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BIOGENIC AMINES IN FISH AND SHELLFISH

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I. INTRODUCTION

The term biogenic amines refers to the nonvolatile amines such as cadaverine, putrescine, spermidine, spermine, tyramine, tryptamine, and histamine produced post mortem in fish and shellfish products. The compounds are reported to originate from the decarboxylation of specific free amino acids in fish or shellfish tissue. Chemically, the biogenic amines are defined as low-molecular-weight aliphatic, alicyclic, or heterocyclic organic bases (Davídek and Davídek, 1995). The use of chemical compounds as objective product standards or indices of quality has long been suggested since these tests are rapid when compared to traditional microbiological analyses and less subject to individual interpretation than sensory analyses.

II. NONVOLATILE AMINE FORMATION

The decarboxylation process can proceed through two biochemical pathways: decarboxylation through endogenous (naturally occurring) decarboxylase enzymes or by exogenous decomposition through enzymes released by the microflora associated with a seafood product. There is general agreement among researchers that the endogenous production of diamines is insignificant when compared to the exogenous pathway (Wendakoon and Sakaguchi, 1992).

Polyamines, present in all biological materials, have been implicated in a wide variety of biological reactions (i.e., DNA, RNA, and protein synthesis) (Tabor and Tabor, 1976; Walters, 1987). They have also been shown to maintain the cell envelope integrity of bacteria (Tabor and Tabor, 1985). Besides, the covalent linking of cadaverine (Kamio *et al.*, 1986) and putrescine (Kamio and Nakamura, 1987) to the peptidoglycan is necessary for normal growth of certain bacteria. Using thin-layer chromatography, Rolle *et al.* (1971) were able to detect ethanolamine, methylamine, dimethylamine, putrescine, and spermidine in the unicellular green algae *Chlorella fusca* and *Scenedesmus acutus.*

III. AMINE DETOXIFICATION

Histamine is formed by the decarboxylation of the amino acid histidine, which is found in high levels in tissues of scombroid fishes (Frank, 1985).

Upon ingestion, histamine is detoxified primarily by the enzymes diamine oxidase and histamine *N*-methyltransferase (Hui and Taylor, 1985). Relatively high doses of pure histamine have been administered to humans with no apparent ill effect (Arnold and Brown, 1978; Douglas, 1980; Ijomah *et al.*, 1992), leading to the suggestion that scombroid poisoning by spoiled fish is caused by histamine acting synergistically with other diamines present in the fish, primarily putrescine and cadaverine (Bjeldanes *et al.*, 1978). Putrescine (1,4-diaminobutane) is the decarboxylation product of the amino acid lysine and cadaverine arises from the decarboxylation of ornithine (Fig. 1). Both putrescine and cadaverine may interfere with the normal histamine detoxification system of the intestine by competing under certain conditions with histamine as substrates for diamine oxidase. Cadaverine and putrescine potentiate peroral toxicity of histamine in the guinea pig (Bjeldanes *et al.*, 1978). Spermine and cadaverine increase histamine transport across the gastrointestinal wall (Jung and Bjeldanes, 1979) with little change in the relative amounts of histamine metabolites produced.

Diamine oxidases belong to a widespread class of enzymes related to polyamine metabolism (Hill, 1971; Smith, 1985). Being a key enzyme in the metabolism of polyamines, essential for the growth and replication of all living cells (Bachrach, 1973; Walters, 1987), they catalize the oxidative deamination of a range of primary diamines (Fig. 2) (Equi *et al.*, 1991). Inhibitors of diamine oxidases are known to possess antimalarial, antitrypanosomal, antibacterial, and antifungal activities together with possible roles in cancer chemotherapy (Equi *et al.*, 1991).

Clinical studies have considered the activity of diamine oxidases to be a useful parameter (Baylin, 1977; Luk *et al.*, 1980) thus, many different assays to determine the activity of diamine oxidases have been proposed. Procedures using radioactive substrate (Okuyama and Kobayashi, 1961; Kusche *et al.*, 1973) achieve high sensitivity and most recently the use of high-performance liquid chromatography has been described (Biondi *et al.*, 1984).

IV. BACTERIAL SPECIES WITH DECARBOXYLASE ACTIVITY

It is known that different bacteria vary significantly in either the quantity of decarboxylase they produce and/or the specific activity (turnover number) of those decarboxylases (Wendakoon and Sakaguchi, 1992). It is also known that the composition of the fishery product significantly affects the amount of decarboxylase a bacterium may release.

Primarily responsible for the decomposition of the scombroid fish, with the ability to decarboxylate histidine to form histamine (Table I), are

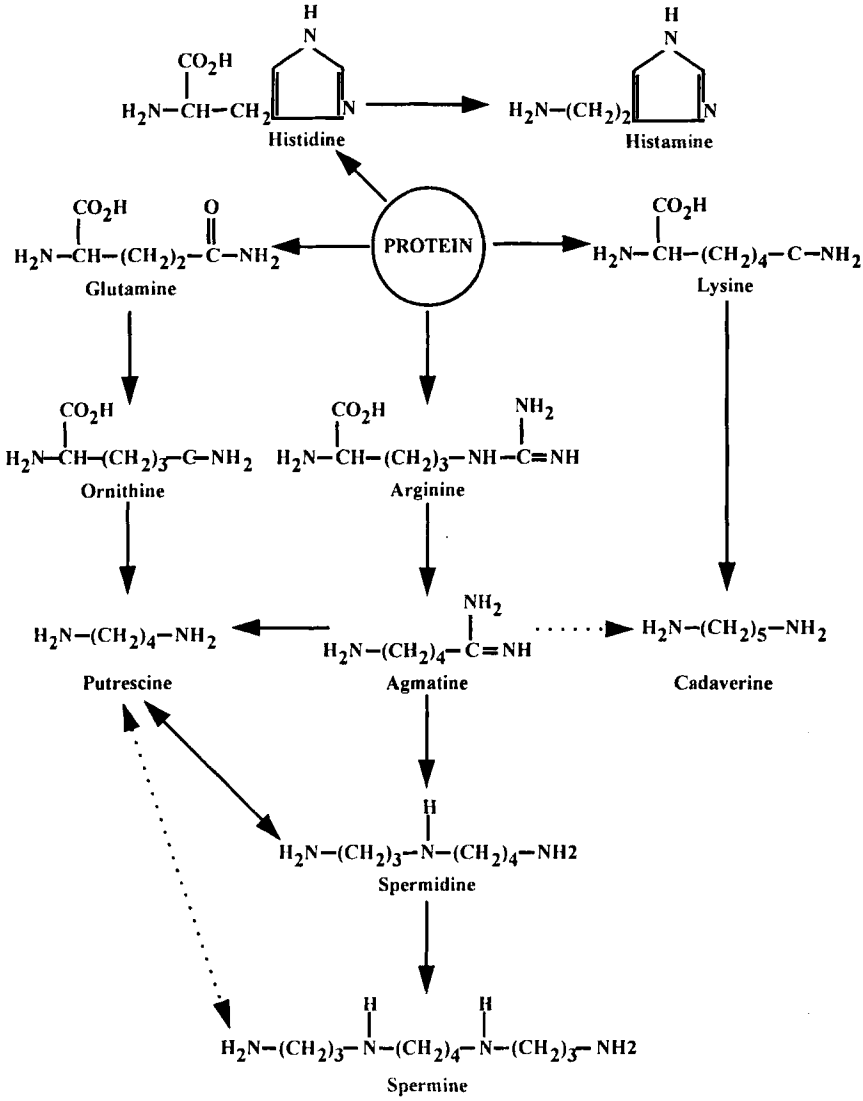

FIG. 1. Biological pathways for the formation of histamine, putrescine, cadaverine, spermidine, and spermine.

the *Enterobacteriaceae* (Frank *et al.*, 1985; Taylor and Sumner, 1986). Histamine-producing bacteria isolated from fish causing scombroid poisoning are *Morganella* (*Proteus*) *morganii, Klebsiella pneumoniae,* and *Hafnia alvei* (Taylor and Speckhard, 1983). *Pleisomonas shigelloides,* a bacterium frequently isolated from fish and aquatic environments, was identified as

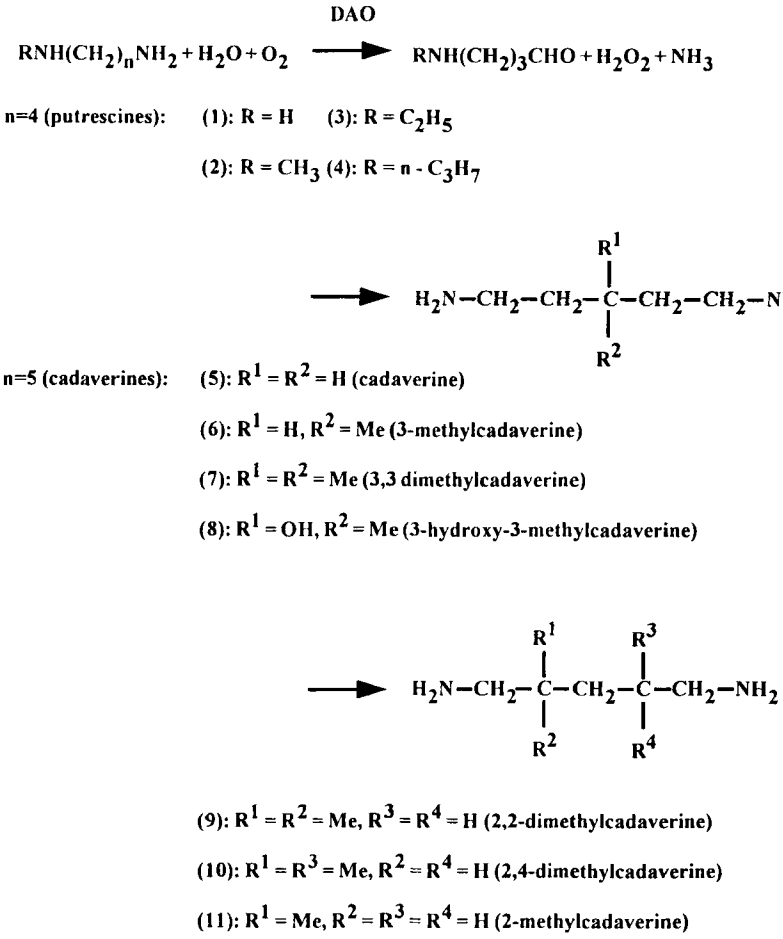

FIG. 2. Deanimation of primary diamines by diamine oxidases (DAO) (based on Equi *et al.*, 1991).

a new histamine former in fish by Lopez-Sabater *et al.* (1994b). In addition, bacteria isolated from skipjack tuna (*Euthynnus pelamis*) and found to produce histamine are *Proteus vulgaris, Proteus mirabilis, Clostridium perfringens, Enterobacter aerogenes,* and *Vibrio alginolyticus* (Arnold *et al.*, 1980; Yoshinaga and Frank, 1982; Frank *et al.*, 1985). Three bacterial species not previously reported to have the potential to produce histamine; *Acinetobacter lwoffi, Pseudomonas putrefaciens,* and *Aeromonas hydrophila,* were isolated from decomposing spanish mackerel (*Scomberomorus maculatus*) together with 11 other bacterial species (*Clostridium perfringes, Enterobacter aerogenes, Enterobacter* sp., *Hafnia alvei, Morganella morganii, Proteus*

TABLE I

MICROBIAL ISOLATES SHOWING HISTIDINE DECARBOXYLASE ACTIVITY[a]

Identification of isolate	Decomposition temperature(s) at which found (°C)	Source
Acinetobacter lwoffi	0, 15	
Aeromonas hydrophila	0, 15	Beef
Citrobacter freundii	15	Skipjack tuna
Clostridium perfringens	15, 30	Skipjack tuna
Edwardsiella sp.	15	Mahi-mahi
Enterobacter aerogenes	15, 30	Skipjack tuna, tuna, mahi-mahi, pork, beef
Enterobacter sp.	30	Food, tuna
Escherichia coli	15, 30	Tuna
Hafnia alvei	15, 30	Tuna, skipjack tuna, mackerel, food
Klebsiella pneumoniae	15	Tuna, mahi-mahi, mackerel
Klebsiella sp.	15	Skipjack tuna, mackerel
Morganella morganii	0, 15, 30	Scombroid fish, pork, turkey, mahi-mahi
Proteus mirabilis	15	Pork, turkey, tuna, skipjack tuna
Proteus vulgaris	30	Beef, pork, turkey, tuna
Proteus sp.	15	Fish, feces, tuna, mackerel, food, milk
Pseudomonas fluorescens/putida	15	
Pseudomonas putrefaciens	0, 15, 30	
Pseudomonas sp.	0, 15	Food
Vibrio sp.	15	Mackerel
Vibrio alginolyticus	0, 15, 30	Skipjack tuna

[a] Activities determined using the carboylase base broth supplemental with histamine.

mirabilis, Proteus vulgaris, Proteus sp., *Pseudomonas fluorescens/putida, Pseudomonas* sp., and *Vibrio alginolyticus*) with decarboxylase activity (Middlebrooks *et al.*, 1988). Histamine forming bacteria in tuna fish (*Thunnus thynnus*) destined for canning are primarily members of the *Enterobacteriaceae* family (Lopez-Sabater *et al.*, 1994a). Among these, the most active and frequent histamine former was *Morganella morganii,* followed in activity by *Klebsiella oxytoca, Klebsiella pneumoniae,* and some strains of *Enterobacter cloacae* and *Enterobacter aerogenes.* All these bacteria produced more than 500 ppm of histamine under experimental conditions. Using gas–liquid chromatography, Suzuki *et al.* (1988) studied the production of polyamines by putrefactive *Pseudomonas* type III/IV. They found that halophilic types produced spermidine in high ratios and that nonhalophilic types produced putrescine but did not produce spermidine. *Photobacterium* was also shown to be involved in the production of histamine, agmatine, and

cadaverine (Okuzumi *et al.*, 1990). Histamine production by *Photobacterium phosphoreum* was greater under anaerobic than aerobic conditions, while the organism grew better under aerobic conditions (Morii *et al.*, 1994).

Behling and Taylor (1982) reported that the minimum temperature for two strains of *Morganella morganii* to produce toxicologically significant levels of histamine in tuna fish infusion broth (30 mg/100 g) was 15°C. This was confirmed by Klausen and Huss (1987) who reported that *M. morganii* grows well at temperatures of 15°C (generation time is 2.6 hr at 15°C, 1.08 hr at 25°C) or above and that the growth is greatly reduced at temperatures below 10°C (generation time is 10.2 hr at 10°C, 14.9 hr at 5°C). They were able to confirm that large amounts of histamine (600–1400 ppm) can be produced by *M. morganii* in mackerel stored at low temperatures (0–5°C) following storage at higher temperatures (10–25°C).

Frank *et al.* (1985) studied the composition and decarboxylase activity of bacteria isolated from decomposed mahi-mahi. They reported that the mesophilic bacteria isolated from fish incubated at 32°C for 24 hr were mainly Gram-negative rods; 89% of these were *Vibrio alginolyticus.* Strong histamine-producer (>100 mg/100 ml) mesophiles were *Morganella morganii* and *Proteus mirabilis.* Weak histamine-producer (<10 mg/100 ml) mesophiles were all *Vibrio alginolyticus.* Psychrotrophic isolates obtained from fish incubated at 0°C for 14 days were essentially Gram-negative bacteria, 9% of which were *Alteromonas putrefaciens,* a weak histamine producer (<1 mg/100 ml) at 5 and 20°C.

To evaluate a microorganism's ability to produce histamine in tuna fish, Omura *et al.* (1978) developed a culture medium from an extract of fresh skipjack tuna (*Euthynnus pelamis*). The medium was later modified by Arnold *et al.* (1980), Yoshinaga and Frank (1982), and Ramesh *et al.* (1989). A further modification in the composition of the broth was further obtained by using sardine instead of skipjack. Variations in composition, due to species and quality of the raw material, were reported by Taylor and Woychik (1982) and Yoshinaga and Frank (1982). Taylor and co-workers (1978b) were able to eliminate some variation by using a medium consisting of trypticase soy broth supplemented with 0.1% histidine monohydrochloride with a pH adjusted to 6.8. To assure maximum histamine formation, Taylor and Woychik (1982) increased the histidine content to 2% and adjusted the pH to 6.3. The medium was further modified (Frank *et al.*, 1985) by adding 0.01% pyridoxal hydrochloride as a cofactor. Early studies determined the bacterial histidine decarboxylase activity by using a Warburg manometer to measure the CO_2 volume released in the culture broth. Nowadays, the histamine produced in the culture broth is measured mainly by high-pressure liquid chromatography (HPLC), spectrofluorometry and thin-layer chromatography (TLC). Sumner and Taylor (1989) developed a

method for detecting histamine-producing lactic acid bacteria, using leuco-crystal violet and diamine oxidase. With this technique it was possible to detect bacteria capable of producing more than 1.2 μmol of histamine per milliliter. However, the technique had only qualitative value due to interference from the culture broth (Sumner and Taylor, 1989). Lopez-Sabater *et al.* (1994b) adapted an enzymatic method to measure histidine decarboxylase activity in bacteria isolated from fish. Quantification of histamine at levels as low as 3 ppm were achieved, with correlation of 0.99 between histamine content and the increase of absorbance in the concentration range between 3 and 30 ppm.

V. AMINES PRESENCE IN THE MARINE ECOSYSTEM

The diamines putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) belong to a group of natural polyamines which serve as stabilizing cations of the macromolecular structure of DNA and RNA and can be expected to be abundant in all living matter (Cohen, 1971, 1978). The abundance of these diamines is documented in all major groups of marine organisms such as algae (Rolle *et al.*, 1971), invertebrates (Manen and Russell, 1973), vertebrates (Cohen, 1971), and microorganisms (Cohen, 1971). Therefore, the presence of diamines in the marine environments can be expected. Höfle (1984) investigated the potential in the marine microbial community to degrade diamines. They reported removal of putrescine and cadaverine from coastal waters supplemented only with these compounds within 48 hr. There was no increase in the bacterial biomass, growth rate, or viability when compared to the control (unsupplemented) cultures. Labeled putrescine experiments indicated that most putrescine carbon is mineralized to CO_2 rather than assimilated by the bacteria. At the concentrations added (500 μg per liter), the diamines were not toxic to the marine bacteria.

VI. AMINES OCCURRENCE IN SEAFOOD

A. FINFISH

Fish muscle can support formation of a wide variety of amine compounds resulting from the direct enzymatic decarboxylation of amino acids. The substrate for the decarboxylase enzymes are free amino acids, therefore amine build-up normally occurs during a decomposition or spoilage process involving formation of free amino acids through proteolysis together with bacterial production and action of an amino acid decarboxylase (Eitenmiller

and De Souza, 1984). Amines frequently found in fish muscle include cadaverine from lysine, putrescine from ornithine, and histamine from histidine. Factors involved in the formation of histamine, related to scombroid food poisoning (histamine intoxication), have been widely studied in several different fish species. Tuna and other fish from the families *Scomberesocidae* and *Scombridae* and a nonscombroid fish, mahi-mahi or dolphin (*Coryphaena hippurus*), are known to contain high levels of histamine when spoiled. Muscle in scombroid fish as well as in mahi-mahi contains high levels of histidine, being readily transformable in toxic levels of histamine. Determination of histamine in such species is difficult due to the lack of rapid detection methods, because histamine can reach toxic levels in the absence of organoleptic indication of spoilage and because there may be an unequal distribution of the toxic agent in the fish.

Kim and Bjeldanes (1979) determined concentrations of cadaverine, putrescine, histamine, spermidine, and spermine in canned wholesome tuna and in canned tuna that had been implicated in an outbreak of scombroid poisoning in humans (Table II). Fresh tuna recently caught contains negligible quantities of histamine, usually less than 1 ppm (Frank *et al.*, 1981). Fernandez-Salguero and Mackie (1987b) determined the levels of higher amines in canned tuna, mackerel, and sardine. They reported very low levels of histamine (below 0.5 mg/100 g) in all the samples analyzed, putrescine cadaverine ranged between 0 and 1.5 mg/100 g and spermidine and spermine between 0 and 2.5 mg/100 g.

A good correlation has been found in Spanish mackerel (*Scomberomorus maculatus*) between the levels of histamine, cadaverine, and putrescine and the time and temperature of decomposition, between the ratios of cadaverine/histamine and putrescine/histamine levels and the temperature of decomposition, and between increasing total microbial counts and rising amine levels (Middlebrooks *et al.*, 1988). No formation of amines during ice storage of mackerel (*Scomber japonicus*) was reported by Wendakoon *et al.* (1990). However, at 20°C, histamine, putrescine, cadaverine, and tyramine were formed in large amounts. The low initial levels of spermidine in both dark and white muscle decreased during storage regardless of temperature. Amine production rate in dark muscle was higher and reached higher levels than in white muscle.

Histamine poisoning (scombrotoxic fish poisoning) has been frequently associated with the consumption of spoiled scombroid fish, which usually have high levels of histidine in their muscle tissue, such as tuna and mackerel (Gilbert *et al.*, 1980). However, pelagic fish, such as sardines, herring, and pilchards, and some types of cheese have been involved in outbreaks of this illness (Taylor, 1986). Thin-layer chromatography analysis of nine samples of smoked herring indicated that putrescine, cadaverine, and spermine

TABLE II

TLC ANALYSIS OF AMINES IN COMMERCIAL (NONDECOMPOSED) AND DECOMPOSED CANNED TUNA FISH[a]

Fish sample	n^b		Range of amines (mg%)				
			Cadaverine	Putrescine	Histamine	Spermidine	Spermine
Commercial canned	34		0 ~ 3.70	0 ~ 2.50	1.0 ~ 17.0	0.47 ~ 7.90	0.25 ~ 2.90
tuna fish		Avg ± SE	1.05 ± 0.60	0.35 ± 0.10	2.74 ± 0.71	3.26 ± 0.33	1.23 ± 0.09
		Median	0.80	0.12	1.97	2.5	1.15
Canned decomposed[c]	15		2.40 ~ 21.0	0 ~ 5.60	9.70 ~ 200	0 ~ 4.90	0 ~ 2.2
tuna fish		Avg ± SE	12.8 ± 1.66	1.53 ± 0.44	116 ± 6.24	2.37 ± 0.50	1.16 ± 0.15
		Median	10.8	1.25	118	2.63	1.30

[a] Data from Kim and Bjeldanes (1979).
[b] Total number of cans analyzed.
[c] Lots D417 and D419 implicated in human poisoning.

were present at levels between 1 and 16 $\mu g\ g^{-1}$ (nine samples), while tyramine, spermidine, and histamine were present at low levels (1–8 $\mu g\ g^{-1}$) in five, five and seven samples, respectively (Shalaby, 1995). Tryptamine and phenylethylamine were not detected in any samples analyzed. In some cases, fish with low contents of histamine have been implicated (Murray *et al.,* 1982), indicating that other substances might be involved, possibly as histamine toxicity potentiators (Bjeldanes *et al.,* 1978; Klausen and Lund, 1986). Biogenic amines such as cadaverine and putrescine have been shown to potentiate the uptake of histamine *in vitro* (Lyons *et al.,* 1983) and inhibit intestinal histamine-metabolizing enzymes *in vivo* (Hui and Taylor, 1985).

Fletcher *et al.* (1995) in a survey of the histidine levels in retail fish in New Zealand, found levels above 1000 mg/100 g of muscle in the white muscle of Albacore (*Thunnus alalunga*) (4280 mg/100 g), Kingfish (*Seriola grandis*) (1580 mg/100 g), and Kahawai (*Arripis trutta*) (1242 mg/100 g). Changes in bacteria, amino acids, and biogenic amines in sardines (*Sardina pilchardus*) stored at ambient temperature were reported by Ababouch *et al.* (1991). Table III shows the bacterial counts for fresh sardines. Bacteria located initially on the skin and gills of the freshly caught sardines invaded and rapidly grew in the sardine muscle, reaching 5×10^8 CFU/g after 24 hr at ambient temperature and 6×10^8 CFU/g after 8 days in ice. The fresh sardines consisted of 64% water, 20% protein (N × 6.25), 2.4% fat, and 2.5% ash. Sardine amino acid content (Table IV) is rich in histidine, mainly free, arginine, phenylalanine, and lysine. During storage, a decrease in the levels of histidine, arginine, lysine, tyrosine, and methionine and an accumulation of the other amino acids, except proline and taurine, was observed in the fish muscle. After 24 hr of storage at ambient temperature, histamine, cadaverine, and putrescine accumulated to levels of 2350, 1050, and 300 ppm, respectively. In ice, histamine and cadaverine reached similar levels after 8 days, while putrescine formation was insignificant. The large amount of cadaverine accumulated in the sardines indicated that proteolysis had taken place during storage as the initial amount of its precursor, free lysine, was low (200 ppm). Arginine can be metabolized into ornithine and/

TABLE III

BACTERIAL COUNTS OF FRESH SARDINES (*Sardina pilchardus*) (CFU/CM^2 OF SKIN OR PER GRAM OF GILLS, VISCERA, MUSCLE, OR MUSCLE + SKIN)[a]

	Skin	Gills	Viscera	Muscle	Muscle + Skin
Total bacterial count	2.5×10^6	1.2×10^5	3.1×10^4	3×10^4	1.3×10^6
Histamine producing bacteria	5×10	3.3×10^5	1.1×10^4	6×10^2	4.4×10^2

[a] Data from Ababouch *et al.* (1991).

TABLE IV
AMINO ACID COMPOSITION OF FRESH SARDINES[a] (*Sardina pilchardus*)

Type of amino acid	Free amino acid content (mg 100 g^{-1})	Total amino acid content (mg g^{-1})
Thr	13.9	10.3
Val	14.6	15.9
Met	4.6	9.2
Cys	0	1.6
Ileu	6.9	13.4
Leu	13.7	22.1
Phe	9.7	12.3
Tyr	3.9	9.4
Lys	20.0	29.0
Trp	0	0
Asp	4.1	17.9
Ser	9.0	9.6
Glu	29.9	40.9
Pro	19.2	10.0
Gly	13.9	15.7
Ala	56.0	17.4
His	288.8	10.6
Arg	54.0	17.7
Taurine	128.6	1.3
Ornithine	7.5	0.085

[a] Data from Ababouch *et al.* (1991).

or putrescine. Ornithine can also be derived from glutamic acid (Lehninger, 1982).

Amine content in red perch and anchovy, determined by extraction with trichloroacetic acid and high-pressure liquid chromatography, are shown in Table V.

The free amino acid content in fishery products is high when compared to terrestrial animals since the primary function of the compounds in aquatic organisms is to serve as osmoregulators. It is also known that the amino acids in fish can be rapidly decarboxylated. Haaland *et al.* (1990) reported that the formation of free amino acids post mortem was temperature dependent. Most amino acids showed higher values at 2°C than at 20°C, and amine formation was higher at 20°C than at 2°C. They also reported that the formation of cadaverine and putrescine was higher in whole (ungutted) fish than in filets taken from stored whole fish. However, the reverse results were observed when gutted fish were studied. Fernandez-Salguero and Mackie (1987a) reported that histamine, cadaverine, and putrescine were produced more rapidly in haddock (*Melanogrammus aeglefinus*) fillets than

TABLE V
AMINE CONTENT (HPLC DETERMINATION) IN RED PERCH AND ANCHOVY SAMPLES[a]

Amine	Average content (mg/kg)	
	Anchovy	Red perch
Putrescine	18	73
Cadaverine	107	109
Histamine	650	ND[b]
Tyramine	111	ND

[a] Data from Feier and Goetsch (1993).
[b] ND, not determined.

in the whole gutted fish and that ungutted fish spoiled more rapidly than fillets. Reported concentrations (Fernandez-Salguero and Mackie, 1987a) of nonvolatile amines formed in haddock and herring during storage in ice and at 5°C are presented in Tables VI and VII. The rates of biogenic amine formation in fish can be summarized as whole ungutted fish > filets from whole ungutted fish; filets > whole gutted fish.

In storage tests (Wei *et al.*, 1995), fresh swordfish was shown to contain spermine, and the longer the storage, the higher in the amount of putrescine, cadaverine, histamine, and spermidine. The order of producing rate of biogenic amine was histamine > cadaverine > spermidine > putrescine.

Amino acid formation is also dependent on the harvesting season and feeding activity prior to capture (Aksnes and Brekken, 1988). Fish harvested in summer during feeding quickly liberated large quantities of lysine and arginine, the microbial precursors of cadaverine and putrescine, respectively, due to the presence of large quantities of intestinal tract enzymes. Haaland *et al.* (1990) concluded that the storage conditions of whole mackerel have little effect upon the nutritional composition of whole fish meal, unless the fish is stored for at least 7 days at 20°C.

Dawood *et al.* (1988) studied the effect of holding (between 0 and 30°C) freshly caught rainbow trout (*Salmo irideus*) for a period of 6 hr prior to chilled storage. Samples of whole and eviscerated fish were analyzed for putrescine, cadaverine, histamine, spermidine, and spermine by high-performance liquid chromatography of their benzoyl derivatives at intervals between 2 and 14 days of storage at 0°C. They observed an increase in the concentration of putrescine (<3.5 to 6–8 μg/g), cadaverine (<1 to 1–4 μg/g), and histamine (<1 to 4.5–13 μg/g) during storage, while the levels of spermidine (initially <5 μg/g) and spermine (initially <6 μg/g) decreased after an initial increase during the first 4 days. Tyramine was

TABLE VI

PRODUCTION OF HIGHER AMINES (MG/100 G TISSUE) IN HERRING AND HADDOCK HELD IN ICE AS FILLETS AND WHOLE FISH[a]

Time of storage (days)	*Putrescine*		*Histamine*		*Cadaverine*		*Spermidine*		*Spermine*	
	Whole fish	Fillet	Whole fish	Fillet	Whole fish	Fillet	Whole fish	Fillet	Whole fish	Fillet
Herring										
0	tr[b]	—	0	—	0	—	tr	—	0.51	—
2	tr	tr	tr	0	2.75	0	tr	tr	tr	0.71
4	tr	tr	0	0	tr	0	tr	tr	tr	0.57
6	tr	tr	0	0	0.57	tr	tr	tr	tr	0.62
8	1.24	tr	1.65	0	3.80	tr	tr	tr	tr	tr
10	1.10	tr	9.67	6.81	7.27	18.77	tr	tr	tr	1.00
12	1.49	0.55	18.99	15.46	14.77	12.68	tr	tr	0.89	0.92
Haddock										
0	tr	—	0	—	0	—	tr	—	1.35	—
2	tr	tr	0	0	0	0	tr	tr	1.39	1.22
4	tr	tr	0	0	0	0	tr	tr	1.08	1.05
6	tr	tr	0	0	tr	0.70	tr	tr	0.89	1.12
8	tr	tr	0	0	1.10	0.79	tr	tr	0.68	1.14
10	0.96	tr	tr	tr	1.06	4.02	tr	tr	1.10	1.10
12	2.85	1.21	tr	1.56	7.38	9.82	tr	tr	1.70	1.41
14	2.44	1.07	tr	2.82	7.40	9.15	tr	tr	1.44	1.53
16	2.28	2.55	0.78	5.11	7.23	28.11	tr	tr	1.70	1.78

[a] Data from Fernandez-Salguero and Mackie (1987a).
[b] tr, <0.5 mg/100 g tissue.

TABLE VII

PRODUCTION OF HIGHER AMINES (MG/100 G TISSUE) IN HERRING AND HADDOCK HELD AT 5°C AS FILLETS AND WHOLE FISH

Time of storage (days)	*Putrescine*		*Histamine*		*Cadaverine*		*Spermidine*		*Spermine*	
	Whole fish	Fillet	Whole fish	Fillet	Whole fish	Fillet	Whole fish	Fillet	Whole fish	Fillet
Herring										
1	tr[b]	tr	0	0	tr	tr	tr	tr	0.58	0.84
3	0.89	tr	1.16	0.82	5.20	3.38	tr	tr	0.63	0.66
5	4.78	0.58	9.88	30.66	47.54	29.64	tr	tr	1.12	0.86
7	6.24	2.28	53.10	52.38	36.66	38.66	tr	tr	1.16	1.16
Haddock										
1	tr	tr	0	0	tr	0	tr	tr	1.29	1.06
3	0.52	tr	tr	0	0.83	3.57	tr	tr	1.18	1.19
5	0.54	0.64	tr	2.31	1.62	5.66	tr	tr	1.38	1.68
7	1.79	1.71	3.57	9.24	8.60	6.41	tr	tr	1.56	2.02
9	2.09	1.99	4.96	7.67	10.18	15.68	tr	tr	1.78	2.51

[a] Data from Fernandez-Salguero and Mackie (1987a).

[b] tr, <0.5 mg/100 g tissue.

not detected in any of the samples analyzed. Lower concentration of amines were found in the samples of eviscerated fish compared to the samples of whole fish.

Capelin (*Mallotus villosus*) is an important raw material used for the production of fish meal and oil in Norway. Aksnes and Brekken (1988) studied the biochemical and microbial changes that occur during autolysis of bulk stored capelin with high contents of feed in the gut. They reported a fast release of arginine, serine, histidine, leucine, lysine, and tyrosine and a slower rate of release for glycine, aspartic acid, and glutamic acid. The amounts of tyrosine, lysine, serine, arginine, and histidine decreased rapidly due to bacterial activity, and the main products obtained from the bacterial decomposition of lysine, histidine, and arginine were cadaverine, histamine, and putrescine, respectively.

Changes in bacterial flora and polyamine content during the storage of minced horse mackerel (*Trachurus japonicus*) meat were studied by Okuzumi *et al.* (1990). At spoilage stages (total aerobic count of 1.1 to 1.3 $\times$ 10^{10} /g) in the samples stored at 5°C, *Pseudomonas* I/II and *Pseudomonas* III/IV-NH, typical spoilage bacteria, were dominant in the bacterial flora and high amine contents of putrescine (2.3–54 mg/100 g), cadaverine (11–15 mg/100 g), and histamine (7.2–12 mg/100 g) were detected. Samples stored at 30°C showed *Vibrio* and *Photobacterium* as dominant bacteria and high contents of histamine (210–1336 mg/100 g) and cadaverine (74–612 mg/100 g) were observed by the time the samples were at spoilage level ($1.8–2 \times 10^9$ /g). Production of polyamines from arginine by *Pseudomonas* I/II, ornithine by *Pseudomonas* III/IV-NH, arginine and lysine by *Photobacterium*, and arginine by certain *Vibrio* was shown. It was suggested that *Pseudomonas* III/IV-NH produced putrescine in samples stored at 5°C and that *Photobacterium* produced agmatine and cadaverine in samples stored at 30°C.

B. SHELLFISH

Formation of nonvolatile amines during spoilage in the muscle of squid (*Todarodes pacificus*) and in a species of octopus (*Paroctopus dofleini dofleini*) was studied by Takagi *et al.* (1971). During 4 days of storage they found cadaverine (10.1–7.6 mg% at 15°C and 27.7–6.0 mg% at 25°C) and very small amounts of putrescine (1.2 mg% at 15°C, 96 hr storage; 0.1 mg% at 25°, 72 hr storage) in the muscle of squid and did not detect either tyramine or histamine. Cadaverine (2.4–6.0 mg% at 15°C and 3.9–4.5 mg% at 25°C), putrescine (3.4–7.1 mg% at 15°C and 1.5–1.0 mg% at 25°C) and small amounts of tyramine (trace at 15°C and 2.6 mg% at 25°C) were found in decomposing octopus muscle, and histamine was not detected.

TABLE VIII

CHANGES IN POLYAMINE CONTENTS (MG/100 G) IN JAPANESE SCALLOP (*Patynopecten yessoensis*) ADDUCTOR MUSCLE DURING STORAGE AT 5°C

Storage time (h)	Tyramine	Putrescine	Cadaverine	Agmatine	Tryptamine	Spermidine	Sensory rating[b]
0	0	0	0	0	0	0.10	1
24	0	0	0	0.38	0	0.09	1
48	0	0.12	0.14	0.40	0	0.08	1
72	0.03	0.49	0.37	0.69	0	0.08	2
96	0.15	2.71	0.88	0.79	0.08	0.08	3

[a] Data from Yamanaka (1989).
[b] 1, acceptable; 2, initial decomposition; 3, advanced decomposition.

Yamanaka (1989) reported that storage at 5 and 15°C of Japanese scallop (*Patinopecten yessoensis*) adductor muscle results in an increase in agmatine, putrescine, and cadaverine, while a significant decrease in arginine, with partial conversion into ornithine and agmatine, is observed. The changes in polyamine contents reported by Yamanaka (1989) during storage of Japanese scallop (*Patinopecten yessoensis*) adductor muscle at 5 and 15°C and the sensory ratings observed are shown in Tables VIII and IX.

In crustaceans, the biogenic amines have a primary function as neurotransmitters and neuromodulators. In addition, some biogenic amines serve also as neurohormones in the hemolymph (Fingerman *et al.*, 1994).

TABLE IX

CHANGES IN POLYAMINE CONTENTS (MG/100 G) IN JAPANESE SCALLOP (*Patynopecten yessoensis*) ADDUCTOR MUSCLE DURING STORAGE AT 15°C[a]

Storage time (h)	Tyramine	Putrescine	Cadaverine	Agmatine	Tryptamine	Spermidine	Sensory rating[b]
0	0	0	0	0	0	0.10	1
12	0	0.05	0.07	2.19	0	0.08	1
18	0	0.72	0.43	5.27	0	0.08	2
24	0	2.16	0.84	8.46	0.98	0.08	3
36	0.15	11.84	2.17	8.59	1.34	0.29	3
48	0.20	12.30	7.23	10.50	2.02	0.60	3
60	1.43	19.28	20.29	14.21	2.10	0.70	3

[a] Data from Yamanaka (1989).
[b] 1, acceptable; 2, initial decomposition; 3, advanced decomposition.

C. SEAFOOD PRODUCTS

Fermented fish products available mainly in Asian countries are particularly rich in histamine (Azudin and Saari, 1988; Wootton *et al.*, 1986). Fermented fish paste, prepared from small fish or shrimp, is used frequently as a condiment for rice dishes in Southeast Asia. Fardiaz and Markakis (1979) tentatively identified the following amines in fish paste: ethanolamine, 2-methylbutylamine, 2-mercaptoethylamine, 2-phenylethylamine, cadaverine, and histamine in concentrations ranging from 0.5 to 64 mg/100 g. Histamine and 2-phenylethylamine were the major amines found with maximal amounts of 64.0 and 60.0 mg/100 g, respectively.

Fermented sardine with rice bran is a traditional Japanese foods produced in a barrel for a period of 6 months to 1 year. In a study by Yatsunami *et al.* (1994), an increase in the numbers of halotolerant and halophilic histamine-forming bacteria from 10^1–10^2 /g to 10^4–10^5 /g after 6 months was observed. Putrescine, histamine, and tyramine content also had a considerable increase in the same time period. The isolates of halotolerant and halophilic histamine-forming bacteria from the raw sardines were identified as *Staphylococcus, Micrococcus, Vibrio, Pseudomonas* III/IV NH, and *Pseudomonas* III/IV-H. The isolates from fermented sardine with rice-bran after 6 months were identified as *Staphylococcus, Micrococcus,* and *Vibrio.*

VII. SCOMBROTOXICOSIS

Histamine poisoning has been reported to be one of the major illnesses among foodborne diseases (Taylor *et al.*, 1989; Morrow *et al.*, 1991). In Britain, between 1976 and 1986, 258 incidents of suspected scombrotoxic fish poisoning were reported (Bartholomew *et al.*, 1987). Originally associated with the consumption of fish belonging to the *Scombridae* and *Scomberosocidae,* scombrotoxicosis is a human intoxication which in severe cases is characterized by rapid onset (10 to 30 min). Symptoms of histamine poisoning include headache, nausea, vomiting, diarrhea, itching, oral burning sensation, red rash, and hypotension (Taylor *et al.*, 1989; Hughes and Potter, 1991; Ijomah *et al.*, 1992). Recently, this intoxication has also been associated with the consumption of certain nonscombroid species (Bartholemew *et al.*, 1987; Hughes and Potter, 1991; Morrow *et al.*, 1991) and in rare ocassions with the consumption of cheese, mainly Swiss cheese (Taylor *et al.*, 1989). The level of histamine which constitutes a toxic level is uncertain as potentiators of toxicity which lower the effective dosage may be present in the fish (Arnold and Brown, 1978; Taylor, 1985). It is generally accepted that the risk of scombrotoxic poisoning in well-iced fish is very

low. Under normal conditions of storage, histamine concentration rarely reaches levels higher than 5 mg/100 g flesh (Arnold and Brown, 1978; Murray *et al.*, 1982). The risk for histamine poisoning is associated not only with the consumption of fresh fish. Due to the heat resistance of histamine (Ijomah *et al.*, 1992), it can be present in cans of tuna fish and other related species (Ienestea, 1971). The presence of histamine in toxic amounts, in sterilized cans, can also be due to the use of fish of poor hygienic quality as raw material or to defective handling of high quality tuna during processing. When incidents of poisoning have been reported for scombroid species the concentrations of histamine have often been found to be high, in excess of 100 mg/100 g flesh (Arnold and Brown, 1978). Scombrotoxic poisoning was attributed to the consumption of canned tuna containing 68–280 mg histamine/100 g fish (Merson *et al.*, 1974).

Possible vasoactive or psychoactive effects due to an excessive oral intake of biogenic amines have been reported (Malone and Metcalfe, 1986; Taylor, 1986; Joosten, 1988). Since destruction of these amines by physical methods such as freezing and heating is very difficult, it is important to prevent their formation. Prevention of amine formation in fish muscle extracts by some spices has been demonstrated (Wendakoon and Sakaguchi, 1992). A possible synergistic effect of clove essential oils and sodium chloride for the inactivation of the growth and amine production of *Enterobacter aerogenes* in mackerel muscle broth at 30°C was suggested by Wendakoon and Sakaguchi (1993).

Contrary to widespread belief, new medical evidence has demonstrated that histamine present in the fish has but a minor role in the aetiology of scombrotoxicosis (Douglas, 1980; Clifford *et al.*, 1989, 1991; Ijomah *et al.*, 1991, 1992). Consequently, two hypotheses have been formulated: (1) that the action of dietary histamine is potentiated by some other component(s) of the toxic fish (Bjeldanes *et al.*, 1978; Taylor and Lieber, 1979; Chu and Bjeldanes, 1981; Lyons *et al.*, 1983; Ijomah *et al.*, 1992) or (2) that the toxin(s) is a mast cell degranulator, and the antihistamine therapy is effective because it eliminates the effect of endogenous histamine rather than the effect of dietary histamine (Clifford *et al.*, 1991; Ijomah *et al.*, 1991; Ijomah *et al.*, 1991).

Several reports of *in vivo* and *in vitro* studies suggest that the absorption, metabolism, and/or potency of one biogenic amine might be modified in the presence of a second amine (Bjeldanes *et al.*, 1978; Taylor and Lieber, 1979; Lyons *et al.*, 1983). Histamine taken in combination with wholesome tuna is reported to yield toxic effects in people even at moderate doses (100–180 mg/100 g tuna) (Motil and Scrimshaw, 1979). Diamines and polyamines inhibit the binding of histamine to mucin, therefore histamine could be released from intestinal mucin and increase the amount of histamine

absorbed in the intestinal lumen (Jung and Bjeldanes, 1979; Chu and Bjeldanes, 1981), resulting in an increase in histamine toxicity. Histamine taken with a meal (bread, milk and butter) has been reported to be absorbed to a greater extent than histamine consumed by itself (Mitchell and Code, 1954).

Storage temperature has an important role in the production of histamine (Behling and Taylor, 1982). It is generally agreed that temperatures of 0°C or below inhibit histamine formation. However, the effect of storage at temperatures between 2 and 10°C is not that clear. Several studies have reported that at these temperatures there is little or no formation of histamine (Hardy and Smith, 1976; Smith *et al.*, 1980; Klausen and Lund, 1986) while others have reported production of low levels of histamin at temperatures below 10°C (Baldrati *et al.*, 1980; Morii *et al.*, 1986). The differences in these reports could be due to the type and level of microbial flora of the fish used in the studies (Behling and Taylor, 1982).

VIII. AMINE FORMATION AS AN INDICATOR OF FRESHNESS IN SEAFOODS

The potential use of amine concentration as a criteria to assess freshness in finfish and shellfish has been discussed by many researchers. The volatile amines trimethylamine (TMA) and dimethylamine (DMA) have been widely used as indicator of freshness of marine fish (Castell *et al.*, 1971). Tables X and XI give the concentration of TMA and DMA in herring and

TABLE X

CONCENTRATION OF TMA AND DMA (MG N/100 G) IN HERRING AND HADDOCK HELD IN ICE AS FILLETS AND WHOLE FISH[a]

Time of storage (days)	Herring				Haddock			
	Whole fish		Fillets		Whole fish		Fillets	
	TMA	DMA	TMA	DMA	TMA	DMA	TMA	DMA
0	1.46	0.50	—	—	0.45	0.20	—	—
2	3.79	0.45	0.95	0.20	0.45	0.25	0.47	0.50
4	2.28	0.30	1.28	0.40	1.37	0.50	0.72	0.20
6	5.40	0.25	2.71	0.40	1.42	1.13	1.61	0.35
8	19.64	0.20	7.49	0.40	3.68	2.05	6.58	1.00
10	20.79	0.20	35.84	0.54	6.87	4.43	23.12	0.80
12	22.88	0.20	33.52	0.56	7.56	4.39	37.45	0.50
14	—	—	—	—	9.99	3.99	43.47	0.55
16	—	—	—	—	17.10	3.27	41.47	0.60

[a] Adapted from Fernandez-Salguero and Mackie (1987a).

TABLE XI

CONCENTRATION OF TMA AND DMA (MG N/100 G) IN HERRING AND HADDOCK HELD AT 5°C AS FILLETS AND WHOLE FISH[a]

Time of storage (days)	Herring				Haddock			
	Whole fish		Fillets		Whole fish		Fillets	
	TMA	DMA	TMA	DMA	TMA	DMA	TMA	DMA
1	1.86	0.70	1.49	0.20	0.70	0.18	0.80	0.50
3	20.34	0.45	9.56	0.30	5.45	1.13	8.69	0.50
5	50.25	0.50	39.85	0.40	10.31	1.62	39.31	0.20
7	57.68	0.45	53.62	0.44	37.71	1.90	50.45	0.90
9	—	—	—	—	40.86	4.88	50.01	1.30

[a] Adapted from Fernandez-Salguero and Mackie (1987a).

haddock during refrigerated storage (Fernandez-Salguero and Mackie, 1987a).

Luong and Male (1992) developed a biosensor system to measure the hypoxanthine concentration ratio as an indicator of fish freshness. Hypoxanthine is the autodegradation product formed from adenosine 5'-triphosphate in fish tissue and is responsible for the bitter "off-taste" characteristic of fish which has lost its freshness.

Dawood *et al.* (1988) reported that the concentration of putrescine and cadaverine in the flesh of chilled-stored rainbow trout (*Salmo irideus*) exceeded 1.10 ppm by the second day of whole fish storage at 0°C. Therefore, they indicated that determination of these two diamines could be used to assess fish freshness. Putrescine and ornithine have been reported as potential indicators of freshness of scallop adductor muscle (Yamanaka, 1989). Spermidine and spermine are minor components in fish, changing only slightly during the period of storage over which fish is acceptable for human consumption (Ritchie and Mackie, 1980). Putrescine and cadaverine have a steady increase once bacterial spoilage begins, thus, they are considered potential indicators of fish quality (Fernandez-Salguero and Mackie, 1987a). Yamanaka *et al.* (1987) examined tyramine, putrescine, cadaverine, agmatine, and tryptamine variations in the muscle of common squid during storage at 0, 3.5, and 15°C. Initially, even in the fresh muscle, agmatine was detected in small amounts. The agmatine concentration increased with storage time, exceeding 30 mg/100 g at the stage of initial decomposition and reaching 40 mg/100 g at the stage of advanced decomposition. They concluded that agmatine, formed from arginine, can be potentially a useful indicator for freshness of common squid. At 22°C a good correlation between the spoilage

of imitation crab meat and the amounts of total volatile acids, total volatile bases, cadaverine, putrescine, histamine, aerobic plate count, and protelytic count has been shown (Hollingworth *et al.*, 1991). However, at 4 and 10°C, neither the chemical nor microbial indicators were adequate to assess quality of the product. Ethanol has proven to be a useful chemical indicator, especially in canned salmon (Hollingworth *et al.*, 1987). An increase in the levels of putrescine, cadaverine, tyramine, and tryptamine was observed as the decomposition of sardine (*Sardinops melanosticta*) and saury pike (*Cololabis saira*) progressed during storage at 5 or 20°C (Yamanaka *et al.*, 1986). Maximum formation was observed for cadaverine, reported to be the most useful index for decomposition of fish: below 15 mg/100 g of meat at the passable stage, between 15 and 20 mg/100 g of meat at the stage of initial decomposition, and over 20 mg/100 g of meat at the stage of advanced decomposition.

While the presence of histamine in fish muscle is a good indication that decomposition has taken place, its occurrence is extremely variable. Its production is a function of time, temperature, and the type and level of microbial flora present. Variations within individual fish, depending upon the section of fish from which the meat sample originated (Lerke *et al.*, 1978; Frank *et al.*, 1981), as well as variation between and within species (Edmunds and Eitenmiller, 1975) have been reported. Frank *et al.* (1984) reported that decomposed skipjack tuna frequently had histamine levels of <5 mg/100 g. In a survey of histamine levels in commercially processed scombroid fish products by Taylor *et al.* (1977), over 90% of these products had histamine levels below 5 mg/100 g. Therefore, although histamine may confirm the presence of decomposed tissue, it is not a good indicator of the degree of decomposition in fish products. A chemical index (Fig. 3) that correlates well with sensory evaluation for canned tuna of varying qualities was proposed after the work of Hui and Taylor (1983) and Mietz and Karmas (1977). The index comprises levels of histamine, putrescine, cadaverine, spermidine, and spermine. Production of putrescine, cadaverine, and histamine and loss of spermidine and spermine were observed during the decomposition of raw and commercially canned tuna fish. Using the HPLC method described by Hui and Taylor (1983), histamine levels less than 5 mg/100 g can be detected. To increase sensitivity, fluorescence detection instead of uv detection can be used (Seiler, 1970).

$$\text{quality index} = \frac{(\text{mg/kg histamine} + \text{mg/kg putrescine} + \text{mg/kg cadaverine})}{(1 + \text{mg/kg spermidine} + \text{mg/kg spermine})}$$

FIG. 3. Chemical quality index (based on Mietz and Karmas, 1977).

A study of the decomposition of raw surimi and a surimi-derived flaked artificial crab stored at 4, 10, and 22°C (Hollingworth *et al.,* 1990) indicated that total volatiles acids and total volatile bases content has the most potential as chemical indicators of decomposition compared to ethanol, cadaverine, putrescine, and histamine content.

IX. RECOMMENDED LIMITS OF AMINE CONTENT

The U.S. Food and Drug Administration (FDA) in 1982 established a defect action level for histamine in scombroid fish products of 20 mg/100 g, which indicates prior mishandling, and a hazard action level of 50 mg/100 g, which is considered a potential health hazard. Although the relationship between the level of histamine and the toxicity of a fish sample is not clear, based on the available present knowledge, it is considered that a histamine content >200 ppm in fish can be considered to be toxicologically significant and that a content of >50 ppm indicates that the fish has been exposed to higher temperature (Arnold and Brown, 1978; Murray *et al.,* 1982). In France a maximum permitted concentration of histamine in marine products has been set at 10 mg/100 g (as applied to fresh tuna) (Gouygou *et al.,* 1992).

The FDA proposed in 1995 that its compliance policy guide be revised on decomposition and histamine to: (1) lower the histamine action level for decomposition to 50 ppm from 100 ppm; (2) extend application of the new action level to raw and frozen tuna and mahi-mahi; (3) eliminate the provision that findings of less than 200 ppm need to be confirmed by organoleptic testing; (4) allow other species to be considered as decomposed when histamine levels reach, 50 to 500 ppm; and (5) consider fish, other than tuna, implicated in histamine poisoning outbreaks as health hazards when histamine levels reach 500 ppm. It is anticipated that final action on this proposal will be announced after 1996 (Craven *et al.,* 1995; National Fisheries Institute, Inc., 1995).

A. QUALITY CONTROL PURPOSES

Pelagic fish

Histamine < 5 mg/100g
(Murray *et al.,* 1982)

Canned skipjack tuna

Putrefactive amines 0.4 to 0.5 μg/g
(Sims *et al.,* 1992)

Canned tuna fish
Histamine < 50 ppm
(Lönberg *et al.*, 1980)

B. TOXIC LEVEL

Histamine > 100 mg/100 g flesh (Arnold and Brown, 1978)

The European Union has established regulations for species of fish belonging to the *Scombridae* and *Clupeidae* families and fixed 100 ppm of histamine as the limit of acceptance. A three-class plan for maximum allowable levels of histamine in fresh fish (n = 9; c = 2, m = 100 ppm; M = 200 ppm) and enzymatically ripened fish products (n = 9; c = 2, m = 200 ppm; M = 400 ppm) from *Scombridae* and *Clupeidae*) families (where n = number of units to be analyzed from each lot, m and M = histamine tolerances, and c = number of units allowed to contain a histamine level higher than m but lower than M) has been initiated (Anonymous, 1991).

Histamine levels higher than 2000 ppm have usually been reported in sardine, mackerel, and tuna fish cans (Kim and Bjeldanes, 1979; Lönberg *et al.*, 1980; Schulze and Zimmermann, 1980; Ababouch *et al.*, 1986). Taylor *et al.* (1978a) reported that only 4% of mackerel and tuna fish cans showed a histamine level lower than 10 ppm. Ababouch *et al.* (1986) observed that 7% of tuna fish samples contained levels higher than 500 ppm of histamine as compared to 3.7 and 3.2% for sardine and mackerel, respectively. Sailfish filets (*Istiophorus platypterus*) were involved in a 1994 food poisoning outbreak in Taiwan. Samples were collected from the victims' residues and analyzed for amines by a HPLC gradient elution technique. Analysis (see Table XII) indicated that tryptamine, histamine, spermine, TMA, spermidine, and cadaverine were prominent in all samples; other amines were not detectable (<2.5 mg/100 g) (Hwang *et al.*, 1995).

TABLE XII
CONCENTRATIONS OF BIOGENIC AMINES IN SAILFISH TISSUE CAUSING SCOMBROID POISONING[a]

Source	Amine level (mg/100 g)				
	Cadaverine	Tryptamine	Spermidine	Histamine	Trimethylamine
Victim	14.5 ± 0.3	208 ± 8	50.0 ± 3.9	168 ± 8	24.5 ± 3.3
Victim	11.0 ± 0.4	185 ± 11	20.0 ± 1.6	180 ± 9	19.0 ± 0.5

[a] Adapted from Hwang *et al.* (1995).

X. DETERMINATION OF BIOGENIC AMINES IN FISH

Various analytical techniques, including thin-layer chromatography, amino acid analyzers, liquid chromatography, gas chromatography, and enzymic tests have been developed for the determination of amines. Histamine has been usually determined by a specific fluorometric reaction with orthophthalaldehyde (Shore, 1971) and its concentration in fish with high levels of free histidine have been widely used as an index of spoilage and of potential scombrotoxic poisoning (Taylor, 1983). Histamine and the diamines have been determined by ion-exchange procedures in decomposed mackerel (Hatano *et al.*, 1970).

The fluorometric procedure is the official AOAC method most commonly used for the determination of histamine (AOAC Methods, 1980). The method involves extraction of the fish with methanol, separation of histamine from amino acids by passing the extract through an ion-exchange column, and reaction with *o*-phthalaldehyde under controlled conditions, followed by fluorometric measurement. This method has proven to be accurate and sensitive, with a detection limit of approximately 1 mg histamine/100 g fish sample (Gouygou *et al.*, 1992). Drawbacks are that it is rather slow (maximum four or five samples per hour) and requires strict handling.

A. LIQUID CHROMATOGRAPHY

Walters (1984) described a liquid chromatography (LC) system using a bonded cation-exchange column to resolve and detect histamine from histidine, cadaverine, and putrescine from fish. Detection of histamine by LC is difficult since histamine does not have enough ultraviolet absorbance or fluorescence nor is the molecule suitable for electrochemical detection. The introduction of a postcolumn reaction with *o*-phthalaldehyde to form a fluorescent derivative increased the detection sensitivity and specificity. The limit of detection was 1.5 ng histamine with a linear response in the range between 7 and 750 ng. Prior work by Hatano *et al.* (1970) used cation-exchange chromatography with a commercially available amino acid analyzer for amine analysis of samples containing between 0.1 and 1.0 μmol of the amines with high accuracy (within 3.5%) and short time (8 hr).

B. THIN-LAYER CHROMATOGRAPHY

Low levels of spermidine, spermine, putrescine, cadaverine, and histamine can be determined with the TLC method developed by Spinelli *et al.* (1974). The method uses the fluorescent derivatives of the amines formed with dansyl chloride (5-(dimethylamino)-1-naphtalene sulfony chloride)

(Abdel-Monem and Ohno, 1975). Shalaby (1995) described the use of TLC with a multiple development technique to resolve the dansyl derivatives of histamine, cadaverine, putrescine, phenylethylamine, tyramine, tryptamine, spermine, and spermidine from fish, cheese, and meat samples. The procedure allowed for the detection in 14 samples of as little as 5 or 10 ng of the dansyl derivatives of the amines within 2 hr.

C. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Polyamines are usually quantified by HPLC with precolumn (Morier-Teissier *et al.,* 1988; Yen and Hsieh, 1991) or postcolumn (Redmond and Tseng, 1979) derivatization by benzoyl chloride for measuring ultraviolet absorbance, dabsyl chloride for measuring visible absorbance, or dansyl chloride or *o*-phthalaldehyde for fluorescent detection (Abdel-Monem and Ohno, 1975; Desiderio *et al.,* 1987). The dansyl derivatives of the polyamines have been detected with a 280-nm ultraviolet detector (Abdel-Monem and Ohno, 1975) and Mietz and Karmas (1977) and Hui and Taylor (1983) used a 254-nm detector and a gradient elution high-pressure liquid chromatographic system to separate the dansyl derivatives. The method was used to determine the quality of canned tuna and cheese. Later, Mietz and Karmas (1978) developed a chemical index to determine decomposition in rockfish, salmon, lobster, and shrimp. The dansylated polyamines, putrescine, cadaverine, spermidine, and spermine, and the amine histamine were quantified and an index formula was developed. Results from the chemical analysis of 21 samples (rockfish, salmon, and lobster) correlated well with the organoleptic analysis performed by 23 sensorists. The chemical index classified samples correctly 90.5% of the time versus an 83.9% correct classification by organoleptic determination. A good correlation between organoleptic and chemical index was also found in the analysis of shrimp composites.

The dansyl derivatives, which have a napthtalene structure, are excellent derivatives for primary amines (Mietz and Karmas, 1978). They are easily formed and detected by a uv (HPLC) detector in amounts as little as 10 ng, therefore the method does not require high sensitivity or fluorometric detectors. Gradient elution improves the separation, allowing for a broad range of derivatives to be separated in a relatively short time (40 min). Use of reverse-phase microparticular (5–10 μm) columns can further improve the HPLC separation (Gouygou *et al.,* 1992). Desiderio *et al.* (1987) described the use of a reversed-phase HPLC method for the quantification of putrescine, cadaverine, spermidine, and spermine from brain extracts as the dansyl derivatives.

Maruta *et al.* (1989) described a rapid liquid chromatographic assay of urinary polyamines (putrescine, spermidine, spermine, and cadaverine) involving electrochemical detection with postcolumn-immobilized enzyme, polyamine oxidase, from soybean seedlings. The polyamines were separated by isocratic ion-pairing reversed-phase chromatography and then enzymatically converted, with release of hydrogen peroxide, via the postcolumn reactor with immobilized polyamine oxidase. The hydrogen peroixide was detected by electrochemical oxidation on a platinum electrode. Detection limits for the injected polyamines were 0.3, 0.5, 0.6 and 4 pmol for putrescine, spermidine, spermine, and cadaverine, respectively, with linear ranges of two to three orders of magnitude.

Rosier and Van Peteghem (1988) described a rapid method for the extraction, derivatization, and determination by HPLC of the 5-dimethylaminonaphthalene-1-sulphonyl (dansyl chloride) derivatives of putrescine, cadaverine, histamine, spermidine, and spermine from fish. Comparison of this procedure to earlier methods reflected considerable reduction in the time needed for sample preparation (from approx 8 to 0.5 hr) and cost (use of water and methanol instead of acetonitrile).

Morier-Teissier *et al.* (1988) used precolumn derivatization with *o*-phtalaldehyde and thiol combined with HPLC to measure spermine, spermidine, putrescine, and cadaverine. The derivatives were quantified by electrochemical detection instead of fluorescence, with an optimum potential from the working electrodes of + 0.65 V. A good linear relationship ($r = 0.99$) existed between polyamines concentration and the peak height over the range 1 pmol to 10 nmol when the reaction time was carefully controlled. Spermidine gave the best sensitivity, with a signal/noise of 2 for 200 fmol. It was slightly lower for putrescine and cadaverine and seven to eight times lower for spermine.

D. GAS–LIQUID CHROMATOGRAPHY

Yamamoto *et al.* (1982) developed a quantitative method for the determination of putrescine, cadaverine, spermidine, and spermine in foods. Separation of the amines from foods was achieved by eluting through a cation-exchange resin column and then converted to their (ethyloxy) carbonyl derivatives by the reaction with ethyl chloroformate in aqueous medium before application to the gas chromatograph with a flame ionization detector. They used 1,8-diaminooctane as an internal standard and performed the separation and determination of the resulting derivatives on a 1.5% SE–30/0.3% SP-1000 on Uniport HP column (0.5 m) under the temperature-programmed condition. Staruszkiewicz and Bond (1981) developed a GLC procedure for the quantitative determination of the diamines putrescine

and cadaverine using their perfluoropropionyl derivatives. Extraction of the amines from foods was performed with methanol and hexanediamine used as internal standard. A dry residue of the hydrochloride salts of the amines was prepared and derivatized with perfluoropropionic anhydride by heating for 30 min at 50°C. An alumina column was used to separate the reaction mixture and the derivatives were eluted with a 30% solution of ethyl acetate in toluene. GLC separations were performed on a 3% OV-225 column held at 180°C. Using this procedure, less than 1 μg diamine/g tissue could be quantitated using either an electron capture detector or a nitrogen-specific detector. Later, Farn and Sims (1987) applied the procedure on controlled spoilage studies of skipjack and yellowfin tuna and found, under the conditions of the test, that putrescine, cadaverine, and histamine developed in the raw fish only after severe temperature abuse (36 hr at 21°C). They concluded that whenever putrescine and cadaverine levels were found in amounts above background levels in canned tuna, it indicated that the original raw material had reached a stage of advanced decomposition prior to heat processing. A good correlation was observed between a panel of well trained sensory judges and the chemical analysis for putrefactive amines as an indicator of protein decomposition in canned skipjack tuna (Sims *et al.*, 1992). The resolution by capillary gas chromatography of various substituted analogues of putrescine as their *N,N'*-perfluoroacyl derivatives was facilitated by the use of chiral stationary phases (Gaget *et al.*, 1987). Bonilla *et al.* (1995) evaluated the use of a "cold on-column" GC injection technique in conjunction with a new base-deactivated fused silica capillary column for the direct analyses of putrescine and cadaverine by gas chromatography. They reported excellent resolution of the amines as well as symmetrical peaks. Good linear response was observed between concentrations of 20 and 400 ppm.

E. ENZYMIC TEST

Lerke *et al.* (1983) developed a rapid screening method to detect histamine in fish. The qualitative procedure uses a two-step sequential enzyme system. In the first step, the enzyme diamine oxidase catalyzes the breakdown of histamine with production of hydrogen peroxide. Detection of hydrogen peroxide is then performed by the formation of crystal violet from the leuco base in the presence of peroxidase at 596 nm. The method could be used to detect histamine in raw or heat-processed fish. Later, Lopez-Sabater *et al.* (1993) modified the procedure to achieve histamine quantification. Further modification of the Lerke method by Rodriguez-

Jerez *et al.* (1994) resulted in a rapid and reliable technique for histamine determination. Determination of the optimum wavelength depending on incubation time at a constant temperature of 37°C resulted in a recommendation of a wavelength of 580 nm, with an incubation time of 15 min. At these conditions the linearity was observed among 1 and 25 ppm (μg/g) of histamine.

F. ENZYME ELECTRODE

Enzyme electrodes are formed by the joining of an electrochemical sensor and an enzymatic layer. Enzyme electrodes for oxidases are obtained by coupling an enzymatic film of immobilized oxidase with an oxygen electrode (Clark electrode) (Gouygou *et al.*, 1992).

Oxidase catalyzed reation:

$$XH_2 + O_2 \rightarrow X + H_2O_2,$$

where XH_2 is the reduced form of substrate and X is the oxidized form.

Decrease in oxygen concentration, due to the enzymatic reaction in the film, is monitored amperometrically by the Clark electrode (Romette *et al.*, 1982).

G. TEST STRIPS

The test strips were obtained from an immobilized enzyme film containing both lentil seeds diamino oxidase and horse radish peroxidase (Gougouy *et al.*, 1992). The films were made by coentraping the molecules of enzyme and an inactive matrix protein (gelatin or bovine serum albumin) with a bifunctional reagent (glutaraldehyde). A solid-phase assay (dipstick test) for histamine in tuna based on the coupling of diamine oxidase to a peroxidase/dye system was reported by Hall *et al.* (1995). The assay was linear to 1.0 m*M* histamine, and the minimum detectable concentration was 0.07 m*M*, which corresponds to 2.3 mg% in tuna extracts. Putrescine did react slightly with the dipstick; however, determinations in fresh and spoiled tuna gave good agreement with the modified AOAC fluorometric method.

H. CAPILLARY ZONE ELECTROPHORESIS

Wang *et al.* (1994) reported on the use of capillary zone electrophoresis for the routine determination of biogenic amines in fresh fish samples. They obtained electrophoregrams of fluorescamine-derivatized histamine,

cadaverine, and putrescine in methanol and trichloroacetic acid under hydrostatic injection conditions. Although efficient peak separation of the standards was achieved within 7 min, the reproducibility of the injection varied among analyses due to difficulties in regulating the amount of amine entering the capillary. They were able to improve the reproducibility maintaining an efficient peak separation by using an electrokinetic injection technique.

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