HISTAMINE IN TUNA IN THE PACIFIC ISLAND REGION

by

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ABSTRACT

Fisheries are of crucial importance to many Pacific Island countries and for several, one of the largest earners of foreign exchange. The most significant fish and fishery products exported from the region are tuna and tuna products, i.e. sashimi, canned tuna and dried tuna. With the introduction of US Seafood HACCP Rule 1995 there is a strong incentive for countries that export seafood products, particularly to North America and Europe, to maintain trade access to these large and extremely valuable markets. Even considering the larger businesses, the degree of HACCP preparation is very limited. SPC and FAO have made some efforts to evaluate and commence the development of HACCP in the Pacific Island region. More training, assistance to industry and governments, and research is still needed. Good regional data are not always readily available to justify currently used endpoints or to permit appropriate detection of the hazard. Research is needed to determine the appropriateness and effectiveness of current quality control criteria in reducing the incidence of scrombrotoxin in the Pacific Island region. To this end the University of the South Pacific has refined a highly quantitative chemical test for histamine analysis. A critical review of the USFDA stand on histamine control in tuna reveals that contentious issues exist concerning temperature and time limits, sampling regimes, critical limits and whether to control histamine by a HACCP program or by SSOPs. Recommendations for future research include validation of histamine test kits, regional studies into temperature and time affects on histamine production in fresh tuna and studies on histamine production in tuna jerky products.

INTRODUCTION

Tuna fisheries are of crucial importance to many Pacific Island countries and for several, one of the largest earners of foreign exchange. The annual commercial catch for tuna in the region in 1992 was in excess of one million tonnes, of which 67% was skipjack and 24% was yellowfin.

The main tuna exports are fresh/frozen tuna (especially sashimi grade tuna), dried tuna and caned tuna. The high prices offered in the highly competitive and demanding sashimi markets justifies the special treatment of the product and strict adherence to stringent trading requirements. Successful tuna jerky operations are Teikabuti Fishing Company, Tarawa, Kiribati and Ocean Traders at Pacific Harbour Fiji. Both have found successful export markets in Australia, New Zealand, Hawaii and Japan. Canned tuna has had a very important role in Fiji and throughout the Pacific in the past half century. There are large canneries established at a number of Pacific Island countries including: Fiji, American Samoa and the Solomon Islands. Canned tuna is extremely popular, with various species used such as albacore, skipjack, bluefin and yellowfin.

The importance of tuna exports is apparent if we look at Pacific islands by country. In Tonga most major exporters export fresh and frozen tuna or bottom fish (Gillet, 1997). In Samoa most major exporters export fresh, frozen and smoked tuna. According to the Secretariat for the Pacific Community (SPC) Tuna Fishery Yearbook (1995), Fiji Islands domestic long line fleet (less the US/Vietnamese vessels) landed 2,465 mt of tunas and other species in 1995. Most major exporters export fresh, frozen and smoked tuna. In the Solomon Islands fresh, frozen, smoked and canned tuna make up the major share of fish exports by weight and by value; although the reef fish exports have proven to be lucrative exports also. Sixty percent of the canned tuna production is exported to the United Kingdom, virtually all of the fresh tuna and katsuobushi is exported to

Japan and the frozen tuna are exported to a variety of locations including Japan, Thailand, Fiji, American Samoa, Philippines, and Latin America (Gillet, 1997).

HAZARD ANALYSIS AND CRITICAL CONTROL POINT

Introduction

On December 18, 1995 the USFDA passed a regulation Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products. This is now known as the "Seafood HACCP Regulation". The US Food and Drug Administration (FDA), for instance, hopes to prevent 30,000 to 60,000 cases of illnesses attributed to the consumption of seafood by implementing the US Seafood HACCP Rule on the safe and sanitary handling and processing of all fish and fish products.

Current status of HACCP in Pacific Island Countries

Introduction

Food processors all over the world, particularly in the least developed countries are having to come to terms with a different more rigorous and effective quality assurance systems. The promising evolution of the export fish trade from the Pacific Island region is considered to be under threat as a result of regulatory changes that were introduced by the major fish importing countries. The loss of these important markets, in particular the US, could have potentially lead to closures of businesses and loss of employment opportunities (McDorman, 1998). The incentive for countries that export seafood products, particularly to North America and Europe, is not only to maintain trade access to these large and extremely valuable markets, but also to become even more competitive and successful in the international market place (Roberts, 1998).

Tonga

There is currently no testing of fish exports. There are no laboratory facilities at the Ministry of Fisheries, although some laboratory facilities exist in the Ministry of Health where officials state that coliform bacteria and salmonella tests can be performed (Gillet, 1997).

Samoa

There is some testing of food and water carried out by the National Health Laboratory, but it is apparently not in response to a legal requirement. The Director of the Laboratory and a technologist stated they occasionally carry out tests on food and water.

Fiji

According to Ministry of Health officials, the testing requirements and procedures are those requested by the overseas client. The Ministry of Health uses the laboratory facilities of the Ministry of Agriculture, Fisheries, Forests, and ALTA at Koronivia, but those facilities do not have international accreditation. PAFCO maintains laboratory facilities at their cannery that, according to the General Manager, are able to do histamine analysis. Those facilities are not accredited but if testing in an accredited laboratory is required, samples can be forwarded to the B C Packers lab in Canada.

The Institute of Applied Science (IAS) at the University of the South Pacific (USP) has testing facilities. Although they have no accreditation at present, they expect to obtain such certification in about a year when they transfer to new facilities. The Director of IAS expects they could take on a

verification/auditing role and because USP is a regional body, this function could be extended to other countries.

Federated States of Micronesia

The only analytical test the National Food Inspectors are able to carry out is that for E.coli and this test is only carried out when contamination is suspected. The Food Safety Act has provision for the appointment of Food Analysts to "perform laboratory and field tests upon food and other articles". According to the National Food Inspectors, there are plans to build a national laboratory in Chuuk for the testing of food and water. Officials of the Pohnpei Fisheries Corporation (PFC) stated that the PFC laboratory is able to do most standard food and water quality tests (but not histamine tests) and that PFC has had some role in training government health workers.

Solomon Islands

There do not appear to be any specific testing requirements under the Pure Food Act (to date, no regulations under the Act have been made). Under Section 23 of the Act, a food exporter can apply to have products inspected and analysed by a laboratory certified by an approved laboratory certification scheme". The specific tests are not stipulated.

The Environmental Health Division is responsible for the Public Health Laboratory (distinct from the hospital laboratory). According to the Director of the Environmental Health Division, the laboratory is able to carry out the HACCP-related tests, water analysis (BOD, COD), and histamine tests.

General conclusions

There are varying degrees of dependence on marketing of fishery products from Pacific Island Countries to countries where HACCP has been put in place.

Table 1. Dependence on EU, Canada, and US Markets.

Country	Market Dependence (%of total value of exports)
Tonga	72%
Western Samoa	95%
Fiji	73%
FSM	10%
Solomon Islands	30%

Although there were exceptions, in 1997 it appeared that in most countries the government fishery departments had passed little or no information about HACCP to the private sector fish exporters. Only a few government fisheries officials charged with HACCP responsibilities had a good understanding of HACCP principles or specific details of US FDA HACCP regulations.

Table 2 gives a subjective evaluation of the degree of preparation by the major fish exporters in five countries. The Table considers only the major exporters, few, if any, of the smaller exporters (especially those operated by Pacific Islanders) had made any HACCP preparations.

Table 2. HACCP preparations in a number of Pacific Island countries.				
	Number of exporters			
Country	contacted	with well-advanced HACCP preparations	with some substantial HACCP preparations	with little or no substantial HACCP preparations
Tonga	5	1	1	3
Western Samoa	5	1	1	3
Fiji	7	2	0	5
FSM	4	1	0	3
Solomon Islands	4	1	0	3
Total	25	6	2	17

Even considering the larger businesses, the degree of HACCP preparation is very limited. It appears as though only four exporting firms have any sort of HACCP plans in place (two of these are industrial-scale canneries), with two additional firms making some progress on plan formulation and implementation (Gillet, 1997).

Advances of Pacific Island countries towards HACCP

A Regional Workshop hosted by SPC and FAO on the Implementation of HACCP-based Quality Assurance for Seafood was held in Fiji in 1997. The workshop provided valuable grounding in the legal and technical aspects of HACCP implementation by the USA (and to a lesser extent, the EU) and at identifying strategic options which governments could pursue in meeting the US requirements.

In response to recommendations made at the Regional workshop in Fiji, FAO Sub-Regional Office in Apia (FAO SAPA) and SPC Coastal Fisheries Program, continued to collaborate by organising certified HACCP workshops that met the US HACCP training curriculum as drawn up by National Seafood HACCP Alliance for Training and Education.

The workshops ensured that recognised HACCP trained individuals are available to exporters to undertake key activities described in the HACCP Rule. Such activities include developing, reviewing and modifying as necessary written HACCP plans and procedures; monitoring, reviewing and maintaining written records; undertaking basic verification procedures; etc. In all 60 participants were certified (18 in Samoa, 24 in Fiji, 18 in Tonga and 18 in the Federated States of Micronesia). Most of those certified were staff belonging to export operations. A number of different Government departments were also represented.

Assistance to seafood exporters to meet HACCP requirements

In 1997 five Fijian, five Samoan and three Tongan seafood exporters were assisted by an Australian HACCP consultant who evaluated the processing establishments and helped develop Good Manufacturing Practice (GMP), Sanitation Standard Operating Procedures (SSOP), HACCP plans and training advise.

All companies that received assistance in 1997 had implemented HACCP at least to a basic but satisfactory level of compliance and that there were genuine commitments to improved processing. One or two companies had made genuine attempts to meet all requirements and almost every company had significantly improved their premises during 1998 (Roberts, 1998).

Future Efforts

- further visits to seafood exporters
- more national HACCP workshop are needed

- assistance to establish effective national Controlling Authorities
- development of a protocol on how best to establish an effective Controlling Authority, training of inspectors and auditors
- more in-depth training to exporters
- top-up training to the senior HACCP staff
- training for production line staff
- more applied and pure research on seafood safety and quality in the region

Handling, processing and distribution methods can be monitored by a Hazard Analysis Critical Control point (HACCP) system. However good data are not always readily available to justify currently used endpoints or to permit appropriate detection of the hazard. Research is needed to determine the appropriateness and effectiveness of current quality control criteria in reducing the incidence of human disease (Ahmed, 1992).

HISTAMINE

There has been considerable international attention paid to histamine in canned, chilled, frozen and dried fish. High histamine levels in seafood products exported from the Indo-Pacific region have, in the past, resulted in economic losses and now a number of importing countries have introduced regulations on maximum allowable histamine content.

Temperature/Time

The mechanism of histamine formation is similar between species. Histamine will develop if there is a fish with copious amounts of free histidine, and there are bacteria (from whatever source) that can convert histidine to histamine, and there is time where the fish are stored at elevated temperatures, its about that simple.

High levels of histamine (approaching 50 mg% and beyond) are normally associated with high temperature/shorter time abuse of the fish. This is even mentioned on p 69, Chapter 7 in the Fish and Fishery Products Hazards and Controls Guide (USFDA, 1998). The higher temperature abuse results in the bacteria that decarboxylate histadine to histamine being able to proliferate and produce substantial amounts of the active agent - the enzyme histidine decarboxylase.

High levels of histamine indicate that the fish has been stored under conditions that could give rise to toxicity, and effective temperature control during handling and processing is the main and practical way of minimising the hazard. The main problem associated with distribution of seafood products is time-temperature abuse. Most bacteria are not capable of growth at temperatures <4°C, thus proper cooling of seafoods during transportation becomes an important consideration. Chilled product should be loaded at internal temperatures below 4°C and frozen product at or below –18°C (Ahmed, 1992).

Any time above 4.4°C significantly reduces the expected safe shelf-life. For this reason, fish should not be exposed to temperaturs above 4.4°C for more than four hours, cumulatively, after chilling on board the harvest vessel. The safety of this limit is dependent upon proper handling at sea. Fish that have been handled particularly well on-board the harvest vessel may be able to safely withstand somewhat more exposure to elevated temperatures during post-harvest handling (USFDA, 1998). Fish should not be exposed to temperatures above 4.4°C for more than twelve hours, cumulatively, after chilling on board the harvest vessel. An uninterrupted period of exposure should not exceed six hours. Intermittent refrigeration breaks the cycle of rapid bacterial growth and slows the formation of histamine. The safety of these limits is again dependent upon proper handling at sea (USFDA, 1998).

The only realistic place or point where we can see high levels of histamine forming would be between the time the fish are caught and die on board the fishing vessel, until the temperature is reduced to

approximately 4.4 - 5.0°C. Fresh fish should be kept at the 4.4-5°C temperature or below until ready for processing.

Relative to the use of 4.4°C as a critical limit for backbone temperature in incoming fish, Section K, p 4175 of the preamble to the Proposal to Establish Procedures for the Safe Processing and Importing of Fish and Fishery Products (USFDA, 1994) states the harvesters goal should be to bring the fish to an internal temperature of 4.4°C or below as soon as possible after the fish dies to minimise the risk of histamine production. Cooling fish below 10°C, greatly reduces the growth of populations of the bacteria which are the most likely to cause histamine formation. Once bacterial growth has begun, temperature at or below 5°C halts bacterial growth, although enzymatic histamine formation may slowly continue (p 95)". The agency goes on to say that they were dropping the temperature recommendations in the proposal to 4°C, to make it consistent with other temperature recommendations in the proposal, and invited comments on the appropriateness of this temperature. In reviewing the preamble to the Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products (USFDA, 1995) there are no comments discussed by FDA where there was an objection to the use of this temperature. The agency does acknowledge, however, that a large number of comments were received relative to the appropriateness of the 4.4°C temperature proposed. The agency was not going to discuss the comments received on the appropriateness of 4.4°C as a refrigeration temperature.

The Refrigerated Foods and Microbiological Criteria Committee (RFMCC) (1988) supports the use of 4.4°C as an appropriate refrigeration temperature. The RFMCC is aimed primarily at establishing good manufacturing practices for finished products which are to be stored and distributed under refrigeration, it basically supports the use of 4.4°C (and below, as appropriate) as being recommended for products requiring refrigeration.

Burns (1985) states that the rate of histamine formation decreases markedly at temperatures below 10°C. The International Committee on Microbiological Specifications for Foods (1996) appears to offer some degree of corroboration with this by indicating the control of the histamine hazard in scombrotoxic species is enhanced by bringing the temperature of the fish to 10°C within at least 2 hours after death and maintaining it at <10°C. These authors also stated that from the standpoint of spoilage (i.e. quality factors), the fish temperature should be maintained at 0°C. Ahmed (1992) recommends bringing the temperature below 15°C and, preferably below 10°C in four hours, a time factor that was not listed by the USFDA in the proposed seafood HACCP rule reference quoted above. They also state that from the standpoint of spoilage (i.e. quality factors) the fish temperature should be maintained at 0°C.

Perhaps the only practical way to determine the time/temperature and histamine/decomposition relationship is to conduct some actual real-time studies. It is noteworthy, moreover, that the ICMSF states that what they term a "dangerously toxic condition" occurs in scombrotoxic species only when they are held for several hours in the temperature range >15-20°C. Another possibility is to conduct studies where conditions on board the fishing vessels and/or during transportation to the factory could be realistically simulated. Once the time/temperature relationship to a level of histamine of 50 mg% or greater was determined one might then have established a scientific basis for critical limits. The same would apply if a particular backbone temperature could be related to a particular level of time/temperature abuse, leading to high histamine levels. Exceeding such critical limits, established in this manner, would present, therefore, a real possibility that scombrotoxic levels of histamine could be present, and would necessitate true HACCP-type corrective action.

It should be noted that the time required to lower the internal temperature of fish after capture will be dependent upon a number of factors, including (USFDA, 1998):

- The harvest method;- delays in removing fish from a longline may significantly limit the amount of time left for chilling and allow the fish to heat up as it struggles; the size of the sets;
- The size of the fish;
- The chilling method; how fast is they chilled after catch?

CRITICAL LIMIT

The USFDA has established decomposition limits for histamine in raw, frozen tuna and mahi-mahi, canned tuna and related species of 50 ppm (5 mg/100g or 5 mg%) or less. They have also specified a health hazard (scombrotoxic) limit of 500 ppm (50 mg/100g or 50 mg%) and above (USFDA, 1996).

In 3 August 1995 Federal Register notice, the decomposition limit was lowered by the USFDA from 100 ppm **and** evidence of organoleptic decomposition; to the current 50 ppm **or** evidence of organoleptic decomposition. The notice also eliminates the provision in the original CPG that states findings of less than 200 ppm histamine need to be confirmed by organoleptic evaluation. Chemical analysis now is deemed to be sufficient for confirmation.

Histamine is considered a poisonous or deleterious substance because, when ingested at sufficiently high levels, it is known to cause scrombroid poisoning, FDA noted. The agency is not changing the 500 ppm action level at this time because the threshold toxic dose of histamine is not known.

Furthermore, the presence of other amine decomposition products in fish may have a synergistic effect on histamine toxicity. The synergism may dramatically lower the threshold toxic dose."

> "Although the agency intends to use this defect action level in deciding whether to recommend regulatory action, it does not consider that the fact that a fish or fishery product has a histamine level below 50 ppm establishes that the fish or fishery product is acceptable. Other spoilage mechanisms are possible that do not result in the formation of histamine. Thus a finding of histamine levels between 20 and 50 ppm should be viewed as indicating that the fish or fishery product has deteriorated and should cause a producer to further evaluate or test the product."

The critical concentration for poisoning through histamine in spoiled fish is then about $100mg100g^{-1}$ of muscle. Amounts found as high as 450 to $500mg100g^{-1}$ do not, however, always produce poisoning. It is believed that related substances (e.g. TMAO, TMA) could intensify histamine activity or even be more toxic than histamine.

In most cases histamine levels in illness-causing fish have been above 200 ppm, often above 500 ppm. However, there is some evidence that other chemicals (*e.g.* biogenic amines, such as putrescine and cadaverine) may also play a role in the illness (USFDA, 1998).

Finally, it should be emphasized that although the USFDA seafood HACCP regulation does not require documentation supporting the establishment of critical limits (or hazard analysis for that matter), the Codex "recommend it for science as well as for due diligence." While it is important to have documentary support for any limits - critical or otherwise - is essential that it be there for true critical limits because, by definition, compliance with critical limits is necessary for direct public health reasons. Qualified persons with expert knowledge in the establishment of the specific critical limit(s) in question, and having any necessary facilities to establish such limits.

DECOMPOSITION LIMIT OR SAFETY LIMIT

Historically, the major problems involving histamine in tuna have been the result of reaching or exceeding the limits for decomposition, rather than the critical limit for safety (Cole, 1998). Why then use HACCP to control factors other than those affecting the safety of the product. This happened in the food industry in the US in the 1970s, with unfortunate results. We are seeing evidence of the same inclination again today, among certain organisations. What happens eventually is that since HACCP is such a science-based, intensely focused system, it quickly loses that necessary focus when it is used to control quality and economic factors. In the 1970s several firms in the US tried turning HACCP into a Total Quality Control System and it

became so burdensome and so unwieldy, that a large segment of the industry gave up on it. While HACCP is a science-based system, it does not need to be made complicated beyond the realm of human comprehension.

In cases of high temperature abuse fish may still appear organoleptically sound, which means it could be used in processing. Staruszkiewicz (undated), it was stated that for fresh/frozen fillets of mahi-mahi, allowed to decompose at temperatures above 21°C, sixty-five (65%) of those which contained scombrotoxic levels of histamine were found acceptable by organoleptic expert analyst, based solely on odours in the cleaned fillets.

It is important to keep in mind that the hazard of scombrotoxin must be in product which can continue to be processed and is deemed fit for consumption by the consumer, that is, scombrotoxic levels of histamine **combined with** acceptable organoleptic quality. Since decomposition is one of the most often reported problems with seafood, and since scombrotoxin-formation in certain species of fish is considered by the USFDA to be a hazard which is "reasonably likely to occur"; the question arises as to whether both quality and safety related decompostion might be considered under a HACCP plan.

Lower levels of histamine are usually associated with the abuse of fish at lower temperatures and for longer periods of time. This also often results in organoleptic decomposition and is, of course, why fresh fish, kept under refrigeration (not frozen) spoils after a few days. Even if the histamine levels increase over time at these lower temperatures, the fish normally become noticeably spoiled organoleptically, before scombrotoxic levels of histamine (>=50 mg% or 500 ppm) are reached. If the lot cannot be used in processing because of quality deterioration, then, rationally, it becomes a quality control point, not a critical control point. You can only rationally have a critical control point **if** the fish can be further processed; **and** the hazard (500 ppm) is **not** prevented, eliminated or reduced to acceptable levels; **and** the product can reach the consumer with the hazard potentially, or, in fact, present.

Most tuna processors attempt to control histamines from a decomposition standpoint (a quality issue), rather than the critical limit of 500 ppm. With the advent of the Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products (USFDA, 1995), tuna processors have not been sure whether to control histamine in a sanitation standard operating procedure (SSOP) or in a HACCP plan. Most have tended to locate their controls for histamine in a HACCP plan, while listing decomposition limits as their critical limits. This is contrary to the intent of HACCP, which is to control the hazards which most critically and directly affect the safety of the product. This confusion was compounded, no doubt, by information contained in USFDA (1998), for example, in Chapter 7 "Scombrotoxin (Histamine) Formation (A Chemical Hazard)" Table 7-3 lists Receiving as critical control points; the critical limits however, appear to be pertinent to decomposition not safety, at least for Receiving.

With respect to establishing a critical limit for incoming raw tuna, where the preventative measure is other than time/temperature control, the only other reasonable possibility that presents itself would seem to be to establish 500 ppm as the critical limit. If 500 ppm were the limit then a sufficient number of fish in each incoming lot for histamine would have to be analysed, to assure that at the 95% confidence level that there were no fish with histamine levels above the critical limit. This, however, might require a large number of samples to achieve that confidence level.

DETECTION

Organoleptic evaluation and chemical testing are the accepted methods for detecting histamine. There is a lot of mileage in a chemist looking for rapid methods of measurement (D. James, pers. comm., 1998). Neogen's Veratox test Kit is a recently developed quick, portable and reasonably quantitative method for determining histamine. Another good area to be developed would be a meat probe specific for *Histadine decarboxylase* concentration. Computer modelling in predictive microbiology may also have the potential to be developed as an accurate detector of histamine.

Chemical testing is an effective means of detecting the presence of histamine in fish flesh. However, the validity of such testing is dependent upon the design of the sampling plan. For this reason, chemical testing alone will not normally provide adequate assurance that the hazard has been controlled (USFDA, 1998).

Association of Official Analytical Chemists (AOAC) methods are available for histamine analysis. The University of the South Pacific's (USP) Institute of Applied Science (IAS) has been developing a method of histamine analysis that combines and modifies two of the known methods. This quantitative method is to be developed for use with miniaturised chromatographic columns capable of very precise applications (P. Ravi, pers. comm., 1998).

Principle

The sample is extracted with methanol as histamine is soluble in alcohol and water. The extract is passed through a purified ion-exchange column. O-Phthaldialdehyde solution is reacted with eluate to form fluorescent histamine derivatives. Flourescence intensity is measured on HPLC using a fluorescence detector. Quantification is done using external standards.

Standards

- Stock solution (1000 ppm) - 0.1656 g of histimine dihydrochloride dissolved and made to 100 ml in water.
- Dilution 1 – 10 ml to 100 ml gives 100 ppm.
- Dilution 2 – 5 ml (100 ppm) to 100 ml gives 5 ug/ml.
- Working solutions – 0.0, 0.1, 0.2, and 0.5 ml of 5 ppm solution is diluted to 25 ml giving 0.0, 0.02, 0.06 and 0.1 ug/ml histamine. Reagents must be added to produce fluorescent derivatives before diluting to mark.

Sample treatment

- Fish sample is homogenised in food processor/blender.
- 5 g of duplicate sample is weighed accurately but separately into 50 ml centrifuge tubes.
- Blanks and a few recoveries are also included. Recoveries are a third fish sample with 1.0 ml of 1,000 ppm stock added and followed through the procedure.
- 20 ml methanol is added to all solutions, shake vigorously 5 min, centrifuge for 5 min at 2,500 rpm. Transfer extract to 50 ml volumetric flask via funnel. Repeat extract twice more similarly each time using 15 and 10 ml of methanol. Finally make volume of extract to 50 ml using methanol.
- Convert the resin from Cl^- form to OH^- form by treatment with 2M NaOH. Swirl resin (in ratio 15 ml NaOH for each gram of resin) with NaOH for at least 15 min then decant NaOH. Repeat process one more time then wash resin with excess distilled water each time until pH of water is < 9.0.
- Fill resin into chromatographic columns (20 cm X 1.0 cm diameter). Resin height should be about 10 cm. Plug the end of the column using cotton wool. Store filled resin under distilled water and never let it go dry.
- Load 1.0 ml of extract onto the column and immediately direct column flow into a 50 ml volumetric flask containing 5 ml O.1 N HCl. Collect eluate till about 35 ml using water to elute.
- Once about 35 ml remove purified solution and dilute to 50 ml using 0.1 N HCl.

Derivatisation

- Should be done with care.
- Pipette 2.0 ml of purified and diluted solution into a 25 ml volumetric flask.
- Add 4.0 ml 0.1 N NaOH solution and mix.
- Add 0.2 ml of O-Phthaldialdehyde solution (OPA) (1% solution in methanol m/v).

- Allow 5 min for OPA to react.
- Add 2.0 ml of citric acid solution (0.2 M in water), mix and make volume to 25 ml using 0.1 N HCl.
- [Note: standards are derivatised exactly the same way by taking appropriate volumes of stock 1000 ppm histamine solution into 25 ml volumetric flasks].
- Inject appropriate volumes (usually 30 to 40 ul) of derivatised standards and sample solutions.
- [HPLC specifications: mobile phase is water filtered and degassed; flow rate is 1.0 ml/min; detector is a scanning fluorecence 470, excitation wavelength 340 nm, emmission wavelength is 425 nm, sensitivity 64 Gain 100; integrator – water 746, chart speed 0.25 cm/min, attenuation 32 or 16.
- Calculate peak height as follows:

$$\frac{\text{Peak Height Sample}}{\text{Peak Height Standard}} \times \text{Standard conc. ug/ml} \times \frac{\text{Vol Std Inj}}{\text{Vol Samp. Inj}} \times \text{DF} \times \frac{\text{Vol of Extract}}{\text{Wt. Fish Samp.}}$$

RESULTS

Fresh tuna analysed from the Pacific Fishing Company (PAFCO) tuna cannery, Levuka, Ovalau, Fiji had histamine levels of 4.0 to 25.0 mg/kg tuna. Only two batches of old canned tuna (i.e.; material that had passed its "use by" date) gave unacceptable results of levels of 300 mg/kg.

More work is still being done on this method to verify it. Quality assurance and quality control procedures are still being incorporated into testing schemes. The four recoveries tested in a batch of fifteen produced a test accuracy in the range 89 to 102 %.

Blanks gave peaks due to fluorescing OPA. Therefore this needs to be taken into consideration in the calculation.

Replicates were within < 10 % variation.

Both the AGX and Dowex resin were tested. There was no difference between resins as long as the resin treatment is correct and the resin is not overloaded with sample.

It was found that there was no difference to the peak height when standards were either passing through, or not passing through, the columns. Therefore it was concluded that standards need not be passed through columns in the future (P. Ravi, pers. comm., 1998).

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