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Histamine levels in seventeen species of fresh and processed South African seafood

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Abstract

Histamine levels were determined in fresh and processed seafood from a representative range of 10 outlets after several incidents of scombroid seafood poisoning occurred. Species included seventeen fresh and processed scombroid- and non-scombroid fish, marine mollusks and crustaceans. Histamine levels in fresh seafood were generally low (0–9 ppm) with the exception of one sample of snoek (scombroid fish; >50 ppm) and one sample of yellowtail (non-scombroid fish; >50 ppm). Both species are rich in free histidine (1.5–5.3 ppb), a precursor of histamine. Processed seafood had, in general, low histamine concentrations (0–3 ppm) with the exception of fish meal (76 ppm), salted herring (47 ppm), one sample of smoked snoek (>50 ppm) and dried tuna (8000 ppm). In total, 5 of 80 examined samples (6%) contained histamine concentrations above the legal limit of 50 ppm. Experimental formation of histamine was demonstrated to be strongly temperature- and time-dependent. Samples were not contaminated with *Vibrio* spp., *Pseudomonas* spp., *Klebsiellas* spp. or *Enterobacteria*.

The data confirm that *Thyrsites atun* (snoek) and *Seriola lalandi* (yellowtail) are the primary fish species in South Africa posing a risk for consumers, as was documented in several scombrotoxism outbreaks.

Keywords: Histamine; Seafood; Scrombroid poisoning; Scombrotoxism; South Africa; Free histidine

1. Introduction

South Africa is the largest fishing nation in Africa with a total catch of 643,812 t in the year 2000 of which about 80% is exported (Burger, 2004). Mainly *Merluccius capensis* (hake), *Sardinops sagax* (pilchard), *Thunnus* spp. (tuna), *Thyrsites atun* (snoek – a mackerel), and *Seriola lalandi* (yellowtail, a pelagic caraganid fish) are consumed in Cape Town and exported. This ensures a continuous supply of the city and its surroundings with fresh seafood harvested from the Atlantic and Indian Oceans. South Africans consume large amounts of fresh and processed seafood: one million cans of pilchards alone are sold every day in the country (Burger, 2004). This is in addition to other canned seafood and popular products, such as smoked snoek and dried tuna (tuna "biltong"). The vicinity of the sea should ensure the supply of fresh seafood to the restaurants. There are, however, frequent incidents of adverse reactions to seafood, very often consisting of yellowtail (see Table 1). Medical reporting of scombroid toxication is infrequent in South Africa and, to our knowledge, there are no reported cases for the rest of the continent. This under-reporting is possibly the result of the lack of analytical laboratories able to detect histamine in Africa. Another reason may be that scombroid toxication is often mistaken for seafood allergy (Lopata & Potter,

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Table 1
Reported incidents of histamine poisoning in South Africa

Date	Number of cases	Fish species	Diagnosed by	Reference
1986	70	Unknown	Unknown	Taylor (1986)
1992	22[a]	Yellowtail	Symptoms/histamine quantification	Müller et al. (1992)
2004	19[b]	Yellowtail	Symptoms	Anonymus (2004a)
2004	1	Yellowtail	Symptoms	Anonymus (2004b)
2004	1	Tuna	Symptoms/histamine quantification	This study

[a] Ten incidents.
[b] One incident.

2000). Information on potential cases of scombrotoxism is anecdotal and often only available from newspaper reports. An example is an article in "The CapeTowner", which reported the most recent incident, in which 19 guests of a restaurant simultaneously developed rashes and heart palpitations after eating yellowtail (Anonymus, 2004a, 2004b). Such symptoms, amongst others (see Lehane & Olley, 2000), are indicative of so-called 'scombroid poisoning' which is caused by ingestion of more than 1 mg histamine per kg bodyweight (Taylor, 1986). A histamine level of 50 ppm is an indicator of decomposition (FDA, 1995) and several countries have set legal limits of histamine concentrations that are regarded as safe for human consumption: Australia, 200 ppm (Australian Food Standards Code, 2001), Europe, 100 ppm (EC, 2003), USA, 50 ppm (FDA, 1998) and South Africa, 100 ppm (South African Bureau of Standards, 2001). In this study, we apply the stricter FDA level.

There are three pre-requisites for the elevation of the post-mortem histamine concentration in fish: (1) a sufficiently high content of free histidine, (2) the presence of bacterial histidine decarboxylase, and (3) environmental conditions, such as high temperatures (Lehane & Olley, 2000).

The labelling 'scombroid poisoning' derives from the fact that symptoms of the poisoning were often observed after eating scombroid fish (tuna- and mackerel species). However, the term is misleading since fish from other families, such as Salmon (Salmonidae), have also been regularly reported to cause histamine poisoning (Gessner, Hokama, & Isto, 1996; Scoging, 1991). Many of these fish are large pelagic species that continuously swim at a high velocity and have, therefore, developed a high proportion of red musculature. Many of these red-muscled fish, that cause histamine poisoning, contain high concentrations of the free amino acid histidine (Taylor, 1986), although their white muscles contain more histidine than the red muscles (Takagi, Iida, Murayama, & Soma, 1969). Histidine is the substrate of the enzyme histidine decarboxylase, which converts histidine to histamine by so-called spoilage bacteria (Love, 1980). Such bacteria include members of the genera *Vibrio, Photobacterium, Klebsiella, Morganella* and others (Gram, 2002).

The temperature optima for most of these bacteria are in the range 20–30 °C (McMeekin, Olley, Ross, & Ratskowsky, 1993), although some histamine-producing bacteria have temperature optima below 10 °C (for example *Vibrio* species). Thus, elevated histamine levels in seafood indicate an interruption of the cooling chain at least at one stage (Richie & Mackie, 1979).

The present study was executed to quantify histamine levels in fresh and processed seafood. In addition, storage experiments were conducted with the most popular fish species (hake, snoek and yellowtail) to investigate the increase of histamine levels at 4 and 30 °C.

2. Materials and methods

2.1. Sample collection

A representative range of sources in the Cape Town area (South Africa) was chosen for sampling: an up-market seafood restaurant and shop, a fresh fish outlet, a 'Fish & Chips' shop, a retailer, an up-market retailer, the processing factory of a large fishing company, a street vendor, a producer of dry fish and a fishmeal factory. Origins of the samples are listed in Table 2. Canned seafood was bought off the shelf from a food retailer (sample numbers 70–80). Sample numbers are used to refer to the origin of the sample in Figures and Tables of Section 3.

Samples of 200–300 g of fresh and frozen seafood were collected from the respective outlets and carried on ice to the laboratory. Samples for determination of histamine and histidine concentrations, as well as for bacteriological investigation, were stored at −80 °C, except those for storage experiments (see below). Analyses took place no longer than one week after purchase of seafood.

When aliquoting the tissues, only ethanol-sterilized equipment was used to avoid contamination of the samples.

2.2. Histamine determination

Histamine was determined using the Neogen Veratox Histamine ELISA kit. In brief: an aliquot of each sample

Table 2
Origins of collected seafood samples

Sample origin[a]	Sample number	Species/product	
		Common name	Scientific name
Up-market seafood restaurant and shop	13, 14	Tuna	*Thunnus albacares*
	15–17	Yellowtail	*Seriola lalandi*
	25	Butterfish	*Chirodactylus brachydactylus*
	28, 29	Cape salmon	*Atractoscion aequidens*
	35–37	Cape hake	*Merluccius capensis*
	45	Kingklip	*Genypterus capensis*
	53–55	Salmon trout	*Salmo trutta*
	56	Black mussel	*Mytilus galloprovincialis*
	57	Black tiger prawns	*Penaeus monodon*
	58	Calamari	*Loligo vulgaris*
Fresh fish outlet	1	Snoek	*Thyrsites atun*
	10–12	Swordfish	*Xiphias gladius*
	15	Yellowtail	*Seriola lalandi*
	26, 27	Butterfish	*Chirodactylus brachydactylus*
	38	Cape hake	*Merluccius capensis*
	46	Kingklip	*Genypterus capensis*
Fish & Chips shop	2,3	Snoek	*Thyrsites atun*
	18	Yellowtail	*Seriola lalandi*
	30	Cape salmon	*Atractoscion aequidens*
	63	Smoked snoek	*Thyrsites atun*
Retailer	4	Snoek	*Thyrsites atun*
	64	Smoked snoek	*Thyrsites atun*
Up-market retailer	5–7	Snoek	*Thyrsites atun*
	19–24	Yellowtail	*Seriola lalandi*
	31–34	Cape salmon	*Atractoscion aequidens*
	39–42	Cape hake	*Merluccius capensis*
	47–49	Kingklip	*Genypterus capensis*
	52	Norwegian salmon	*Salmo salar*
	60–62	Salted herring	*Clupea harengus*
	65–68	Smoked snoek	*Thyrsites atun*
Fish processing factory	43	Cape hake	*Merluccius capensis*
	44	John Dory	*Zeus faber*
	50	Kingklip	*Genypterus capensis*
	51	Monk	*Lophius upsicephalus*
Street vendor	8, 9	Snoek	*Thyrsites atun*
Dry fish producer	69	Tuna	Unspecified
Fishmeal factory	59	Various	

[a] One source of each kind was sampled.

was homogenized using a blender. Two grammes of the homogenate were transferred into a centrifuge tube and a total volume of 10 ml adjusted with PBS-Tween buffer (pH 7.2). After shaking at room temperature for 30 min, the mixture was centrifuged at 3000*g* and 4 °C for 10 min. An aliquot of the aqueous supernatant was diluted appropriately for testing with the provided kit according to the manufacturer's manual.

2.3. Determination of free histidine

Small blocks of tissue were cut from frozen blocks of fish and subjected to freeze-drying for approximately two days. Thereafter, the samples were ground into a fine powder using a mortar an pestle. Two milligrammes of each sample and 40 µl of ethanol (100%) were vortexed in glass counting vials. The samples were allowed to stand for 1 h at 4 °C and were centrifuged thereafter at maximum speed in a bench-top centrifuge. The supernatants were transferred into fresh Eppendorf tubes, 128 µl of 0.066 M citric acid (pH 2.2) and 32 µl of norleucine (20 nmol) being added to each sample as an internal standard. Free histidine concentration of this solution was determined using a Waters Amino Acid Analyzer HPLC system. The buffer system consisted of (A) 0.2 M Na^+ (pH 3.0) and (B) 1.0 M Na^+ (pH 7.4). A linear gradient ran from 0% to 100% B in 40 min and remained at 100% B during the next 50 min at a flow rate of 1.0 ml min^{-1} to elute the amino acids from a Waters sulfonated cross-linked polystyrene column

(cation exchanger, 25 cm × 0.46 cm) at a temperature of 55 °C. Fluorimetric post-column detection of amino acids with *o*-phthaldialdehyde (OPA) was applied at 338 nm excitation and 425 nm emission.

2.4. Bacteriology

2.4.1. Homogenisation of tissue samples

Tissue aliquots of 25 g were weighed aseptically and placed in sterile plastic bags. 225 g of sterile buffered peptone water were added and the mixture homogenized in a stomacher for 30 s. Dilutions of 1:10 were prepared for testing within the next 45 min.

2.4.2. Vibrio, Pseudomonas, Aeromonas, Enterobacteria

Two cultures were prepared, each consisting of 25 ml of the initial 1:10 dilution and 25 ml of double strength peptone water with added NaCl, respectively. These cultures were incubated for 18–24 h at 36 ± 1 °C and thereafter plated out onto TCBS agar for single colonies. The plates were thereafter examined for *Vibrio*, *Pseudomonas*, *Aeromonas* and various Enterobacteria according to standard procedures.

2.4.3. Klebsiella and Pseudomonas

100 μl of initial 1:10 suspension were inoculated onto the surface of McConkey agar with crystal violet. Agar plates were inverted and incubated at 37 ± 1 °C for 18–24 h. Potential *Klebsiella* colonies and potential *Pseudomonas* colonies were further identified using standard biochemical methods.

2.5. Storage experiments

Exposure experiments were started on the day of sampling. The samples were incubated in sealed containers at 30 °C, whereas the other samples were kept in a refrigerator at 4 °C. Aliquots of the respective samples were taken at the times stated in the Figures, using sterile equipment, and frozen at −80 °C for analysis. Biochemical and bacteriological analyses took place no later than one week after purchase of seafood.

2.6. Chemicals and biochemicals

All chemicals and biochemicals were of analytical grade and were supplied by Sigma–Aldrich or Roche.

3. Results and discussion

3.1. Histamine levels in fresh seafood

Histamine levels in live seafood are generally low, as shown by several authors who measured histamine concentrations in freshly caught fish: values below 1 ppm were found in scombroid species, such as the skipjack (*Katsuwonus pelamis*) (Frank, Yoshinaga, & Nip, 1981; Thadhani, Jansz, & Peiris, 2002) or black skipjack (*Euthynnus lineatus*) (Mazorra-Manzano, Pacheco-Aguilar, Díaz-Rojas, & Lugo-Sánchez, 2000) whereas, in non-scombroid fish, such as in hake, (*Merluccius merluccius*), no histamine was found at all (Baixas-Nogueras, Bover-Cid, Vidal-Carou, & Veciana-Nogués, 2001; Ruiz-Capillas & Moral, 2001). The concentration of histamine, along with those of other biogenic amines, such as putrescine and cadaverine, accumulate, due to bacterial spoilage of fish tissue (Male, Bouvrette, Luong, & Gibbs, 1996; Veciana-Nogues, Vidal-Carou, & Marinè-Font, 1990). Such spoilage is most likely to happen during handling of the catch on fishing vessels or during unloading and storing in the port (Starkusziewicz et al., 2004). The involvement of contaminated tools is evidenced by the fact that gutted fish and particularly fillets have higher bacterial loads and histamine levels than whole fish (Chytiri, Paleologus, Savvaidis, & Kontominas, 2004; Paleologus, Savvaidis, & Kontominas, 2004). The phenomenon of histamine contamination was mostly recorded in scombroid fish, such as tuna and mackerel species (Suyama & Yoshizawa, 1973; Takagi et al., 1969). In the present study, however, histamine levels in scombroid fish are generally low (even in snoek purchased from a street vendor) and in a similar range when the three scombroid fish species are compared (Table 3). The only exception is one snoek sample (purchased from an up market retailer) that had more than 50 ppm histamine. Histamine levels were also low in non-scombroid fish species that were investigated. Samples of butterfish, Cape salmon, hake, kingklip, monk and Norwegian salmon contained little histamine (Table 3). Most samples of yellowtail and salmon trout also contained little histamine, but one yellowtail sample from an up-market retailer had a very high histamine level of 399 ppm and, in one sample of salmon trout, the level was raised to 48 ppm. The only sample taken from John Dory also had levels of histamine above 50 ppm; due to insufficient material, the exact value could not be determined.

In our present study, a few samples of different non-scombroid fish (hake, kingklip, monk) had no histamine (samples 43, 50, 51), indicating that these fish were completely unspoiled. These samples were collected from the processing factory of a large fishing company and indicate that fresh seafood should have no histamine.

It is also interesting to note that, in snoek samples taken from a street vendor (8–9), where one would expect the worst handling and storage conditions, histamine levels were lower (<5 ppm) than in a snoek sample (6) from an up-market food retailer (>50 ppm). In total, only 4 out of the 58 (9%) fresh seafood samples investigated in the present study had histamine levels above 50 ppm.

Table 3
Histamine concentrations in fresh seafood purchased from various outlets in Cape Town (South Africa)

Species	Sample number[b]	Histamine[a] [ppm]
Scombroid fish		
Snoek (*Thyrsites atun*)	1	1.4
	2–3	1.5–2.8
	4	4.5
	5–7	0.7–>50[c]
	8–9	1.2–4.3
Swordfish (*Xiphias gladius*)	10–12	0.8–3.9
Tuna (*Thunnus albacares*)	13–14	2.9–3.8
Non-scombroid fish		
Yellowtail (*Seriola lalandi*)	15–17	2.6–7.4
	18	2.5
	19–24	0.7–399[c]
Butterfish (*Chirodactylus brachydactylus*)	25	8.6
	26–27	0.9–6.0
Cape salmon (*Atractoscion aequidens*)	28–29	1.8–4.6
	30	7.2
	31–34	1.1–3.1
Hake (*Merluccius capensis*)	35–37	0.8–3.9
	38	6.1
	39–45	0.8–4.8
	43	0
John Dory (*Zeus faber*)	44	>50[c]
Kingklip (*Genypterus capensis*)	45	1.0
	46	0.7
	47–49	2.5–3.8
	50	0
Monk (*Lophius upsicephalus*)	51	0
Norwegian salmon (*Salmo salar*)	52	2.8
Salmon trout (*Salmo trutta*)	53–55	3.8–48.4
Marine invertebrates		
Black mussel (*Mytilus galloprovincialis*)	56	5.1
Black tiger prawns (*Penaeus monodon*)	57	0.5
Calamari (*Loligo vulgaris*)	58	0

[a] Histamine concentrations are given as individual values or, when multiple sampling took place from the same outlet, as range of the observed concentrations.
[b] The sample number is given to identify the outlet where sample was purchased (see Section 2).
[c] Concentration above legal limit of 50 ppm.

In the few samples of marine invertebrates that were investigated, histamine concentrations were very low (Table 3): no or negligible histamine levels were found in calamari, prawn and mussel samples.

Although the histamine levels in fresh seafood tested in the present study are generally acceptable, the high concentration found in one yellowtail highlights the hazard that derives from this popular fish species. Most known incidents of histamine poisoning are associated with yellowtail (see Table 1). The fish are often displayed on ice, a common practice in South Africa that has been previously criticised (Müller, Lamprecht, & Barnes, 1992). The problem with such a display is that the fish is only partially covered while the rest is exposed to ambient temperature.

3.2. Histamine levels in processed seafood

Histamine concentrations were investigated in a variety of processed seafood (Table 4) for which different preservation methods were used (canning, smoking, salting and drying).

It is noteworthy that canned seafood always had low histamine levels in the present study, in contrast to seafood that was processed with methods other than canning. In a variety of cans, representing various fish species processed in different ways, levels of histamine were generally low and in the narrow range of 0–2.8 ppm. In some cans, even in those containing scombroid fish (samples 71, 76, 77; see Table 4), no histamine could be detected. These low values indicate that high quality standards are in place in the processing factories of those companies from which samples have been tested in the present study.

In contrast, wide variations and sometimes high histamine values were observed in salted herring, smoked snoek, dried tuna and fish meal (the latter is used for animal feed). Amongst the six samples of smoked snoek were two samples (from an up-market retailer and a 'Fish & Chips' shop) that had histamine concentrations

Table 4
Histamine concentrations in different processed seafood purchased from various outlets in Cape Town (South Africa)

Product	Sample number[b]	Histamine[a] [ppm]
Fish meal	59	76.2[c]
Salted herring	60–62	0–47
Smoked snoek	63	>50[c]
	64	1.2
	65–68	0.8–36.3
Dried tuna ("biltong")	69	8001[c]
Canned fish		
Sardines in tomato	70	1.5
Pickled fish (hake)	71	0
Curried fish (snoek)	72	0
Pilchard cutlets	73	2.8
Pilchards in tomato	74	2.8
Shredded tuna	75	0.9
Solid pack tuna	76	0.1
Shredded tuna	77	0
Middle cut (makarel)	78	0
Middle cut (pilchard)	79	2.1
Solid tuna	80	2.4

[a] Histamine concentrations are given as individual values or, when multiple sampling took place from the same outlet, as range of the observed concentrations.
[b] The sample number is given to identify the outlet where sample was purchased (see Section 2).
[c] Concentration above legal limit of 50 ppm.

of 36 ppm and more than 50 ppm, respectively. The histamine concentrations in the sampled salted herrings, all from the same up-market retailer, varied widely from no histamine to almost 50 ppm. In a sample of dried tuna, known as tuna ''biltong'' in South Africa, the concentration was extremely high. Serious poisoning symptoms were seen in a 60-year old man, consuming about 25 g of the analysed sample. The sample was tested for histamine concentration and a level of 8000 ppm recorded. The person ingested, therefore, an amount of approximately 200 mg of histamine. This is well in the range of the 70–1000 mg histamine per meal (Cinquina et al., 2004), or 1 mg per kg bodyweight (Taylor, 1986) known to cause ''scombroid poisoning''. It is noteworthy that two dogs that were fed from the same piece of dried tuna, vomited almost immediately after ingestion.

Salting and canning may remove bacterial contamination (Fletcher, Summers, & van Veghel, 1998; Lehane & Olley, 2000) but they cannot destroy the causative toxin (histamine) of scombroid seafood poisoning (Etkind, Wilson, Gallagher, & Cournoyer, 1987). Any histamine found in such products should be, therefore, an indication of the conditions to which the seafood was exposed before processing. In contrast, favourable conditions for bacterial growth and hence, histamine production, continue at least for a certain period of time during the process of smoking and drying. There are similar reports of high histamine concentrations from dried seafood products. Dried sardines with a histamine concentration of 3000 ppm, for example, caused a histamine poisoning incident in Japan, involving one person (Kanki, Yoda, Ishibashi, & Tsukamoto, 2004).

The histamine concentration in fish meal is relatively high at 76 ppm, which is somehow expected when considering that less strict standards for raw material and processing apply than for human food (Macan et al., 2000).

3.3. Free histidine levels and bacterial contamination in fresh and processed seafood

In selected samples of fresh and processed seafood, concentration of histamine was compared to levels of free histidine and to bacteriological contamination (Table 5) with bacterial species thought to be involved

Table 5
Concentrations of free histidine and bacterial contamination in fresh and processed seafood purchased from various outlets in Cape Town (South Africa)

Species	Sample number[b]	Histamine[a] [ppm]	Free histidine [ppb]	Bacteria[c]
Fresh seafood				
Snoek (*Thyrsites atun*)	6	>50	1.58	Negative
	8	4.3	1.43	Negative
Tuna (*Thunnus albacares*)	14	2.9	3.0	n.i.
Yellowtail (*Seriola lalandi*)	15	7.4	5.32	Negative
	17	2.6	5.04	n.i.
	19	399	4.38	Negative
Cape salmon (*Atractoscion aequidens*)	28	4.6	0	n.i.
	31	2.7	0	n.i.
Hake (*Merluccius capensis*)	35	3.9	0	Negative
	39	4.8	0	Negative
John Dory (*Zeus faber*)	44	>50	n.i.	Negative
Kingklip (*Genypterus capensis*)	45	1.0	0	n.i.
	47	2.5	0	n.i.
Norwegian salmon (*Salmo salar*)	52	2.8	n.i.	Negative
Salmon trout (*Salmo trutta*)	53	3.8	0.52	Negative
	55	48.4	0.3	Negative
Processed seafood				
Fish meal	59	76.2	n.i.	Negative
Smoked snoek	63	>50	n.i.	Negative
	65	0.8	n.i.	Negative

n.i. = not investigated.
[a] Histamine concentrations are given as individual values.
[b] The sample number is given to identify the outlet where sample was purchased (see Section 2).
[c] The presence of the following bacteria was tested: *V. cholerae*, *V. parahaemolyticus*, *Pseudomonas aeruginosa* and *Klebsiella* spp. (for details see Section 2).

in the formation of histamine from free histidine (see Section 1).

In fresh seafood, measurable histidine levels were observed in scombroid fish (snoek, tuna), as well as in the non-scombroid yellowtail. The concentrations were highest in yellowtail (4.4 to 5.3 ppb). In the other fresh seafood samples investigated, the histidine concentration was either low (salmon trout) or not detectable (Cape salmon, hake, kingklip). These results are in agreement with previous findings that pelagic fish, that have a high proportion of red muscles contain more histidine than those fish in which white muscles dominate (Suyama & Yoshizawa, 1973).

The bacteriological methods applied in the present study were most likely too restricted to detect all the potential histamine-producing bacteria in the investigated samples (see Table 5 and Figs. 1 and 2): no bacterial contamination could be found in samples that had elevated histamine concentrations (6, 19, 44, 55), not even in those with significant histamine levels (6, 19). The same was true for processed seafood. Despite having high histamine levels, no contamination with the bacterial species investigated could be found in fishmeal or smoked snoek from two different outlets. The nature of the involved bacterial species needs further investigation with a broader spectrum of methods. The conditions in the present study were restricted to the detection of *Vibrio*, *Pseudomonas*, *Aeromonas* and *Enterobacteria* (see Section 2). This probably excluded other potential species with a similar temperature optimum (for example *Morganella*, *Hafnia*) but also those that have lower temperature optima (for example *Photobacterium* species, see Lehane & Olley, 2000).

3.4. Time course of histamine concentration in fish at different ambient temperatures

Samples from three different fish species (the non-scombroid hake and the scombroid species snoek and tuna) were exposed to two different ambient temperatures immediately after purchase and histamine levels were observed (Fig. 1).

The histamine level in the hake sample at 4 °C increased only marginally from 1.7 to 5.3 ppm during four days. No bacterial contamination was observed during this time. The increase in the histamine concentration was more pronounced in the hake sample that was incubated at 30 °C. The level increased from an initial 0.8 to 32.3 ppm four days later. In aliquots of that sample taken on days 1 and 2, contamination with a histamine-producing bacteria (*Klebsiella pneumoniae*) of 200 and 500 cfu g^{-1} was observed. The histamine formation is slow when compared to tissue from scombroid fish and yellowtail (see below) and may be limited by a lack of free histidine in hake (see Table 5). Our findings resemble those of other authors in a different hake species, *Merluccius merluccius* (Baixas-Nogueras et al., 2001; Ruiz-Capillas & Moral, 2001).

At 4 °C ambient temperature, the histamine level in snoek rose steadily from an initial 2.8 to more than 500 ppm after four days of exposure. When the same sample was incubated at 30 °C, the histamine level increased from 2.8 to more than 500 ppm in only one day of exposure, although none of the analysed bacteria were found.

A similar pattern was found in tuna that was exposed to 30 °C: from an initial histamine concentration of 2.9 ppm, the level rose to more than 500 ppm during

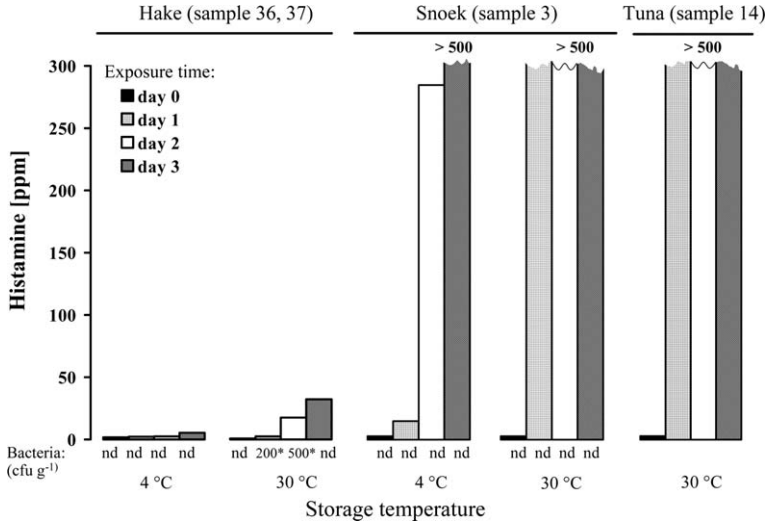

Fig. 1. Course of histamine concentration in tissue samples taken from *Merluccius capensis* (hake), *Thyrsites atun* (snoek) and *Thunnus albacares* (tuna) during incubation at different ambient temperatures (4 and 30 °C) immediately after purchase from different sources. Bars indicate individual histamine concentrations. **Klebsiella spp.* #The sample number is given to identify the outlet where sample was purchased (see Section 2).

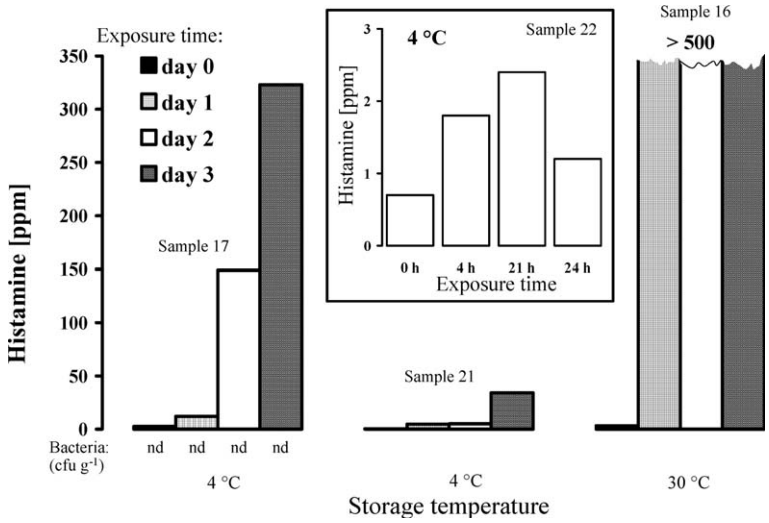

Fig. 2. Course of histamine concentration in tissue samples, taken from *Seriola lalandi* (yellowtail) during incubation at different ambient temperatures (4 and 30 °C) immediately after purchase from different sources. Insert depicts course of histamine concentration during the initial 24 h in the sample obtained from outlet 5. Bars indicate individual histamine concentrations. #The sample number is given to identify the outlet where sample was purchased (see Section 2).

one day of exposure and remained above 500 ppm thereafter. Bacterial contamination was not investigated.

The time-course of histamine production in these two species was expected because of the high concentration of free histidine present in scombroid fish (Suyama & Yoshizawa, 1973) and it is similar to that in other scombroid species (Ben-Gigirey, Vieites Baptista de Sousa, Villa, & Barros-Velasquez, 1998; Hwang, Chang, Shiau, & Cheng, 1995).

When a yellowtail sample was exposed to 4 °C, the histamine concentration increased from 2.6 ppm at the beginning of the experiment to more than 300 ppm after four days of exposure (Fig. 2). An aliquot of the same yellowtail was exposed to 30 °C. The histamine concentration was initially 3.3 ppm and increased to more than 500 ppm after only one day of exposure. Bacterial contamination was not detected.

Histamine concentration in a yellowtail sample from a different outlet behaved differently from the previous sample. When exposed to 4 °C, the level increased from 0.5 ppm at the beginning to only 34 ppm after four days of exposure. This again highlights the influence of freshness and conditions of subsequent storage on histamine production. A sample from the same outlet, but from a different sampling day, was used to record histamine levels in more detail during 24 h of exposure to 4 °C (see insert in Fig. 2). In this sample, histamine concentration rose from 0.7 ppm at the beginning of the experiment to 2.4 ppm after 21 h, after which it declined to 1.2 ppm.

Although not a scombroid fish, yellowtail is a large pelagic fish with a high ratio of red muscles accompanied with a high concentration of free histidine (see above). This explains why yellowtail, similar to scombroid species such as tuna and mackerel (see above), is likely to accumulate high histamine concentrations under conditions favourable for histidine-carboxylating bacteria.

4. Conclusion

Our results demonstrate that few seafood samples in this study were above the legal limit for histamine levels. It also seems clear that the type of seafood outlet is not indicative of a possible histamine contamination but rather the freshness of the sample. Some popular fish species (*T. atun*, *Thunnus* spp., *S. lalandi*) are more prone to histamine formation than most other species analyzed (e.g. *M. capensis*). Future research needs to concentrate particularly on non-scombroid species to understand the mode of histamine formation and to provide a scientific basis for regulatory policies.

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