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Biogenic amine formation and bacterial contribution in fish, squid and shellfish

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ABSTRACT

Forty-one species of fish, squid and shellfish were analyzed for biogenic amine (BA) contents. Most of the fish samples showed lower BA contents, whereas some samples showed higher contents than the allowable levels. Shellfish and squid samples had negligible BA levels. Four fish species containing high BA levels were analyzed for changes in histamine contents during storage. In the most samples, the histamine contents remarkably increased up to 36.6–2123.9 mg/kg after 24 h of storage at 25 °C, while the contents began to gradually increase after 2–3 days of storage at 4–10 °C. The dominant microbial group was enterobacteria throughout the storage period. Meanwhile, out of total 119 strains isolated from different fish species showing high BA levels, 23 strains identified as *Enterobacter aerogenes* produced large amounts of histamine, putrescine and cadaverine, and 33 strains identified as two different *Enterobacter* spp. produced less histamine but large amounts of putrescine and cadaverine.

1. Introduction

Biogenic amines (BAs) are mainly formed in foods by microbial decarboxylation of amino acids and transamination of aldehyde and ketones (Askar and Treptow, 1986; Brink, Damirik, Joosten, & Huis in't Veld, 1990; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Maijala, Eerola, Aho, & Hirn, 1993; Santos, 1996). BAs are of importance due to risk of food intoxication and serve as chemical indicators of fish spoilage (Alberto, Arena, & Manca de Nadra, 2002). Generally, oral administration of BAs does not provoke adverse reactions, because amine oxidases in the human intestine have a role in rapidly detoxifying these compounds. However, food intoxication may occur if the amine-metabolizing capacity is over-saturated and/or the metabolic activity is impaired by specific inhibitors (Taylor, Guthertz, Leatherwood, Tillman, & Lieber, 1978). Putrescine and cadaverine can enhance histamine toxicity through interfering with histamine detoxification system. Moreover, biogenic polyamines, such as putrescine, cadaverine, spermidine, spermine and agmatine, are potential carcinogens to be converted to nitrosamine when exposed to nitrite (Bills, Hildrum, Scanlan, & Libbey, 1973). Common symptoms of BA intoxication are migraine, brain haemorrhage, heart failure, hypertension, urticaria, headache, flushing, abdominal cramps, hypertension, and hypotension as well (Rice, Eitenmiller, & Koehler, 1976). An upper limit of histamine for human consumption has been suggested to be 100 mg/kg food, and 2 mg/l alcoholic beverage and 100–800 mg/kg of tyramine and 30 mg/kg of phenylethylamine have been reported to be toxic doses in foods, respectively (Brink et al., 1990). Total BA levels of 1000 mg/kg in food are also considered dangerous for human health (Taylor, 1985).

In most early studies on BA formation in fish, researchers have focused on histamine poisoning and concluded that the families Scombridae and Scomberesocideae are commonly implicated in incidents of histamine poisoning. Indeed, various scombroids, including mackerel, tunas, saury, bonito, seerfish and butterfly kingfish, have been implicated in cases of histamine poisoning. However, non-scombroid fish, such as sardine, pilchards, anchovies, herring and marline, has also been implicated in cases of histamine poisoning (Taylor, 1985, 1986). Meanwhile, regarding the formation of overall BAs in fish, there have been several works with sardine, tuna horse, mackerel and anchovy (El Marrakchi, Bennour, Bouchriti, Hamama, & Togafait, 1990; Wendakoon, Michiyo, & Sakaguchi, 1990; Yamanaka, Shimakura, Shiomi, & Kikuchi, 1986). However, to the best of our knowledge, less report is available on other fish species. The objective of this research was therefore to extensively determine the levels of eleven different BAs in various fish, squid and shellfish species, and to evaluate the influence of storage temperature on histamine contents in different fish species, especially containing high levels of histamine and tyramine. In addition, to pursue microbial species responsible for BA formation in fish, we isolated and identified BA-producing strains showing strong amino acid decarboxylase activity.

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2. Materials and methods

2.1. Samples and strains used

A total of 229 fresh samples, belonging to 41 different species of fish (20 species), squid (4 species) and shellfish (17 species), were purchased from retail markets in Korea from July, 2007 to June, 2008, and transported in ice to the laboratory within 4 h. Upon arrival, samples were immediately subjected to BA analyses or storage experiments. The samples of amberjack, mackerel, saury and Spanish mackerel containing high levels of histamine and tyramine were stored at different temperatures, −20, 4, 7, 10 and 25 °C, to investigate changes not only in the BA contents, but also in both bacterial load and water activity value. The samples were withdrawn every day during 7 days of storage.

A total of 119 strains (30 strains from each fish species) were isolated from mackerel, saury, Spanish mackerel and amberjack containing high BA levels. The strains were grown on Trypticase soy agar (Difco, Becton Dickinson, Sparks, MD, USA) for 48 h at 25 °C and then stored in a freezer by using glycerol (60%, v/v).

2.2. Preparation of standard amine solutions

Stock solutions of 1,7-diaminoheptane (internal standard) and BAs were separately prepared at 10,000 mg/l concentration in distilled water. Working solutions at 100 or 1000 mg/l concentrations were prepared by diluting 100 μl or 1000 μl of each stock solution in distilled water to bring to a final volume of 10 ml. All standard chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany).

2.3. Preparation of extracts from samples and bacterial cultures

Extraction of both samples and bacterial cultures and HPLC determination of BAs were carried out according to the procedures developed by Ben-Gigirey, De Sousa, Villa, & Barros-Velazquez (1998, 1999). Briefly, 10 ml of 0.4 M perchloric acid containing a known amount of 1,7-diaminoheptane used as an internal standard was added to either 3 g of fish samples or 1 ml of bacterial culture, and the mixture was homogenized with Ultra-Turrax (IKA Labortechnik, Staufen, Germany) and centrifuged at 3000*g* at 4 °C for 10 min. The supernatant was collected, and the residue was extracted again with an equal volume of 0.4 M perchloric acid solution. Both supernatants were combined, and the final volume was adjusted to 30 ml with 0.4 M perchloric acid. The extract was filtered through Whatman paper No. 1.

2.4. Derivatization of extracts and standards

Derivatization of BAs was carried out according to the procedures developed by Ben-Gigirey et al. (1998). One milliliter of extract or standard solution was mixed with 200 μl of 2 M sodium hydroxide and 300 μl of saturated sodium bicarbonate. Two milliliters of a dansyl chloride solution (10 mg/ml) prepared in acetone were added to the mixture, and then incubated at 40 °C for 45 min. Residual dansyl chloride was removed by the addition of 100 μl of 25% ammonium hydroxide. After incubation for 30 min at room temperature, the mixture was adjusted to 5 ml with acetonitrile. Finally, the mixture was centrifuged at 2500*g* for 5 min, and the supernatant was filtered through 0.2 μm-pore-size filters (Millipore Co., Bedford, MA, USA). The filtered supernatant was kept at −25 °C until assayed by HPLC.

2.5. Chromatographic separations

Chromatographic separation of BAs was carried out according to the procedures developed by Ben-Gigirey et al. (1998) with minor modifications. An HPLC unit (Waters 2690, Waters, Milford, MA, USA) equipped with a Waters 996 photodiode array detector and Millennium 2010 software was employed. A Nova-Pak C_{18} 4 μm column (150 mm × 4.6 mm, Waters) was used, with ammonium acetate (0.1 M; Merck; solvent A) and acetonitrile (Merck; solvent B) as the mobile phases at the flow rate of 1 ml/min. The program was set for a linear gradient starting from 50% of solvent B to reach 90% of the solvent at 19 min. The sample volume injected was 20 μl and the sample was monitored at 254 nm. The detection limits for standard amine solutions were: approximately 0.1 mg/kg for β-phenylethylamine hydrochloride, cadaverine dihydrochloride, dopamine hydrochloride, histamine dihydrochloride, putrescine dihydrochloride, serotonin hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, tryptamine hydrochloride and tyramine hydrochloride, and 0.2 mg/kg for noradrenaline hydrochloride. Recoveries of biogenic amines from fish samples varied, ranging from 72.3% (dopamine) to 98.2% (putrescine), as presented in Table 1. Typical chromatograms of biogenic amines in standard solution (a) and in fish samples (b, c) are shown in Fig. 1.

2.6. Biogenic amine-producing activity of bacterial strains

To determine BA-producing activity of total 119 strains isolated from fish samples, the strains were cultured in 5 ml tryptic soy broth (Difco) with 0.5% L-histidine hydrochloride, L-lysine hydrochloride, L-ornithine hydrochloride, and L-tyrosine disodium salt (as precursors) supplemented with 0.0005% pyridoxal-HCl (Sigma) at 25 °C for 48 h. These broth cultures were subjected to the procedures for extraction, derivatization, filtration, and chromatographic separation, as described above.

2.7. Microbiological analyses

Thirty grams of fish samples were homogenized for 5 min with HMF-1600 homogenizer (Hanil Co., Seoul, Korea). One gram of the homogenate was homogenized again in a stomacher with 9 ml of sterile 0.1% peptone water and decimally diluted in sterile 0.1% peptone water. The diluted samples were subjected to microbial

Table 1
The recoveries of biogenic amines from selected fish samples.

Biogenic amines	Percent recovery (%)[b]	
	Amberjack (*Seriola dumerilii*)	Alaska pollack (*Theragra chalcogramma*)
Trp[a]	83.5 ± 1.6	83.4 ± 1.3
Phe	93.8 ± 0.4	95.7 ± 0.6
Put	97.0 ± 1.3	98.2 ± 0.9
Cad	96.4 ± 1.0	96.7 ± 0.6
His	96.6 ± 0.8	94.1 ± 0.3
Ser	93.3 ± 1.1	96.1 ± 0.5
Tyr	97.4 ± 1.3	96.8 ± 1.0
Spd	90.0 ± 1.7	91.7 ± 0.7
Nor	84.1 ± 1.8	83.6 ± 1.0
Dop	88.8 ± 1.7	72.3 ± 1.3
Spm	93.3 ± 1.1	90.0 ± 0.4

[a] Trp: tryptamine, Phe: β-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Ser: serotonin, Tyr: tyramine, Spd: spermidine, Nor: noradrenaline, Dop: dopamine, Spm: spermine.

[b] Data were taken as a mean ± standard deviation calculated from triplicates. Percent recoveries were calculated using the following formula: [(the amine amount found in spiked sample - the amine amount found in non-spiked sample)/theoretical amount of added spike] × 100.

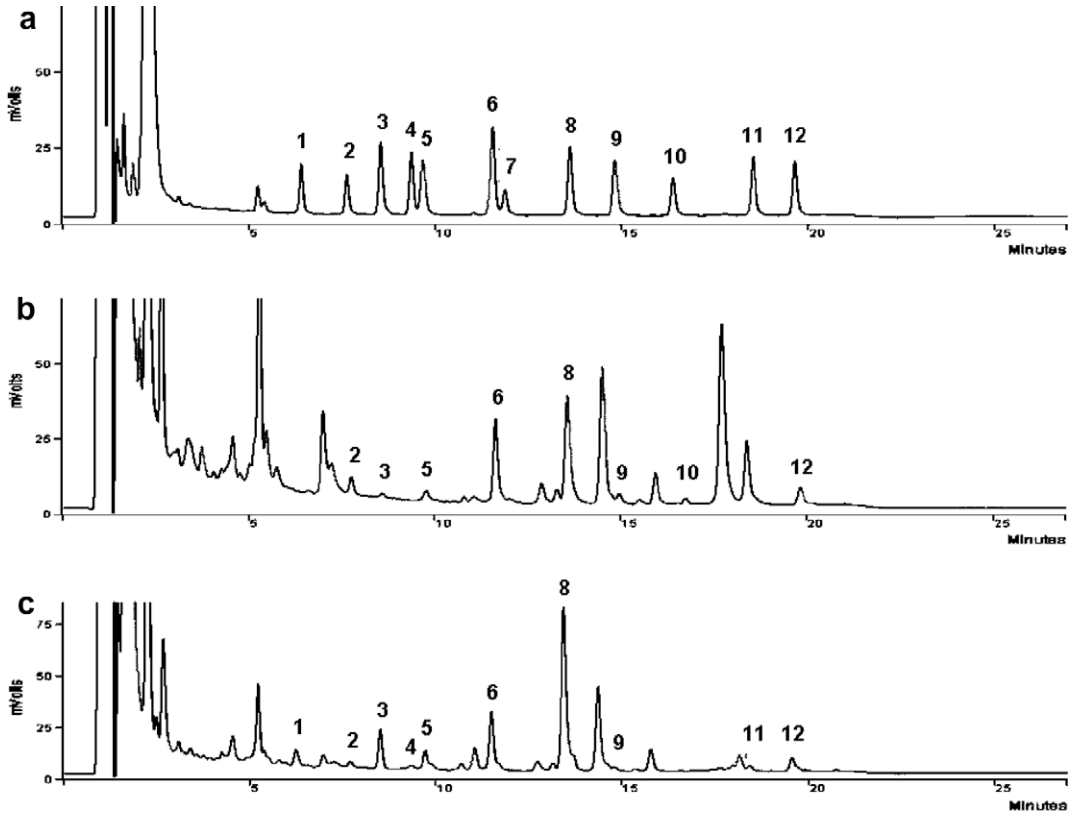

Fig. 1. Typical HPLC chromatograms of biogenic amines in standard solution and in fish samples. (a) standard solution, (b) mackerel and (c) amberjack. 1, tryptamine; 2, β-phenylethylamine; 3, putrescine; 4, cadaverine; 5, histamine; 6, 1,7-diaminoheptane (internal standard); 7, serotonin; 8, tyramine; 9, spermidine; 10, noradrenaline; 11, dopamine; 12, spermine.

analyses as follows: total aerobic mesophilic bacteria on Trypticase soy agar (Difco); BA-forming bacteria on Niven's medium (Niven, Jeffreg, & Corlett, 1981); Enterobacteriaceae on Violet red bile glucose agar (Conda Laboratories, Madrid, Spain); Pseudomonads on Cetrimide agar (Difco); *Vibrio* spp. on Thiosulfate citrate bile salts sucrose agar (Difco); *Escherichia coli* on MacConkey sorbitol agar (Difco). The number of colonies that appeared after incubation for 24–48 h at 30 °C was counted.

2.8. Water activity measurements

Thirty grams of the samples homogenized were also subjected to the determination of water activity. The water activity was measured at 25 °C by using an electric hygrometer (Novasina, Talstrasse, Switzerland).

2.9. Identification of bacterial strains

Bacterial identification was carried out according to the scheme developed by Shewan, Hobbs, and Hodgkiss (1960). Gram staining, motility test, catalase production, oxidase production, and glucose fermentation were performed for initial classification.

To identify to species level, a colony was picked up with a sterilized toothpick, suspended in 0.5 ml of sterilized saline solution in a 1.5 ml centrifuge tube, and centrifuged at 2000*g* for 10 min. After removal of supernatant, the cell pellet was suspended in 0.5 ml of InstaGene Matrix (Bio-Rad, Hercules, CA, USA), which was incubated at 56 °C for 30 min and heated 100 °C for 10 min in a water bath. After heating, the supernatant was used as template DNA for PCR amplification of 16S rDNA. One microliter of template DNA and 1 μl of 27F/1492R primer pair were added in 20 μl of PCR reaction solution. *E. coli* genomic DNA served as a positive control. The reaction program consisted of 35 amplification cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 60 s, and elongation at 72 °C for 60 s.

Unincorporated PCR primers and dNTPs from PCR products were removed by using Montage PCR clean up kit (Millipore). Subsequent sequencing reaction with purified PCR products (approximately 1400 bp) were performed by using a primer pair, 27F and 1492R, and Big Dye terminator cycle sequencing kit (Applied Bio-Systems, Foster city, CA, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing instrument (Applied BioSystems). The sequences were analyzed by BLAST of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/) for similarity matches in the databases.

2.10. Statistical analyses

Data were subjected to one-way analysis of variance (ANOVA). All statistical analyses were performed using the Statistical software, SPSS Version 12.0 for windows (SPSS Inc., Chicago, IL, USA). Value of $P < 0.05$ was used to indicate significant differences.

3. Results and discussion

3.1. Biogenic amine contents in fish, squid and shellfish from retail markets in Korea

The highest amounts of histamine reported previously in fish species were 1270 mg/kg in mackerel (Shalaby, 1996) and 399 mg/kg in amberjack (Auerswald, Morrom, & Lopata, 2006). In the present study, however, these fish species contained much lower levels of histamine than previous reports, showing less than

Table 2
The contents of biogenic amines in different fish species from retail markets in Korea.

Species	Scientific name	No. of samples	Biogenic amines (mg/kg)											
			Trp[a]	Phe	Put	Cad	His	Ser	Tyr	Spd	Nor	Dop	Spm	Total
Alaska pollack	*Theragra chalcogramma*	8	ND[b]-1.7[c](0.2 ± 0.6)[d]	ND-8.5(2.8 ± 3.3)	8.1-209.1(36.3 ± 69.9)	ND-41.8(6.3 ± 14.4)	ND	ND	ND-15.4(1.9 ± 5.4)	ND-37.6(7.1 ± 13.1)	ND-6.6(0.8 ± 2.3)	ND	ND-2.1(0.5 ± 0.8)	8.1-309.6(56.0 ± 110.1)
Amberjack	*Seriola dumerilii*	6	ND-1.4(0.2 ± 0.6)	ND-38.3(12.0 ± 15.9)	ND-126.0(34.7 ± 54.1)	ND-38.5(6.7 ± 15.6)	ND-12.5(5.2 ± 6.1)	ND-81.6(35.2 ± 37.3)	41.4-221.8(102.7 ± 71.3)	ND-85.1(17.0 ± 33.5)	ND-131.2(29.7 ± 50.7)	2.3-137.1(29.7 ± 50.7)	ND-20.1(8.5 ± 8.8)	66.2-639.9(299.3 ± 347.1)
Angler	*Lophiomus setigerus*	6	ND	ND-6.5(2.1 ± 3.3)	ND-13.7(7.0 ± 6.2)	ND-27.3(10.3 ± 12.5)	ND	ND	ND	ND-23.8(10.4 ± 10.5)	ND	ND-2.0(1.0 ± 1.1)	5.5-19.1(12.0 ± 5.8)	5.5-67.8(42.8 ± 39.3)
Armorclad rockfish	*Sebastes hubbsi*	8	ND-1.6(0.2 ± 0.6)	ND-19.0(2.6 ± 6.7)	0.7-78.8(27.2 ± 33.6)	ND-64.6(10.5 ± 22.3)	ND-5.1(1.2 ± 2.3)	ND	ND-0.7(0.1 ± 0.2)	ND-5.6(2.0 ± 2.6)	ND	ND	ND-6.9(2.0 ± 2.9)	2.4-168.0(45.8 ± 71.2)
Atka mackerel	*Pleurogrammus azonus*	7	ND	ND-11.5(1.6 ± 4.3)	ND-13.7(5.6 ± 7.0)	ND-12.6(4.9 ± 6.2)	ND-5.9(2.1 ± 2.6)	ND	ND-7.5(2.5 ± 3.3)	ND-8.9(3.7 ± 4.0)	ND	ND	ND-11.2(4.1 ± 5.3)	ND-59.6(24.6 ± 32.7)
Blackspotted croaker	*Protonibea diacanthus*	5	ND	ND-14.0(5.2 ± 10.9)	ND-22.0(12.1 ± 21.3)	ND-63.5(28.8 ± 55.4)	ND-6.0(1.2 ± 5.5)	ND-2.1(0.5 ± 4.2)	ND-3.1(1.1 ± 14.6)	ND-0.4(0.1 ± 2.9)	ND	ND	ND	2.1-103.0(49.1 ± 121.7)
Bonito	*Katsuwonus pelamis*	5	ND	1.6-3.0(0.9 ± 2.7)	ND-2.5(0.9 ± 2.0)	ND-6.2(3.2 ± 6.2)	15.0-30.1(21.6 ± 11.7)	ND	33.1-99.2(62.3 ± 47.4)	ND-3.0(1.2 ± 3.3)	ND-10.2(3.5 ± 8.9)	3.0-22.4(9.4 ± 16.7)	7.5-20.3(14.0 ± 9.3)	101.8-163.9(117.0 ± 108.3)
Chum salmon	*Oncorhynchus keta*	5	ND-3.8(1.6 ± 1.6)	ND-27.9(10.6 ± 15.9)	ND-2.7(1.8 ± 0.9)	ND	ND-6.8(1.4 ± 3.0)	ND	ND-3.7(1.4 ± 1.9)	ND-2.6(1.0 ± 1.4)	ND	ND-25.0(5.0 ± 11.2)	ND-3.8(1.5 ± 2.0)	0.2-42.4(24.3 ± 36.1)
Cutlassfish	*Trichiurus lepturus*	6	ND-29.4(12.3 ± 12.7)	ND	18.1-23.9(21.0 ± 2.5)	ND-44.7(18.7 ± 21.6)	ND-4.7(0.8 ± 1.9)	ND	ND-22.8(7.4 ± 11.4)	ND-5.6(1.2 ± 2.2)	ND	ND-2.0(0.7 ± 1.0)	ND-5.5(1.8 ± 2.8)	27.8-99.1(63.7 ± 56.1)
Eel	*Anguilla japonica*	4	ND	ND	ND	ND	0.5-8.8(5.8 ± 3.7)	0.2-1.4(1.4 ± 0.8)	8.7-35.8(20.3 ± 12.1)	ND	ND	ND	ND-6.5(1.6 ± 3.3)	16.3-45.6(29.1 ± 19.8)
Pacific cod	*Gadus macrocephalus*	6	ND	ND	0.7-6.6(4.2 ± 4.5)	ND-5.8(2.6 ± 5.9)	ND	ND-1.4(0.2 ± 1.2)	ND-9.4(3.7 ± 9.4)	ND-6.7(3.1 ± 7.0)	ND	ND-3.5(1.0 ± 3.3)	ND-10.0(4.3 ± 10.0)	2.1-37.8(19.9 ± 41.4)
Pacific herring	*Clupea pallasii*	5	ND-44.7(13.3 ± 18.4)	0.7-22.3(9.5 ± 7.9)	15.6-81.6(43.9 ± 25.5)	ND-194.4(59.5 ± 79.7)	ND-45.4(9.1 ± 20.3)	ND	4.5-59.8(23.3 ± 21.1)	ND-5.8(3.0 ± 2.7)	ND	ND-2.0(0.4 ± 0.9)	ND-5.6(3.2 ± 2.4)	90.6-330.6(165.4 ± 178.8)
Pacific mackerel	*Scomber japonicus*	6	ND	ND-9.4(2.8 ± 4.1)	ND-26.6(9.8 ± 10.6)	ND	ND-8.0(2.7 ± 4.1)	ND-42.8(7.1 ± 17.5)	9.6-100.8(40.3 ± 37.3)	ND-112.4(35.2 ± 49.4)	3.8-50.9(16.9 ± 18.4)	ND-28.8(4.8 ± 11.8)	ND-13.6(3.8 ± 6.0)	14.0-319.8(123.4 ± 159.2)
Pacific saury	*Cololabis saira*	8	ND-27.4(8.0 ± 11.1)	ND-23.4(7.5 ± 8.0)	1.5-34.8(12.0 ± 10.3)	ND-60.8(29.2 ± 29.5)	ND-19.4(9.6 ± 14.4)	ND-14.6(1.8 ± 5.2)	ND-147.8(56.8 ± 54.6)	ND-119.9(17.0 ± 41.7)	ND-60.6(10.0 ± 20.8)	ND-201.0(35.3 ± 67.7)	ND-17.7(7.6 ± 10.0)	15.9-395.3(194.7 ± 273.2)
Red seabream	*Pagrus major*	9	ND	ND	ND-33.0(7.3 ± 11.3)	ND	ND	ND-1.4(0.2 ± 0.5)	ND	ND-7.5(1.9 ± 3.1)	ND	ND	ND-8.6(2.0 ± 3.5)	0.4-33.0(11.3 ± 18.4)
Ridged-eye flounder	*Pleuronichthys cornutus*	5	ND	ND-2.8(0.6 ± 1.3)	31.9-74.4(51.3 ± 15.9)	ND-223.7(102.1 ± 112.5)	1.3-5.7(3.8 ± 1.8)	ND	ND-1.2(0.3 ± 0.5)	ND-3.8(1.6 ± 1.5)	ND	ND-1.8(0.7 ± 0.9)	ND-8.7(4.1 ± 4.3)	60.1-295.2(164.5 ± 138.6)
Silver pomfret	*Pampus argenteus*	5	ND	ND	ND	ND	ND	ND	ND-5.6(1.2 ± 2.5)	ND-5.5(1.1 ± 2.5)	ND	ND	ND	ND-11.1(2.3 ± 4.9)
Spanish mackerel	*Scomberomorus niphonius*	7	ND-89.4(17.0 ± 33.3)	ND-37.8(10.0 ± 14.0)	ND-105.7(28.5 ± 39.9)	ND-314.3(56.1 ± 114.9)	ND-45.5(6.5 ± 17.2)	ND	ND-189.0(53.3 ± 82.6)	ND-23.8(7.1 ± 8.7)	ND-19.9(2.8 ± 7.2)	ND-54.1(8.9 ± 20.2)	4.6-49.7(11.6 ± 17.7)	673.3(201.7 ± 355.6)
White croaker	*Pennahia argentata*	7	ND-0.8(0.1 ± 0.3)	ND	ND-8.6(4.3 ± 3.5)	ND-15.5(7.6 ± 7.2)	ND-5.6(2.2 ± 2.5)	ND-0.9(0.3 ± 0.4)	ND-8.6(3.3 ± 3.8)	ND-6.2(1.8 ± 2.9)	ND	ND-22.0(3.1 ± 8.3)	ND-6.9(2.1 ± 3.2)	0.6-47.1(24.9 ± 31.9)
Yellow croaker	*Larimichthys polyactis*	7	ND-16.3(4.0 ± 6.9)	ND-7.6(2.1 ± 3.7)	ND-29.8(16.9 ± 13.0)	ND-58.2(20.1 ± 25.8)	ND-5.9(1.6 ± 2.8)	ND-9.0(1.8 ± 3.3)	ND-26.8(7.6 ± 13.1)	ND-5.7(1.6 ± 2.8)	ND	ND	ND	1.3-154.4(55.9 ± 71.4)

[a] Trp: tryptamine, Phe: β-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Ser: serotonin, Tyr: tyramine, Spd: spermidine, Nor: noradrenaline, Dop: dopamine, Spm: spermine.

[b] ND: Not detected (amine level is less than 0.1 mg/kg).

[c] The range from minimum-maximum.

[d] Mean ± standard deviation.

Table 3
The contents of biogenic amines in different squid and shellfish species from retail markets in Korea.

Species	Scientific name	No. of samples	Biogenic amines (mg/kg)											
			Trp[a]	Phe	Put	Cad	His	Ser	Tyr	Spd	Nor	Dop	Spm	Total
Baby octopus	*Octopus ocellatus*	4	ND	ND	17.0-190.3(78.1 ± 77.5)	ND-160.8(59.6 ± 70.4)	ND	ND	ND-1.4(0.4 ± 0.7)	ND-2.8(0.7 ± 1.4)	ND	ND	0.2-13.1(6.1 ± 5.5)	74.6-190.6(144.9 ± 155.5)
Cuttlefish	*Sepia officinalis*	5	ND	ND-1.3(0.3 ± 0.6)	ND-39.9(10.9 ± 16.4)	ND-45.1(9.6 ± 19.9)	ND	ND	ND-5.6(4.6 ± 1.2)	ND	ND	ND	ND-12.0(7.6 ± 5.0)	11.0-91.6(32.9 ± 43.1)
Japanese common squid	*Todarodes pacificus*	7	ND	ND-14.0(2.0 ± 5.3)	ND-10.0(3.8 ± 3.9)	ND-12.4(3.9 ± 4.5)	ND	ND	ND-2.9(0.4 ± 1.1)	ND-5.9(2.6 ± 3.0)	ND	ND	ND-7.9(3.3 ± 4.1)	ND-31.6(16.0 ± 21.9)
Japanese octopus	*Octopus minor*	5	ND	ND-5.8(1.2 ± 2.6)	ND-6.1(2.4 ± 3.3)	ND-6.1(1.8 ± 2.5)	ND	ND	ND-5.4(2.1 ± 2.9)	ND-5.1(1.0 ± 2.3)	ND	ND	ND-8.9(4.2 ± 4.1)	1.5-31.2(12.7 ± 17.8)
Arkshell	*Scapharca broughtonii*	7	ND[b]-9.5[c](1.4 ± 3.6)[d]	ND-11.5(1.6 ± 4.3)	0.8-88.7(30.3 ± 36.4)	ND-150.7(39.7 ± 52.6)	ND-49.4(8.4 ± 18.2)	ND-12.3(2.9 ± 5.1)	ND-22.5(6.3 ± 7.5)	6.3-91.5(32.6 ± 31.4)	ND	ND-39.2(5.6 ± 14.8)	7.7-126.2(40.6 ± 43.6)	20.9-450.8(169.3 ± 217.5)
Bladder moon shell	*Glossaulax didyma*	5	20.1-59.7(42.0 ± 24.4)	ND-5.2(2.6 ± 2.5)	11.1-97.3(60.5 ± 31.4)	ND-8.6(5.2 ± 3.4)	ND	ND	ND-7.5(3.8 ± 3.6)	10.9-20.9(16.1 ± 4.1)	ND	ND	9.8-27.0(19.1 ± 7.6)	76.4-193.1(142.1 ± 68.8)
Butter clam	*Saxidomus purpuratus*	5	ND-3.4(1.4 ± 1.9)	ND-1.9(0.7 ± 1.0)	ND-12.3(3.1 ± 5.2)	ND	ND-7.6(2.8 ± 3.4)	ND	2.0-4.3(2.9 ± 0.9)	ND-2.7(0.9 ± 1.3)	ND-3.3(0.7 ± 1.5)	ND	29.3-55.3(41.7 ± 9.3)	45.0-70.6(54.1 ± 24.4)
Common sea squirt	*Sargassum sagamianum*	4	ND	ND	ND-37.6(12.5 ± 17.7)	ND-52.2(18.7 ± 24.8)	ND-13.8(3.4 ± 6.9)	ND-6.2(1.6 ± 3.1)	ND-18.2(4.6 ± 9.1)	ND-9.0(2.3 ± 4.5)	ND	ND	ND	ND-127.9(42.9 ± 66.0)
Grand jackknife clam	*Solen grandis*	5	ND	ND-5.1(2.0 ± 2.7)	0.5-108.6(22.9 ± 47.9)	ND-1.8(0.6 ± 0.8)	ND-5.2(1.3 ± 2.3)	ND	ND-1.6(0.5 ± 0.7)	ND-51.7(11.1 ± 22.7)	ND	ND	5.1-22.8(10.1 ± 7.4)	12.0-183.2(48.5 ± 84.4)
Granulated ark shell	*Tegillarca granosa*	5	ND	ND	ND-8.0(3.2 ± 4.4)	ND-18.9(4.9 ± 8.2)	ND	ND	ND-14.5(4.5 ± 6.4)	ND-101.9(24.5 ± 43.4)	ND-20.1(10.4 ± 9.9)	ND	ND-36.3(18.5 ± 17.6)	20.5-143.3(65.8 ± 89.9)
Hard clam	*Meretrix petechialis*	4	ND	ND	ND-3.4(0.9 ± 1.7)	ND	ND	ND-34.7(11.7 ± 16.4)	ND-0.5(0.2 ± 0.2)	ND-20.6(9.4 ± 10.9)	ND	ND-30.4(7.6 ± 15.2)	ND-25.3(12.9 ± 10.3)	12.9-65.3(42.6 ± 54.7)
Humpback shrimp	*Pandalus hypsinotus*	5	ND	ND	ND-4.5(1.3 ± 2.0)	ND-43.1(8.6 ± 19.3)	ND-12.5(5.2 ± 5.5)	ND-0.8(0.2 ± 0.4)	ND-6.2(1.5 ± 2.7)	ND	ND	ND	ND-19.5(5.2 ± 8.2)	ND-55.1(22.0 ± 38.0)
Japanese mystery snail	*Cipangopaludina japonica*	5	ND-13.2(5.3 ± 5.5)	ND-43.4(11.0 ± 18.2)	91.2-168.2(134.1 ± 35.4)	ND-26.7(6.3 ± 11.5)	ND	ND	ND	12.4-51.6(34.6 ± 19.0)	ND-9.0(1.8 ± 4.0)	ND	ND-9.0(3.0 ± 3.7)	173.2-223.0(196.2 ± 97.3)
Japanese scallop	*Patinopecten yessoensis*	4	ND	ND	ND-7.8(2.9 ± 3.7)	ND-7.6(2.5 ± 3.6)	ND	ND	ND	ND	ND-2.0(0.5 ± 1.0)	ND	ND-4.9(1.2 ± 2.5)	ND-22.3(7.1 ± 10.8)
Manila clam	*Tapes philippinarum*	6	ND-13.6(3.8 ± 5.1)	ND-43.9(10.2 ± 16.8)	1.2-119.5(33.8 ± 49.4)	ND-75.4(38.3 ± 31.0)	ND-23.1(4.7 ± 9.1)	ND-7.8(1.3 ± 3.2)	ND-23.7(5.4 ± 9.0)	ND-37.6(14.2 ± 13.0)	ND	ND-40.4(6.7 ± 16.5)	12.8-106.8(49.4 ± 31.6)	23.6-309.0(167.9 ± 184.7)
Pacific oyster	*Crassostrea gigas*	5	ND-4.1(2.6 ± 1.6)	ND-4.4(2.8 ± 1.7)	4.6-113.9(39.0 ± 47.6)	1.8-37.9(12.5 ± 15.4)	ND-9.2(1.8 ± 4.1)	ND-15.4(3.1 ± 6.9)	ND-23.5(7.0 ± 9.6)	17.7-54.7(41.6 ± 15.8)	ND	ND	67.0-123.1(101.4 ± 21.0)	177.6-316.3(211.9 ± 123.7)
Sea squirt	*Halocynthia roretzi*	5	ND-9.2(2.9 ± 3.8)	ND-3.6(1.4 ± 1.9)	7.4-174.4(44.6 ± 72.7)	ND-151.8(87.5 ± 61.0)	ND-6.0(2.4 ± 3.3)	ND-93.6(18.7 ± 41.9)	4.4-29.0(15.2 ± 9.6)	ND-16.9(3.7 ± 7.4)	ND	ND	ND-70.8(34.1 ± 30.4)	109.0-369.3(210.4 ± 231.8)
Solitary tunicate	*Styela clava*	5	ND-6.3(2.8 ± 3.1)	ND-1.1(0.2 ± 0.5)	0.2-73.4(20.8 ± 31.2)	3.2-56.6(21.4 ± 21.8)	ND-8.4(2.7 ± 3.8)	ND-10.5(2.1 ± 4.7)	ND-28.4(7.7 ± 11.8)	ND-4.3(0.9 ± 1.9)	ND-15.7(3.1 ± 7.0)	ND-34.0(6.8 ± 15.2)	ND-4.8(2.3 ± 2.2)	18.2-125.9(70.9 ± 103.1)
Swimming crab	*Portunus trituberculatus*	4	ND	ND-1.2(0.3 ± 0.6)	12.0-27.1(17.3 ± 6.8)	6.9-20.1(10.6 ± 6.3)	ND-8.3(2.1 ± 4.2)	ND-0.4(0.1 ± 0.2)	ND-2.6(0.7 ± 1.3)	ND	ND	ND	ND-33.2(8.7 ± 16.4)	19.5-78.8(39.7 ± 35.8)
Turban shell	*Batillus cornutus*	4	ND[b]	ND	ND-50.9(24.4 ± 28.2)	ND-29.5(7.4 ± 14.8)	ND-6.5(1.6 ± 3.2)	ND-11.8(3.0 ± 5.9)	ND-27.3(7.4 ± 13.3)	ND-21.0(5.3 ± 10.5)	ND	ND	ND-28.1(14.0 ± 16.1)	ND-168.6(63.0 ± 92.1)
Venus clam	*Cyclina sinensis*	5	ND-2.6(0.5 ± 1.2)	ND-6.1(2.6 ± 2.6)	1.9-143.6(38.5 ± 59.7)	ND-20.5(5.0 ± 8.9)	ND-9.1(4.2 ± 4.1)	ND-14.5(2.9 ± 6.5)	ND-20.2(5.4 ± 8.4)	ND-64.6(25.3 ± 24.3)	ND-12.3(2.5 ± 5.5)	ND-35.2(7.0 ± 15.7)	41.7-148.9(65.6 ± 46.7)	65.2-383.9(159.6 ± 183.4)

[a] Trp: tryptamine, Phe: β-phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Ser: serotonin, Tyr: tyramine, Spd: spermidine, Nor: noradrenaline, Dop: dopamine, Spm: spermine.

[b] ND: Not detected (amine level is less than 0.1 mg/kg).

[c] The range from minimum-maximum.

[d] Mean ± standard deviation.

20 mg/kg of histamine, respectively, as presented in Table 2. It is noteworthy that, in this study, the highest amount of histamine was 45.5 mg/kg in Spanish mackerel, which is a lower level than the allowable limit (50 mg/kg) suggested by the US Food and Drug Administration for scombroid fish and related product (USFDA, 2001). Meanwhile, some of mackerel, saury, Spanish mackerel and amberjack samples had higher levels of tyramine (ranged between 100.8 and 221.8 mg/kg) beyond the safe level (100 mg/kg) for human health suggested by Brink et al. (1990) and Santos (1996). The high amount of tyramine in fresh fish has been rarely recorded although there has been a report where the fish contained a high level of histamine exceeding 1000 mg/kg (Shalaby, 1996). Squid and shellfish species contained less amounts of both histamine and tyramine than fish species, as presented in Table 3. In detail, the contents of the respective amines detected in fish, squid and shellfish varied as follows: tryptamine, ND (not detected)-89.4 mg/kg; β-phenylethylamine, ND-38.3 mg/kg; putrescine, ND-209.1 mg/kg; cadaverine, ND-314.3 mg/kg; histamine, ND-45.5 mg/kg; serotonin, ND-81.6 mg/kg; tyramine, ND-221.8 mg/kg; spermidine, ND-119.9 mg/kg; noradrenaline, ND-131.2 mg/kg; dopamine, ND-201.0 mg/kg; spermine, ND-49.7 mg/kg; total amines, 11.1-673.3 mg/kg in fish, β-phenylethylamine, ND-14.0 mg/kg; putrescine, ND-190.3 mg/kg; cadaverine, ND-160.8 mg/kg; tyramine, ND-5.6 mg/kg; spermidine, ND-5.9 mg/kg; spermine, ND-13.1 mg/kg; total amines, 31.2-190.6 mg/kg in squid, tryptamine, ND-59.7; β-phenylethylamine, ND-43.9 mg/kg; putrescine, ND-174.4 mg/kg; cadaverine, ND-151.8 mg/kg; histamine, ND-49.4 mg/kg; serotonin, ND-93.6; tyramine, ND-29.0 mg/kg; spermidine, ND-91.5 mg/kg; noradrenaline, ND-20.1; dopamine, ND-40.4; spermine, ND-148.9 mg/kg; total amines, 22.3-450.8 mg/kg in shellfish. It is worth noting that some samples of sea squirt, amberjack and saury showed higher levels of amine neurotransmitters that are considered to be involved in skeletal muscle vasodilatation, increase of blood pressure and cardiac output (93.6 mg/kg for serotonin, 132.2 mg/kg for noradrenaline and 201.0 mg/kg for dopamine, respectively).

Table 4
Distribution of biogenic amine contents in 229 samples of various fish, squid and shellfish species from retail markets in Korea.

Biogenic amines[a] (mg/kg)	Analyzed samples[b]		
	Fish	Squid	Shellfish
β-phenylethylamine > 30	2[c] (0.9)[d]	0 (0)	2 (0.9)
Histamine > 50	0 (0)	0 (0)	0 (0)
Tyramine > 100	7 (3.0)	0 (0)	0 (0)
Total amine contents > 1000	0 (0)	0 (0)	0 (0)
Total	9 (3.9)	0 (0)	2 (0.9)

[a] Hazardous level of each amine in food by FDA and previous paper.
[b] Totally, 20 species of fish (125 samples), 4 species of squid (21 samples), and 17 species of shellfish (83 samples) were analyzed.
[c] Number of samples containing hazardous levels of amines.
[d] Percentage of samples containing hazardous levels of amines.

In Table 4, we showed the distribution of BA contents in total 229 samples, belonging to 41 different fish, squid and shellfish species, which reveals that 11 samples (4.8%) of mackerel, saury, Spanish mackerel, amberjack, manila clam and Japanese mystery snail had BA contents greater than either allowable limit established by USFDA (2001) or unacceptable levels reported by Brink et al. (1990) and Santos (1996).

3.2. Changes in histamine content and microflora during storage at different temperatures

Based on the relatively high contents of histamine in mackerel, saury, Spanish mackerel and amberjack samples, we further investigated changes in histamine contents in the 4 fish species during the storage at −20, 4, 7, 10 and 25 °C. As shown in Fig. 2, the histamine concentration showed no changes in all samples during the storage at −20 °C. The histamine concentrations increased

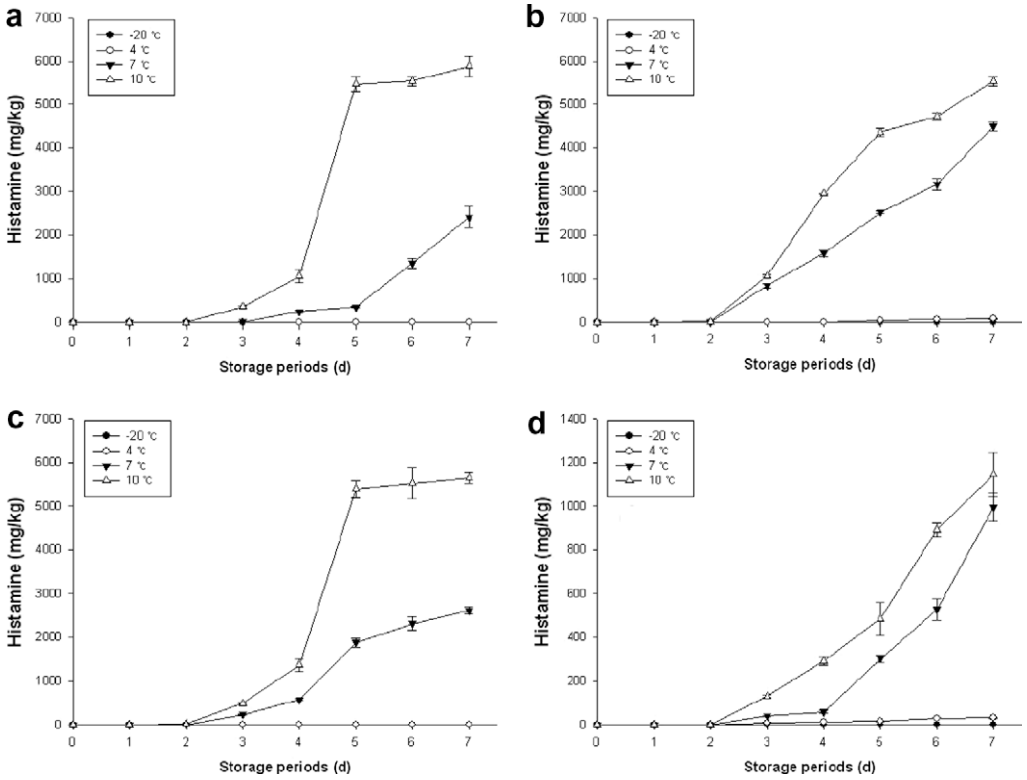

Fig. 2. Changes in histamine contents in four different fish species during storage at different temperatures. (a) amberjack, (b) mackerel, (c) saury and (d) Spanish mackerel. Error bars indicate standard deviations calculated from triplicates.

slightly from 4.2 to 79.3 mg/kg in mackerel, from ND to 2.2 mg/kg in saury, from 10.0 to 32.5 mg/kg in Spanish mackerel, after 3 days of storage at 4 °C and thereafter remained constant. The levels of histamine in amberjack remained either below or close to detection limit (0.1 mg/kg) during the storage at 4 °C. At 7 and 10 °C, the concentrations of histamine in mackerel, saury, Spanish mackerel and amberjack began to increase after 3 and 2 days, respectively, and increased more significantly throughout the storage period, reaching up to 4496.4 mg/kg, 2613.3 mg/kg, 997.6 mg/kg and 2410.7 mg/kg at 7 °C; and 5553.2 mg/kg, 5649.8 mg/kg, 1147.4 mg/kg and 5898.2 mg/kg at 10 °C, respectively. Furthermore, histamine contents in these 4 fish species, which were not detectable at initial time, reached up to 2123.9 mg/kg, 1776.7 mg/kg, 189.9 mg/kg and 36.6 mg/kg, respectively, after 24 h at 25 °C. It is worth noting that there were significant correlations between the storage temperature and histamine contents of the 4 fish species ($P < 0.05$). Based on these results, to prevent the risk of histamine, it might be possible to suggest the upper limit of storage period for fish species of above, as follows: 48 h at 4 °C, 24 h at 7 and 10 °C, and 7 days in a freezing temperature of −20 °C.

As shown in Fig. 3, the microbial changes in amberjack during storage at −20, 4, 7, 10 and 25 °C were analyzed to evaluate the effect of the storage temperature on microflora, because the samples of this fish species showed not only considerably higher histamine levels but also the highest tyramine contents. Regardless of the storage temperature, the dominant microbial group was found to be enterobacteria throughout the storage period. The number of amine-forming bacteria tended to be similar or somewhat less as compared to that of enterobacteria throughout the storage at all temperatures except for 25 °C, at which the number of amine-forming bacteria seemed to be rather greater than that of enterobacteria. Interestingly, the maximum numbers of total plate counts, enterobacteria, and amine-forming bacteria were observed at 10 °C. Taken together, it seemed likely that the most of amine-forming bacteria belong to psychrophilic (and/or mesophilic) enterobacteria. No bacterial load increased in any of the tested microbial group stored at −20 °C (data not shown). Therefore, it can be suggested that BA formation in fish species could be effectively controlled by the storage at −20 °C. Meanwhile, at initial time, total viable counts, amine-forming bacteria, enterobacteria, and pseudomonads were 5.30, 4.82, 5.07, and 4.29 log CFU/g, respectively, but *Vibrio* spp. or *E. coli* were not detected.

In other reports, it has been found that factors affecting histidine decarboxylation are diverse, especially in a spoiling fish. The diversity can be attributed to differences in fish species, handling procedures, and temperatures (Kimata, 1961; Yoshinaga and Frank, 1982). Regarding the fish species, not only Enterobacteriaceae (especially, *Morganella morganii*), but a few strains of *Klebsiella pneumoniae* and *Hafnia alvei* have been known to be the most prolific histamine producers in fish when they are maintained at temperatures greater than 4 °C (Stratton and Taylor, 1991), which is in agreement with our observations. Meanwhile, microbial determinants of amine production in fish have been largely investigated by different groups. Lakshmanan, Shakila, and Jeyasekaran (2002) reported that during the ice storage of fish and shrimp, the predominant amine-forming bacteria were widely distributed not only in Gram-negative genera, such as *Alcaligenes*, *Flavobacterium*, *Acinetobacter*, *Shewanella* and *Pseudomonas*, but also in a Gram-positive genus, *Micrococcus*. In horse mackerel, containing high levels of putrescine, cadaverine and histamine, dominant bacteria were found to be *Pseudomonas*, *Vibrio* and *Photobacterium* (Okuzumi, Fukumoto, & Fujii, 1990). In jack mackerel stored at different temperature, *Proteus vulgaris*, *Aeromonas hydrophila* and *Photobacterium damsela* were found to be responsible for histamine production (Bermejo, Mondaca, Roeckel, & Marti, 2004). Recently, a novel species of *Paenibacillus* that characteristically produces tyramine was isolated from salted and fermented anchovy (Mah, Chang, & Hwang, 2008).

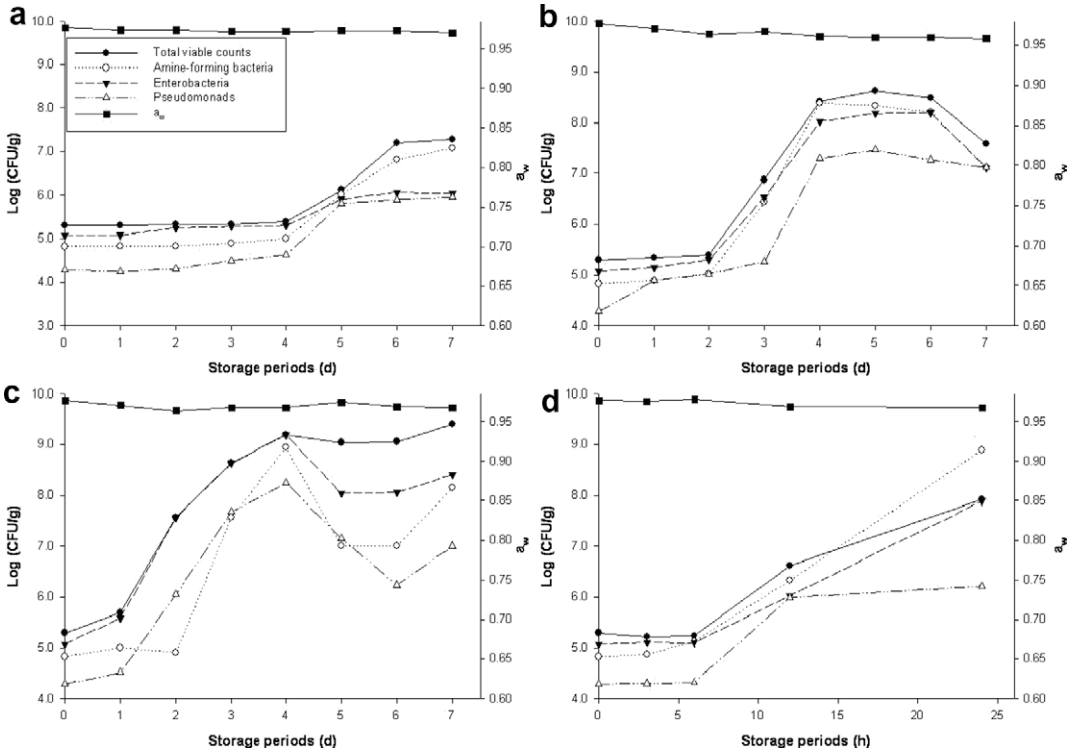

Fig. 3. Changes in microbial flora and water activity during the storage of amberjack at different temperatures. (a) 4 °C, (b) 7 °C, (c)10 °C and (d) 25 °C. All experiments were conducted in triplicate and the data were presented as means.

Table 5
Identification and determination of biogenic amine-producing activity of bacterial strains isolated from 4 different fish samples containing high levels of biogenic amines.

No. of Strain	Identity	Biogenic amines (mg/l)[f]										
		Trp[a]	Phe	Put	Cad	His	Ser	Tyr	Spd	Nor	Dop	Spm
23[b] (19.3)[c]	*E. aerogenes*	ND[d]-3.3[e]	ND-12.8	3249.4-4677.0	1020.9-1587.3	3488.6-4073.3	13.2-37.2	3.8-18.7	ND-14.5	ND-20.8	0.4-10.2	ND
9 (7.6)	*Enterobacter* sp.	ND-3.8	ND-13.6	3325.8-4650.6	954.4-1735.9	ND-14.8	18.0-26.9	41.7-48.1	45.0-56.5	51.9-65.3	ND	ND-9.6
24 (20.2)	*Enterobacter* sp.	ND	ND-23.3	2831.2-4759.5	ND-32.0	ND-31.9	16.7-40.0	16.8-49.6	ND-64.6	ND-61.9	ND	ND-11.7

[a] Trp: Tryptamine, Phe: β-Phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine, Ser: serotonin, Tyr: tyramine, Spd: spermidine, Nor: noradrenaline, Dop: dopamine, Spm: spermine.
[b] Number of isolates identified.
[c] Percentage of isolates identified.
[d] ND: Not detected.
[e] The range from minimum to maximum.
[f] Each amine-producing activity was determined in decarboxylation broth containing 0.5% tyrosine, 0.25% of histidine, ornithine, or lysine, respectively.

3.3. Identification of amine-forming bacteria with strong decarboxylation activity

In order to further pursue the dominant amine-forming bacteria in fish samples to species level, we first determined amino-acid decarboxylation activity of total 119 strains isolated from fish species (see Materials and methods). Out of these strains, 56 strains (47.1%) were found to produce large amounts of histamine (ND-4073.3 mg/kg), putrescine (2831.2-4759.5 mg/kg) or cadaverine (ND-1735.9 mg/kg), and some of them also produced tryptamine, β-phenylethylamine, serotonin, tyramine, spermidine, noradrenaline, dopamine and spermine. Moreover, it was found that while 23 strains had strong ability to produce histamine, ranging from 3488.6 to 4073.3 mg/l, the others produced less histamine. Next, we identified the 56 strains by 16S rDNA sequencing, and the strains were divided into 3 groups and identified as *E. aerogenes* and two different species of *Enterobacter*, as summarized in Table 5. Interestingly, these three groups had different amino acid decarboxylase activity as follows: *E. aerogenes* (23 strains), strong producers of histamine, putrescine and cadaverine; *Enterobacter* sp. (24 strains), strong producers of putrescine; another *Enterobacter* sp. (9 strains), strong producers of putrescine and cadaverine.

There have been several reports describing amino-acid decarboxylation activity of different genera, such as Acinetobacter, Aeromonas, Bacillus, Cedecea, Citrobacter, Clostridium, Escherichia, Klebsiella, Plesiomonas, Proteus, Pseudomonas, Salmonella, Serritia, Shigella and Vibrio, and of some lactic acid bacteria (Brink et al., 1990; Frank, Baranowski, Chongsiriwatana, Brust, & Premaratue, 1985; López-Sabater, Rodríguez-Jerez, Hernández-Herrero, & Mora-Ventura, 1996; Middlebrooks, Toom, Douglas, Harrison, & McDowell, 1988; Rice et al., 1976; Rodríguez-Jerez, Mora-Ventura, López-Sabater, & Hernández-Herrero, 1994; Yoshinaga and Frank, 1982). Moreover, *M. morganii, E. aerogenes, K. pneumonia, P. phosphoreum, P. histaminum,* and *H. alvei* have been described as prolific histamine-forming bacteria (Morii, Cann, & Taylor, 1988; Okuzumi, Hiraishi, Kobayashi, & Fujii, 1994; Taylor, 1986; Taylor, Guthertz, Leatherwood, & Lieber, 1979). In this study, we found that about half of the major amine-forming bacteria isolated from 4 different fish species belong to well-known amine-producing species, *E. aerogenes,* which produced substantially not only histamine but also putrescine and cadaverine. Compared to this, the other strains belonging to two different species of Enterobacter were found to produce much less histamine, but release similar level(s) of putrescine (and cadaverine). These results, together with the fact that putrescine and cadaverine are potential carcinogens to be converted to nitrosamine when exposed to nitrite (Bills et al., 1973), imply that the importance of non- or less histamine producers should not be overlooked. Therefore, it would be greatly important to completely understand bacterial contributions to the formation of overall BAs in foods. This would be a project to be worked on by our and other research group in the near future.

4. Conclusions

This study was conducted to determine the overall amounts of BAs in various fish, squid and shellfish species. Most fish samples were found to contain much less than 50 mg/kg of histamine and 100 mg/kg of tyramine and considered to be safe for human consumption. However, several samples of mackerel, saury, Spanish mackerel and amberjack had considerably high contents of histamine (up to 45.5 mg/kg) and tyramine (up to 221.8 mg/kg). Several fish and shellfish samples also showed relatively high levels of amine neurotransmitters. Furthermore, we found that BA contents can increase significantly during the storage at temperatures above 4 °C. Therefore, it needs to raise safety concerns, considering the levels not only of histamine but also of tyramine, and the levels of neurotransmitter amines, such as serotonin, noradrenaline and dopamine, as well. We also observed that psychrophilic *Enterobacter* spp. are responsible not only for histamine production but also for putrescine and cadaverine production in fish species.

References

Alberto, M. R., Arena, M. E., & Manca de Nadra, M. C. (2002). A comparative survey of two analytical methods for identification and quantification of biogenic amines. *Food Control, 13*, 125–129.

Askar, A., & Treptow, H. (1986). Biogene amine in lebensmitteln. In A. Askar & H. Treptow (Eds.), *Vorkommen, bedeutung und bestimmung* (pp. 21–74). Stuttgart, Germany: Verlag Eugen Elmer.

Auerswald, L., Morrom, C., & Lopata, A. L. (2006). Histamine levels in seventeen species of fresh and processed South African seafood. *Food Chemistry, 98*, 231–239.

Ben-Gigirey, B., De Sousa, J. M. V. B., Villa, T. G., & Barros-Velazquez, J. (1998). Changes in biogenic amines and microbiological analysis in albacore (*Thunnus alalunga*) muscle during frozen storage. *Journal of Food Protection, 61*, 608–615.

Ben-Gigirey, B., De Sousa, J. M. V. B., Villa, T. G., & Barros-Velazquez, J. (1999). Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (*Thunnus alalunga*). *Journal of Food Protection, 62*, 933–939.

Bermejo, A., Mondaca, M. A., Roeckel, M., & Marti, M. C. (2004). Bacterial formation of histamine in jack mackerel (*Trachurus symmetricus*). *Journal of Food Processing and Preservation, 28*, 201–222.

Bills, D. D., Hildrum, K. I., Scanlan, R. A., & Libbey, L. M. (1973). Potential precursors of N-nitrosopyrrolidine in bacon and other fried foods. *Journal of Agricultural Food Chemistry, 21*, 876–877.

Brink, B., Damirik, C., Joosten, H. M. L. J., & Huis in't Veld, J. H. J. (1990). Occurrence and formation of biologically active amines in foods. *International Journal of Food Microbiology, 11*, 73–84.

El Marrakchi, A., Bennour, M., Bouchriti, N., Hamama, A., & Togafait, H. (1990). Sensory, chemical, and microbiological assessment of moroccan sardines (*Sardina pilchardus*) stored in ice. *Journal of Food Protection, 53*, 600–605.

Frank, H. A., Baranowski, J. D., chongsiriwatana, M., Brust, P. A., & Premaratue, R. J. (1985). Identification and decarboxylase activities of bacteria isolated from decomposed mahi mahi after incubation at 0 and 32 °C. *International Journal of Food Microbiology, 2*, 331–340.

Halász, A., Baráth, Á., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science & Technology, 5*, 42–49.

Kimata, M. (1961). The histamine problem. In G. Borgstrom (Ed.). *Fish as food: production, biochemistry and microbiology* (Vol. 1, pp. 29–352). New York, USA: Academic Press.

Lakshmanan, R., Shakila, R. J., & Jeyasekaran, G. (2002). Survival of amine-forming bacteria during the ice storage of fish and shrimp. *Food Microbiology, 19*, 617–625.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Hernández-Herrero, M., & Mora-Ventura, M. T. (1996). Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *International Journal of Food Microbiology, 28*, 411–418.

Mah, J.-H., Chang, Y.-H., & Hwang, H.-J. (2008). *Paenibacillus tyraminigenes*sp. nov. isolated from Myeolchi-jeotgal, a traditional Korean salted and fermented anchovy. *International Journal of Food Microbiology, 127*, 209–214.

Maijala, R. L., Eerola, S. H., Aho, M. A., & Hirn, J. A. (1993). The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. *Journal of Food Protection, 56*, 125–129.

Middlebrooks, B. L., Toom, P. M., Douglas, W. L., Harrison, R. E., & McDowell, S. (1988). Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus maculatus*). *Journal of Food Science, 53*, 1024–1029.

Morii, H., Cann, D. C., & Taylor, L. Y. (1988). Histamine formation by luminous bacteria in mackerel stored at low temperatures. *Bulletin of the Japanese Society of Scientific Fisheries, 54*, 299–305.

Niven, C. F., Jeffreg, M. B., & Corlett, D. A. (1981). Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology, 41*, 321–322.

Okuzumi, M., Fukumoto, I., & Fujii, T. (1990). Changes in bacterial flora and polyamines contents during storage of horse mackerel meat. *Bulletin of the Japanese Society of Scientific Fisheries, 56*, 1307–1312.

Okuzumi, M., Hiraishi, A., Kobayashi, T., & Fujii, T. (1994). *Photobacterium histaminum* sp. nov., a histamine-producing marine bacterium. *International Journal of Systemic Bacteriology, 44*, 631–636.

Rice, S. L., Eitenmiller, R. R., & Koehler, P. E. (1976). Biologically active amines in foods: A review. *Journal of Milk and Food Technology, 39*, 353–358.

Rodríguez-Jerez, J. J., Mora-Ventura, M. T., López-Sabater, E. I., & Hernández-Herrero, M. M. (1994). Histidine, lysine and ornithine decarboxylase bacteria in Spanish salted semi-preserved anchovies. *Journal of Food Protection, 57*, 784–787, 791.

Santos, M. H. S. (1996). Biogenic amines: Their importance in foods. *International Journal of Food Microbiology, 29*, 213–231.

Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International, 29*, 675–690.

Shewan, J. M., Hobbs, G., & Hodgkiss, W. (1960). A determinative scheme for the identification of certain genera of Gram-negative bacteria, with special reference to the Pseudomonadaceae. *Journal of Applied Bacteriology, 23*, 379–390.

Stratton, J. E, & Taylor, S. L (1991). Scombroid poisoning. In D. R. Ward & C. R. Hackney (Eds.), *Microbiology of marine food products* (pp. 331–351). New York, USA: Van Nostrand Reinhold Co..

Taylor, S. L (1985). *Histamine poisoning associated with fish, cheese, and other foods. Monograph, VPH/FOS/85.1*. Geneva, Switzerland: World Health Organization (pp. 1–47).

Taylor, S. L. (1986). Histamine food poisoning: Toxicology and clinical aspects. *Critical Reviews in Toxicology, 17*, 91–128.

Taylor, S. L., Guthertz, L. S., Leatherwood, H., Tillman, F., & Lieber, E. R. (1978). Histamine production by foodborne bacterial species. *Journal of Food Safety, 1*, 173–187.

Taylor, S. L., Guthertz, L. S., Leatherwood, M., & Lieber, E. R. (1979). Histamine production by *Klebsiella pneumoniae* an incident of Scombroid fish poisoning. *Applied Environmental Microbiology, 37*, 274–278.

USFDA (US Food and Drug Administration) (2001). Scombrotoxin (histamine) formation. In Fish and fishery products hazards and controls guide, 3rd ed. (pp. 73-93). Washington, DC, USA: Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.

Wendakoon, C. N., Michiyo, M., & Sakaguchi, M. (1990). Comparison of non-volatile amine formation between the dark and white muscles of mackerel during storage. *Bulletin of the Japanese Society of Scientific Fisheries, 56*, 809–818.

Yamanaka, H., Shimakura, K., Shiomi, K., & Kikuchi, T. (1986). Changes in non-volatile amine contents of the meats of sardine and saury pike during storage. *Bulletin of the Japanese Society of Scientific Fisheries, 52*, 127–130.

Yoshinaga, D. H., & Frank, H. A. (1982). Histamine-producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*). *Applied Environmental Microbiology, 44*, 447–452.