky, nice looking texture) In serving container upon purchase	11.45am 10/10/2000
0010057-04a	Hot Golden Bakery & Cakes Shop 7 Cnr Rifle Range Drive & Kororoit Creek Rd, Williamstown	Finely shredded to pasty Tuna (average) Straight out of tin – Home Brand Sandwich Tuna	12pm 10/10/2000
0010057-05a	Hobsons Bay Bakery 9 Hall Street, Newport	Shredded Tuna (chunky, nice looking texture) In serving container upon purchase	12.25pm 10/10/2000
0010057-06a	Dons Bakery 175 Somerville Road West Footscray	Flaked / Shredded Tuna (chunky, nice looking texture) Straight out of tin (Ocean Blue Tuna in brine)	12.50pm 10/10/2000
0010057-07a	Pacific Bakery 817 Ballarat Road Deer Park	Shredded Tuna (chunky, nice looking texture) In serving container upon purchase	11.30am 12/10/2000
0010057-08a	Bread & Butter Express Shop 35 Sunshine Marketplace 80 Harvester Road, Sunshine	Shredded Tuna (chunky, nice looking texture) In serving container upon purchase	11.50am 12/10/2000
0010057-09a	Don's Café & Pizza Lounge Milleara Shopping Centre Keilor East	Shredded Tuna (chunky, nice looking texture) In serving container upon purchase	12.15pm 12/10/2000
0010057-10a	Union Deli 207 Union Street, Ascot Vale	Finely shredded to pasty Tuna (average) Straight out of tin	12.45pm 12/10/2000
0010057-11a	Blue House Café 19 Pratt Street, Moonee Ponds	Finely shredded to pasty Tuna (average) Straight out of tin	1.00pm 12/10/2000
0010057-12a	Fancy Fillings Shop 2101 Highpoint Shopping Centre Maribymong	Shredded Tuna (chunky, nice looking texture) In serving container upon purchase	1.35pm 12/10/2000

APPENDIX 2

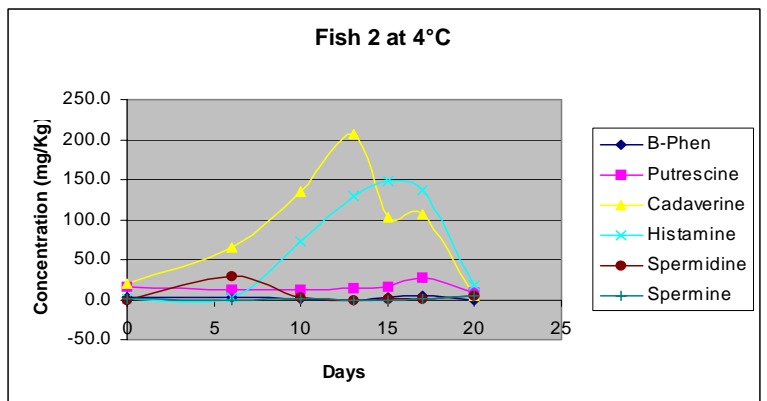
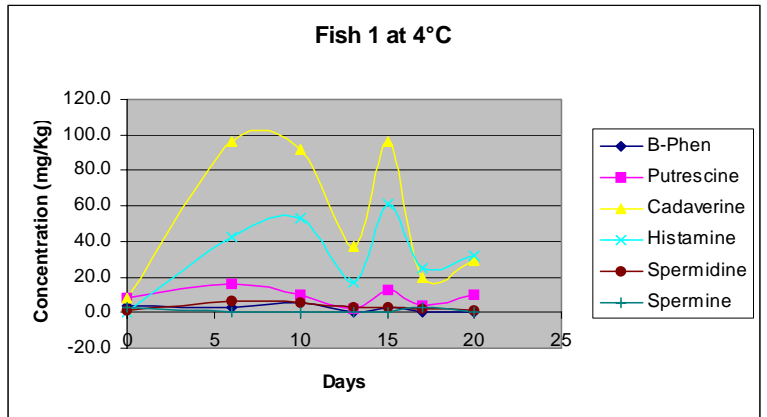
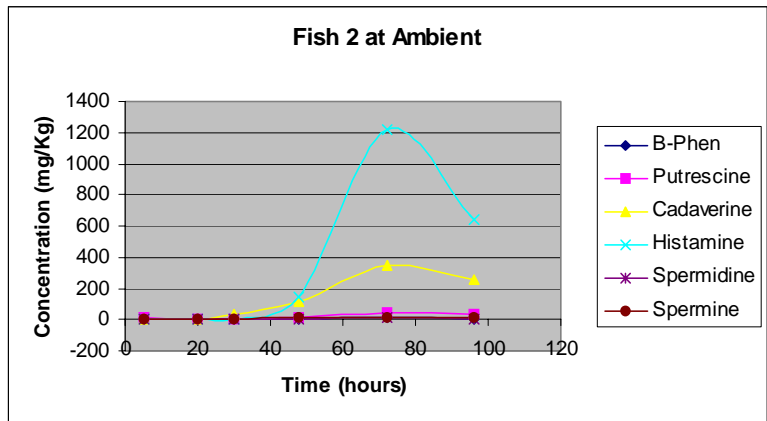
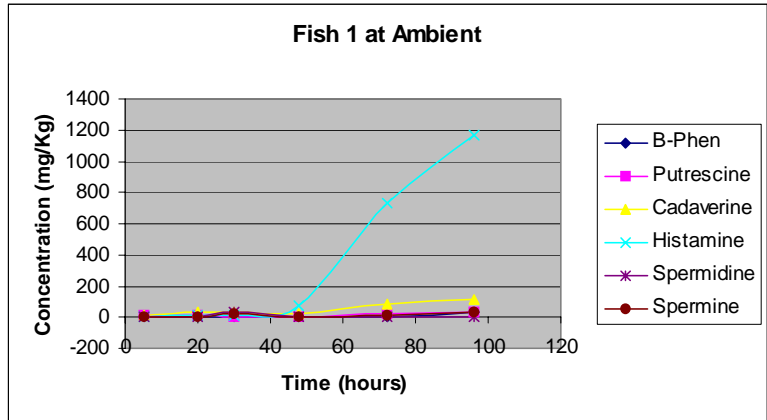
Sampling Protocol

Samples at ambient		Samples at 4°C and 0°C		
Sampling time (hours)	Analysed	Sampling time (days)	4°C Analysed	0°C Analysed
0	X	0	X	X
5	X	2	-	-
20	X	4	-	X
30	X	6	X	-
48	X	8	-	X
72	X	10	X	-
96	X	13	X	X
144	-	15	X	-
192	-	17	X	X
240	-	20	X	X

Samples to be analysed for total volatile nitrogen and biogenic amines.

APPENDIX 3

Supplementary Graphs



APPENDIX 3 cont...

