Chemical, Microbiological, and AromaScan Evaluation of Mahi-mahi Fillets under Various Storage Conditions

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The quality for mahi-mahi stored at 1.7, 7.2, and 12.8 °C for 0, 1, 3, and 5 days was determined using biogenic amine analysis, microbial counts, and sensory evaluation (by a sensory test panel and an AromaScan). Biogenic amines in methanol extracts from mahi-mahi samples were analyzed using capillary electrophoresis (CE) with ultraviolet detection at 210 nm and a gas chromatography (GC) method that can simultaneously determine the contents of putrescine, cadaverine, histamine, spermidine, and spermine within 20 min after pentafluoropropionic anhydride derivatization. A good correlation ($R^2 = 0.99$) was found between CE and GC methods for detecting histamine in mahi-mahi. Fish quality deteriorated and correlated with increasing microbial numbers. Biogenic amines may be useful indicators for mahi-mahi quality and safety. AromaScan was able to correlate quality changes for mahi-mahi in microbiological and sensory analyses.

Keywords: *Mahi-mahi; AromaScan; biogenic amine; cadaverine; histamine*

INTRODUCTION

Fresh seafoods are highly susceptible to spoilage. Decomposition of aquatic food products occurs immediately after harvesting through the action of psychrotrophic bacteria, endogenous enzymes, or abusive handling. Rapid and reliable methods for predicting decomposition would be valuable to seafood processors. Histamine production is usually associated with scombroid poisoning from scombroid fish such as tuna and from nonscombroid fish such as mahi-mahi. Recent interest has focused on using histamine and other biogenic amines as predictors of product decomposition. Hazard Analysis Critical Control Point (HACCP) implementation mandates specifically the monitoring for these safety indicators, yet routine procedures are lacking. In addition, use of biogenic amine indicators to assess quality for most seafoods under actual commercial conditions is limited. Little work has gone into establishing whether a correlation exists between biogenic amine formation and product quality. The seafood industry and regulatory agencies need information to evaluate current guidelines for histamine in seafood and to establish new regulatory guidelines, including action levels for cadaverine and putrescine as indices of aquatic product quality and safety. Therefore, faster and more accurate routine methods that can be used at commercial landings or in the field for screening inferior products before purchase will be beneficial to processors and regulators and will help to reduce potential health risk to consumers.

Volatile acids, acetic acid, succinic acid, ethanol, volatile bases and amines, trimethylamine, changes in lipid content and fatty acid profiles, liberation of hydrogen sulfide, and nucleotide breakdown products have been used as indicators of seafood decomposition (*1*). Additionally, nonvolatile acids, such as amino acids, hypoxanthine, indole, skatole, and histamine, have been used to correlate the degree of decomposition. Histamine, putrescine, and cadaverine levels were shown to correlate with decomposition of fish following evaluation by sensory analysis (*2*, *3*). These biogenic amines are produced in post-mortem shellfish and fish following microbial decarboxylation, and their formation is temperature-dependent. The relationship between bacterial growth and production of cadaverine, putrescine, and histamine in seafood products is well documented (*4*–*6*).

Traditionally, measuring the decomposition or freshness of fish has relied on sensory methods. Mechanisms by which fish and seafood are classified as being fresh or spoiled are subjective processes when assessed by sensory analysis. In addition, the sensory methods are time-consuming and expensive. New methods, such as electronic nose devices, that can be used rapidly, providing information in a matter of minutes and not days, would be useful to food processors. Electronic nose devices are designed to measure the headspace above a liquid, solid, or vapor. The FDA Laboratory in Atlanta, GA, applied the system to test seafood decomposition and demonstrated satisfactory correlation with results from sensory evaluation by panelists. Considering the processors' need for rapid quality evaluation of product entering the plant and during processing, electronic noses could provide this information in a rapid time frame. Therefore, the objectives of this study were to determine the quality of mahi-mahi after storage at 1.7, 7.2, and 12.8 °C for 0, 1, 3, and 5 days using biogenic

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amine analysis, microbial counts, human sensory evaluation, and AromaScan and to determine the applicability of these methods for quality determination of mahi-mahi. A temperature of 7.2 °C represents the temperature for regular household refrigerators. Storage of mahi-mahi at 12.8 °C would guarantee production of histamine on the test samples, whereas storage at 1.7 °C would maintain fresh fish quality, thus contributing adequate samples for comparison purposes.

MATERIALS AND METHODS

Mahi-mahi Preparation and Storage. Fresh mahi-mahi (dolphin fish, *Coryphaena hippurus*) fillets were obtained from a commercial fishery from the Gulf of Mexico through the help of Dr. W. S. Otwell, University of Florida. The fillets were packed in ice and transported to the University of Florida, Gainesville, FL, for testing. Three groups of fresh mahi-mahi fillets (six fillets per group, 27 × 12 cm per fillet) were processed and stored at 1.7, 7.2, and 12.8 °C. Samples were examined at 0, 1, 3, and 5 days to determine the effect of various time−temperature storage conditions on the quality of these aquatic food products by sensory, aroma, bacteriological, and chemical evaluations.

Microbiological Analysis. On each testing day, a 20 g portion was cut from each fillet and homogenized at high speed for 2 min in a sterile blender with 9 volumes (1:9, w/v) of sterile Butterfield's phosphate buffer (BPB). The homogenates were serially diluted with sterile BPB, and 0.1 mL aliquots of the diluents were surface plated on quadruplicate aerobic plate count (APC) agar (Difco, Detroit, MI) plates containing 1.5% NaCl. A pour plate method was also used for some homogenates. Bacterial colonies were counted after the plates were incubated at 25 °C for 48 h. Bacterial numbers were expressed as log colony-forming units (CFU)/g of sample.

Histamine Analysis by Capillary Electrophoresis (CE). The method of Mopper and Sciacchitano (*7*) with slight modification was followed. On each test day, duplicate 10 g portions from each fillet were blended separately in half-pint Mason jars with 25 mL of 50% methanol at low speed for 30 s and then at high speed for 1 min. The homogenate was transferred from the jar to a 50 mL volumetric flask with two rinsings using small portions of 50% methanol. The volumetric flask was placed in a 60 °C water bath for 15 min, then cooled to ambient temperature, and the volume was adjusted to 50 mL using 50% methanol. The sample was filtered through a Whatman No. 1 filter paper (Fisher Scientific, Orlando, FL) and then through a 0.8 μm filter for injection into the CE. Blank controls and spiked samples were extracted similarly. Spiked samples were prepared by adding histamine standard solutions to fresh fish samples at a final histamine concentration of 25, 50, 125, 250, or 500 ppm. Histamine standard solutions (0.5−100 ppm of free histamine) were prepared from a 1000 ppm stock solution by dissolving 169 mg of histamine dihydrochloride (Sigma, St. Louis, MO) in 100 mL of 50% methanol.

CE was performed on a BioFocus 2000 Capillary Electrophoresis System (Bio-Rad, Hercules, CA) using a coated capillary cartridge of 24 cm × 25 μm i.d. Before injection, the capillary cartridge was rinsed with 0.1 N NaOH for 1 min, then with water for 2 min, and finally the running buffer (0.02 M, pH 2.5 sodium citrate buffer) for 3 min. Cartridge temperature was maintained at 35 °C. Sample solutions were injected (8.4 nL aliquot) into the capillary by low-pressure injection at 20 psi·s. A constant voltage at 10 kV was applied, and detection of histamine was performed by monitoring the absorbance at 210 nm. Data were stored in a Pentium PC and processed using an integration BioFocus program (Bio-Rad).

Histamine standards were analyzed together with extracts of various fish (blank, spiked, and test) samples. During analysis, a standard solution was also injected intermittently along with test samples to check chromatographic consistency. Each sample was injected twice. Peak areas of histamine standard solutions were used to prepare a standard curve. From the standard curve, histamine concentrations in test samples were calculated. At the end of each experiment, the CE was run with a shut-down cycle.

Histamine Analysis Using the AOAC Fluorometric Method. Two decomposed mahi-mahi fillets that had been stored at 12.8 °C for 2 days and then at 7.2 °C for another 2 days were extracted with 50% methanol as described above. The extracts were subjected to column chromatography through a Dowex 1-X8 cartridge column following the AOAC (*8*) procedure. The eluates were processed and reacted with *o*-phthalic dicarboxaldehyde (OPT) and then checked for fluorescence intensity using a fluorometer at an excitation wavelength of 350 nm and emission wavelength of 444 nm (*8*). Histamine contents in these fillets were determined by comparing the fluorescence intensity of the test samples with the standard curve prepared from histamine standard solutions.

Biogenic Amine Analysis Using Gas Chromatography (GC). Biogenic amine stock solutions (1 mg/mL) were prepared by dissolving 183 mg of putrescine dihydrochloride, 171 mg of cadaverine dihydrochloride, 166 mg of histamine dihydrochloride, 175 mg of spermidine trihydrochloride, and 172 mg of spermine tetrahydrochloride (Sigma, St. Louis, MO) into respective 100 mL volumetric flasks with 0.1 N HCl. Five biogenic amine standard mixtures were prepared by adding a specified volume of each biogenic amine stock solution into a 10 mL volumetric flask and then diluting to volume with 0.1 N HCl.

The same mahi-mahi extracts used for CE analysis of histamine were used for GC analysis of biogenic amines. The method of Staruszkiewicz and Bond (*9*) was modified to derivatize biogenic amines from mahi-mahi samples. To each 10 mL of fish extraction solution (or 1 mL of biogenic amine standard mixture solution) in a 50 or 100 mL round-bottom flask was added 0.5 mL of 1 N HCl. This was evaporated to dryness on a rotary evaporator at 50 °C. One milliliter of 30% ethyl acetate in toluene and 300 μL of pentafluoropropionic (PFP) anhydride (Sigma) were added. The flask was stoppered, and the residue was mixed and then heated at 50 °C for 30 min. The solution was swirled at least once during this time. After 30 min, the solvent and reagent were evaporated under nitrogen at 50 °C. The residue was dissolved in 1 mL of 30% ethyl acetate in toluene and stored in the freezer until GC analysis. All samples were filtered through a 0.2 μm nylon acrodisc 13 syringe filter (Fisher Scientific, Orlando, FL) before GC injection. The spiked mahi-mahi, which was prepared by adding the biogenic amine mixture to fresh fish samples, was processed similarly to the test samples.

A Perkin-Elmer 8500 gas chromatograph with a flame ionization detector (FID) was used for amine analysis. Separations were achieved using a 15 m × 0.32 mm DB-1 column (J&W Scientific, Folsom, CA) with a film thickness of 3 μm, which was fitted with a fused silica 1 m × 0.32 mm untreated guard column (Supelco, Bellefonte, PA). Optimal analytical conditions were as follows: injection port temperature, 300 °C; detector temperature, 325 °C; injection volume, 1 μL; column flow, 4 mL/min; split on at 0.1 min after injection. The inlet was operated at a constant flow of helium at 8.0 psi. Temperature programming was carried out at initial temperature, 140 °C (held for 0 min), increased 5 °C/min to 178 °C (held for 0 min), and ramped, at 10 °C/min, to a final temperature of 300 °C (held for 5 min). An H_2 pressure of 12 psi and an air pressure of 22 psi were used for the FID.

A set of biogenic amine standard mixtures was analyzed together with test fish samples. During analysis, a standard solution was also injected intermittently with test samples to check chromatographic consistency. Each sample was injected twice. The peak height of each biogenic amine (putrescine, cadaverine, histamine, spermidine, and spermine) from standard mixture solutions was used to prepare standard curves for each amine. From these standard curves, the amine concentrations in mahi-mahi were calculated.

Sensory Evaluation of Mahi-mahi Fillets by Panelists. A 10-point sensory scale was used for judging the progressive changes in quality attributes of mahi-mahi fillets at each

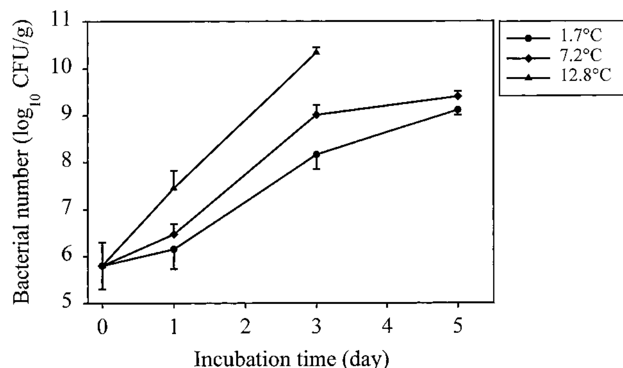

Figure 1. Time-related changes of bacterial loads on mahi-mahi fillets stored at 1.7, 7.2, and 12.8 °C.

sampling interval (days 0, 1, 3, and 5). A 10-member panel (3 females, 7 males), ages 24−48 years, from the Department of Food Science and Human Nutrition at the University of Florida, met for two training sessions using fresh and stale samples in order to review the attributes and establish consistency in sensory evaluation. All samples were coded with random three-digit numbers. Panelists wearing disposal gloves evaluated the samples by feeling, smelling, and checking appearance. Changes in fillet color, texture, and appearance, as well as formation of odors, were used for descriptive commentating. The degrees of defect were rated from 1 to 10 for each category, where 1−4 = fresh, 4−6 = initial decomposition, and 6−10 = advanced decomposition. The final grading (A, B, or C) was made from the sensory evaluation sheets following those described by the National Marine Fisheries Service (NMFS) Fishery Products Inspection Manual (*10*).

Determination of Fish Freshness Using AromaScan. An AromaScan (AromaScan, Inc., Hollis, NH) equipped with an array of 32 electrically conducting organic polymer sensors was used to determine freshness of mahi-mahi fillets. Portions (10 g) of mahi-mahi samples were placed in an analysis bag; the bag was evacuated and then filled with carbon-filtered air. The bag's absolute humidity was 5 g/m^3; the reference humidity was 10 g/m^3. The headspace in the sample bag was allowed to equilibrate at 35 °C for 10 min prior to analysis and then a 2 min analysis time was performed to collect data. Prior to analysis, the polymer sensors were allowed to react with reference air (dried by silica gel to ~15 g/m^3) for 0.5 min. Carbon-filtered ambient air was used as the reference air. After each analysis, the sensors were washed (1 min) with the headspace from a wash bottle filled with 2% 2-propanol and then allowed to react with reference air for 2.5 min before the next sample was analyzed. Data for each sample were collected from a 60-s slice between 60 and 120 s of the total analysis cycle. Computer mapping was performed for all samples at each time−temperature storage condition using AromaScan A32S Windows software v. 1.3.

RESULTS AND DISCUSSION

Microbial Analysis. Stored mahi-mahi fillets showed time- and temperature-related increases in bacterial loads. Fillets stored at 7.2 and 12.8 °C showed a dramatic increase in bacterial counts, reaching >9 log_{10} CFU/g in 3 days (Figure 1). Fillets stored at 1.7 °C did not reach this level until day 5. When the bacterial number reached 7 log CFU/g or higher, the fillet was considered to be spoiled. Mahi-mahi fillets stored at 12.8 °C were considered by panelists as not acceptable for consumption by day 1 (based on microbiological levels). Fillets stored at all other temperatures were also considered unacceptable for consumption by day 3.

CE Determination of Histamine. The CE electropherograms for histamine standard and muscle extracts of blank, histamine-spiked, and test mahi-mahi fillets showed no interference with the histamine peak (Figure 2). The migration time of pure histamine was 2.7 min. Linear relationships (R^2 > 0.999) occurred with peak areas and concentrations over 0.5−100 ppm of histamine standard solutions. An 87.5% recovery rate was achieved from histamine-spiked mahi-mahi fillets over the concentration range of 25−500 ppm. Average coefficients of variation were 4.6% for intra-assay and 7.7% for inter-assay with histamine standard solutions.

High levels of histamine (154−817 ppm) were found in fish after 5 days of storage at 12.8 °C (Table 1). Less than 10 ppm of histamine was found in mahi-mahi fillets stored at 1.7 °C for 5 days. A high variation in histamine content was found among the six mahi-mahi

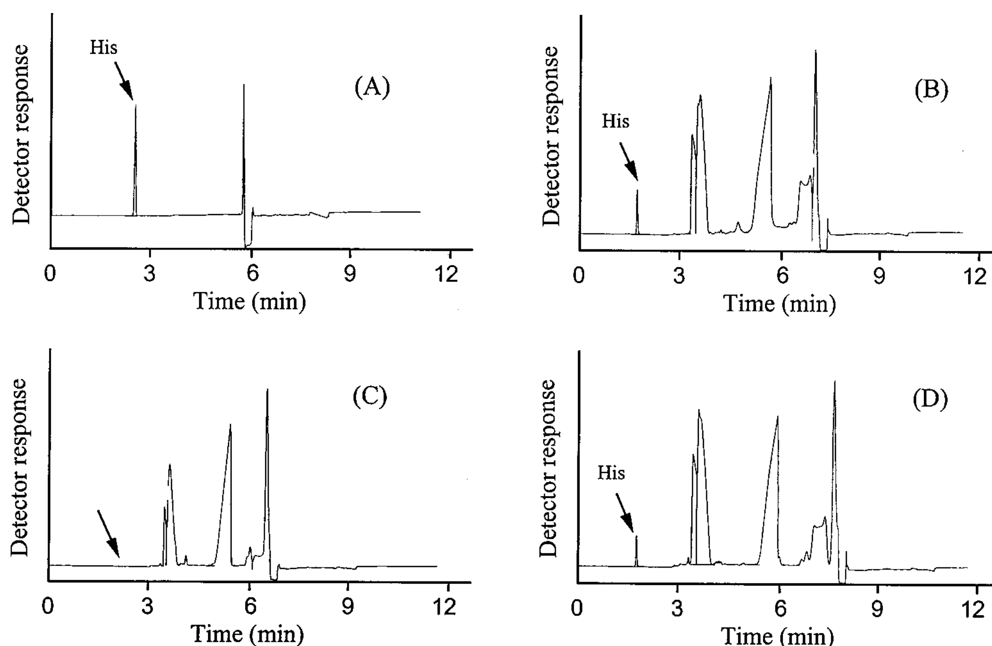

Figure 2. Typical electropherograms of (A) histamine standard solution and mahi-mahi extracts from (B) histamine-spiked fillets, (C) blank fillet, and (D) test fillets.

Table 1. Histamine Concentrations (Parts per Million) in Mahi-mahi Stored at Different Temperatures for 5 Days As Analyzed by CE

temp (°C)	day	fillet replicate I	II	III	IV	V	VI	mean ± SD
	0	ND[a]	6.4	ND	ND	ND	ND	1.1 ± 2.6
1.7	1	ND	ND	ND	ND	6.3	ND	1.1 ± 2.6
7.2		ND	ND	ND	ND	ND	ND	0.0 ± 0.0
12.8		ND	13.2	ND	ND	ND	ND	2.2 ± 5.4
1.7	3	ND	ND	ND	ND	5.5	ND	0.9 ± 2.2
7.2		ND	ND	ND	ND	ND	ND	0.0 ± 0.0
12.8		19.9	16.6	15.7	11.4	41.8	14.2	19.9 ± 11.1
1.7	5	ND	ND	ND	ND	8.2	ND	1.6 ± 3.7
7.2		ND	4.1	4.3	8.7	15.8	0.8	5.6 ± 5.9
12.8		202.4	153.8	187.1	392.3	816.5	224.2	329.4 ± 252.8

[a] ND, not detected.

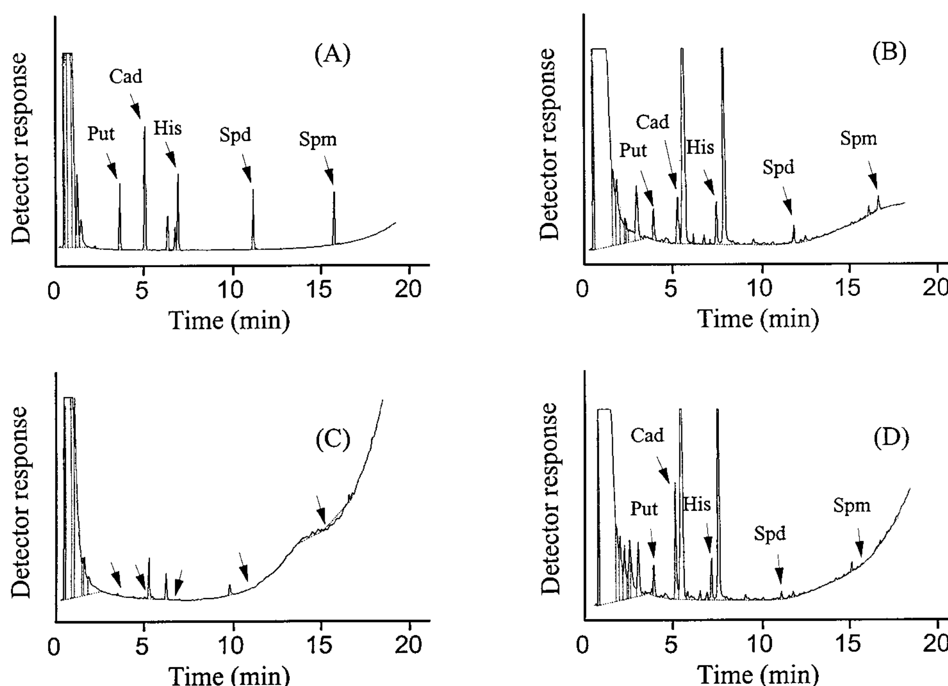

Figure 3. Gas chromatograms of PFP derivatives for (A) biogenic amine standard mixture and mahi-mahi extracts from (B) biogenic amine-spiked filet, (C) blank fillet, and (D) test fillets.

fillets stored at the same temperature for the same period of time. This inconsistency in histamine content even within the same species could be due to variations of how the fish is processed and decomposed (*11*).

GC Determination of Biogenic Amines. The GC method can simultaneously determine the contents of putrescine (Put), cadaverine (Cad), histamine (His), spermidine (Spd), and spermine (Spm) in mahi-mahi fillets within 20 min following PFP anhydride derivatization of the methanol extracts. The migration times were 3.6, 5.1, 7.1, 11.6, and 16.5 min for Put, Cad, His, Spd, and Spm, respectively. The standard curves for these five amines were linear ($R^2 > 0.99$) over the selected concentration ranges.

Gas chromatograms for PFP derivatives of biogenic amine standards and muscle extracts of the blank, spiked, and test mahi-mahi fillets showed no interference with these biogenic amine peaks (Figure 3). The recovery rates of amines were 99.5, 95.8, 86.3, 93.3, and 64.9% for Put, Cad, His, Spd, and Spm, respectively. The average coefficients of variation for the intra-assay of the amine standard solution were 5.4, 4.0, 10.7, 5.2, and 6.4% for Put, Cad, His, Spd, and Spm, respectively, whereas they were 8.6, 14.7, 17.7, 4.0, and 7.4%, respectively, for the inter-assay.

Levels of putrescine and cadaverine increased as decomposition progressed (Table 2). Putrescine was detected at low level for initial decomposition but increased rapidly at advanced decomposition. Cadaverine was detected in small amounts initially, but the quantity increased as decomposition progressed. Formation of putrescine and cadaverine in mahi-mahi stored at 1.7 and 7.2 °C was faster than that of histamine. Levels of putrescine and cadaverine at these two temperatures were also higher than that of histamine at each interval. In contrast, the level of histamine was higher than those of putrescine and cadaverine in mahi-mahi stored at 12.8 °C for 5 days.

Histamine was not detected in fresh mahi-mahi (Table 2). Its content began to increase when decomposition started to occur (7.2 °C for 3 days). Histamine increased dramatically (from 158 to 764 ppm) after the fillets were stored at 12.8 °C for 5 days. Fillets stored at 1.7 °C for 5 days had less than 5 ppm of histamine, whereas those stored at 7.2 °C for 5 days did not exceed 15 ppm.

Table 2. GC Analysis of Biogenic Amine Content (Parts Per Million) in Mahi-mahi Stored at Different Temperatures for up to 5 Days

temp (°C)	day	Put[a]	Cad	His	Spd	Spm	Put + Cad + His	grade[b]
1.7	0	0.0 ± 0.0	1.4 ± 2.8	0.0 ± 0.0	0.1 ± 0.2	2.7 ± 1.0	1.4 ± 2.8	A
1.7	1	0.3 ± 0.4	2.0 ± 2.8	0.0 ± 0.0	1.4 ± 1.0	3.4 ± 1.6	2.2 ± 3.1	A
7.2		0.5 ± 0.7	4.8 ± 4.0	0.0 ± 0.0	1.0 ± 1.0	3.9 ± 0.4	5.3 ± 4.2	A
12.8		1.0 ± 1.0	3.1 ± 3.9	0.0 ± 0.0	0.7 ± 0.5	4.6 ± 2.7	4.1 ± 4.3	A
1.7	3	1.6 ± 1.1	1.8 ± 2.1	0.0 ± 0.0	0.1 ± 0.2	2.2 ± 0.6	3.5 ± 2.9	A
7.2		3.3 ± 3.6	2.4 ± 1.2	0.0 ± 0.0	0.2 ± 0.3	2.0 ± 1.8	5.7 ± 4.6	B
12.8		5.9 ± 8.7	7.3 ± 10.5	3.2 ± 3.6	1.0 ± 0.9	2.1 ± 1.3	16.4 ± 17.5	C
1.7	5	9.1 ± 8.2	6.2 ± 2.8	1.1 ± 2.6	0.4 ± 0.4	0.9 ± 0.8	16.4 ± 10.6	B
7.2		7.8 ± 5.1	8.2 ± 5.2	4.0 ± 6.2	0.7 ± 0.5	1.3 ± 1.1	20.0 ± 12.9	C
12.8		14.2 ± 14.1	26.7 ± 28.5	318.4 ± 239.6	0.5 ± 1.2	0.0 ± 0.0	359.4 ± 249.4	C

[a] Mean ± standard deviation ($n = 6$). [b] Grades: A, sensory rating 1−4 (fresh); B, sensory rating 4−6 (initial decomposition); C, sensory rating 6−10 (advanced decomposition, rejected).

When panelists considered mahi-mahi to be not suitable for consumption (grade C product), the contents of histamine in these fillets stored at 7.2 and 12.8 °C were 4.0 and 3.2 ppm, respectively (Table 2). These fillets had similar levels of putrescine and cadaverine as histamine; 7.8 and 5.9 ppm of putrescine and 8.2 and 7.3 ppm of cadaverine were found in samples stored at 7.2 and 12.8 °C, respectively. At the time of rejection, mahi-mahi fillets stored at 7.2 °C had a sum >20 ppm for these three biogenic amines (Put + Cad + His). However, the combined content of Put + Cad + His was 16.4 ppm in samples stored at 12.8 °C for 3 days. The Put + Cad + His content did not exceed 20 ppm in mahi-mahi stored at 1.7 °C for 5 days. Therefore, the combined content of Put + Cad + His can be used as an indicator of mahi-mahi spoilage.

Spermidine and spermine were present in smaller amounts in mahi-mahi. These two polyamines are usually present in fresh fish because they are necessary for cellular growth (*12*). The level of spermine decreased slightly as decomposition progressed, whereas the level of spermidine remained the same or decreased slightly (Table 2). Therefore, mahi-mahi decomposition had little influence on spermidine and spermine contents. This finding is in agreement with that of Kim and Bjeldanes (*13*), who reported that spermidine levels decreased slightly as tuna decomposed, whereas spermine levels remained the same for both good and decomposed tuna. Klausen and Lund (*14*) reported that spermidine was present in a rather constant amount in herring (2−4 ppm) and mackerel (4−6 ppm) stored at 2 and 10 °C.

Veciana-Nogués et al. (*15*) reported that spermidine and spermine were the prevailing biogenic amines found in fresh tuna samples from zero time. Putrescine and cadaverine were also found but at very low levels (<0.5 ppm), whereas histamine was not detected. Mietz and Karmas (*11*) also reported that, as rockfish and salmon started to decompose, the contents of histamine, putrescine, and cadaverine increased while those of spermidine and spermine decreased.

Cadaverine levels in decomposed mahi-mahi were higher mostly than that of putrescine (Table 2). Dainty et al. (*16*) evaluated the bacterial sources of putrescine and cadaverine in chill-stored vacuum-packaged beef and concluded that cadaverine, which is probably formed through the decarboxylation of lysine, does not require any metabolic input from other organisms. However, formation of putrescine requires growth of arginine-utilizing lactic acid bacteria that are presumed to produce ornithine, which is subsequently decarboxylated by Enterobacteriacea. Spoiled fish were found usually to have a much lower content of putrescine (<10 mg/100 g) than cadaverine (*9*, *13*). The presence of limited quantities of ornithine in fish tissue also contributes to this difference in cadaverine and putrescine levels in decomposed samples.

Table 3. Time-Related Changes in Sensory Ratings by a Sensory Panel for Mahi-mahi Fillets Stored at Different Temperatures

temp (°C)	day	parameters				
		appearance	color	texture	odor	mean ± SD[a]
	0	1.5	1.5	1.5	1.5	1.5 ± 0.0
1.7	1	1.8	2.5	2.0	2.0	2.1 ± 0.3
7.2		2.7	2.5	2.0	2.0	2.3 ± 0.3
12.8		3.3	3.5	2.0	3.7	3.1 ± 0.8
1.7	3	2.8	4.0	3.5	3.0	3.3 ± 0.5
7.2		4.8	5.3	5.8	6.0	5.5 ± 0.5
12.8		7.8	5.8	7.0	7.9	7.1 ± 0.9
1.7	5	5.7	4.4	3.7	3.7	4.4 ± 0.9
7.2		7.2	5.8	5.3	7.7	6.5 ± 1.1

[a] Mean ± standard deviation ($n = 6$).

A good correlation ($R^2 = 0.99$) was found between CE and GC methods for the detection of histamine levels in mahi-mahi fillets stored at 1.7, 7.2, and 12.8 °C for 0, 1, 3, and 5 days. Histamine content in the same fish samples determined by GC were slightly lower than that determined by CE. Histamine in methanolic extracts of mahi-mahi can be analyzed directly by CE without cleanup and derivatization. This method can be used for on-site quantitation of histamine and screening of seafood. The GC method that can simultaneously analyze five putrefactive amines is a useful procedure for assessing these chemical indicators in the spoilage of seafoods.

Good correlation was found between the sum of Put + Cad + His and bacterial count. The bacterial counts of mahi-mahi fillets were higher than 7 log CFU/g when >20 ppm of Put + Cad + His was found. This result indicated that microbial counts played an important role in the spoilage of seafood. High levels of Put + Cad + His were found in mahi-mahi fillets only when decomposition had occurred.

Accuracy and precision for the AOAC fluorometric method were comparable to those of CE and GC methods for histamine determination in mahi-mahi samples. Extracts of two decomposed mahi-mahi fillets that had been stored at 12.8 °C for 2 days and then at 7.2 °C for another 2 days were analyzed by the CE, GC, and AOAC fluorometric methods. Concentrations of

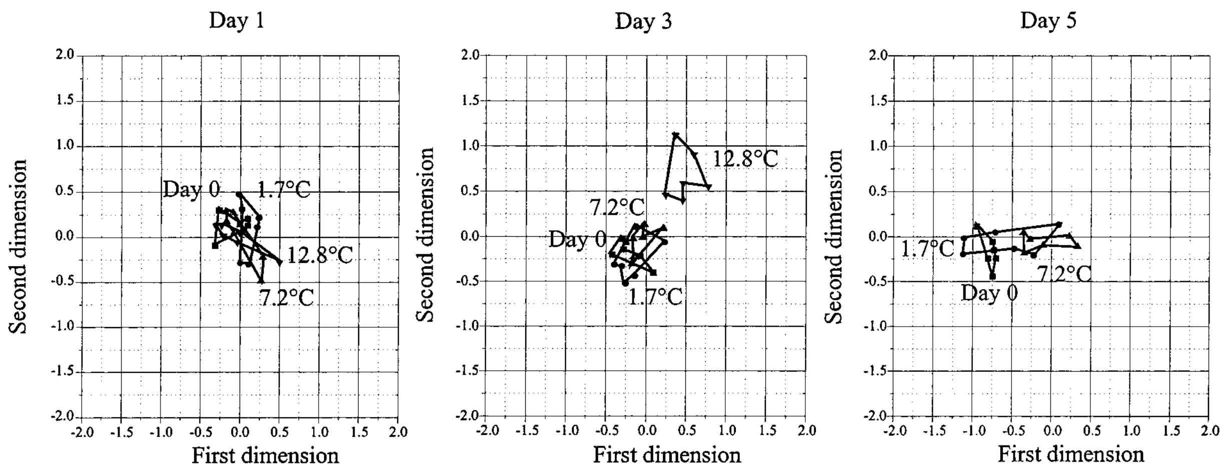

Figure 4. Comparison of AromaMaps for mahi-mahi fillets stored at different temperatures on days 1, 3, and 5 of storage. All fillets were compared to day 0 controls.

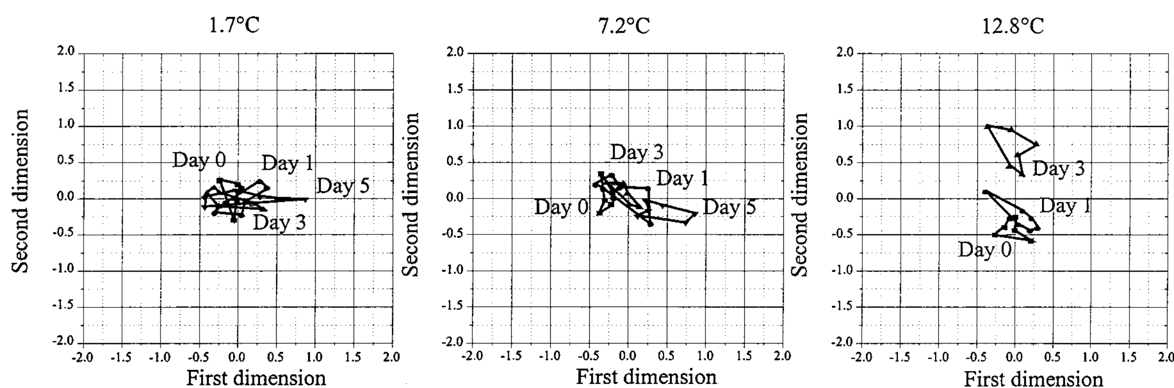

Figure 5. Comparison of AromaMaps for mahi-mahi fillets stored at 1.7, 7.2, and 12.8 °C. All fillets were compared to the day 0 controls.

histamine found in the first extract were 123.3, 115.3, and 112.6 ppm by CE, GC, and fluorometric analysis, whereas for the second extract, they were 202.4, 173.5, and 165.5 ppm, respectively.

Sensory Evaluation of Mahi-mahi Fillets. Sensory evaluation by the panel showed that some of the mahi-mahi fillets stored at 12.8 °C started to release moderate fish odor after 1 day (Table 3). Some of the fillets stored at 7.2 °C started to exhibit moderate discoloration and excessive odor after 3 days. Fish fillets stored at 1.7 °C exhibited only slight fish odor and moderate discoloration even after 5 days of storage. Mahi-mahi fillets stored at 12.8 °C were deemed unacceptable (sensory rating >6) after 3 days, whereas fillets stored at 7.2 °C were considered unacceptable after 5 days. Mahi-mahi fillets stored at 1.7 °C remained acceptable even after 5 days.

Good correlation (R^2 = 0.892) was found between sensory rating and bacterial number. Most mahi-mahi fillets were rejected by sensory analysis when >50 ppm of Put + Cad + His was found. Fillets were classified as grade B or C products when >20 ppm of Put + Cad + His was detected. This indicated that when decomposition in fish had occurred, higher levels of biogenic amines were found. Sensory analysis was not a good indicator for the presence of high levels of biogenic amines in fish. Monitoring the biogenic amine content in high-risk fish species remains important for assurance of seafood quality and safety. Biogenic amines may be used as indicators for mahi-mahi quality and safety. López-Sabater et al. (*17*) suggested that a lack in the relationship between histamine content and sensory attributes can help explain the high incidence of scombrotoxicity.

AromaScan Analysis of Mahi-mahi Fillets. Results of AromaScan analysis of mahi-mahi stored at 1.7, 7.2, and 12.8 °C for three time intervals (1, 3, and 5 days) are shown in Figure 4. The mappings of the three temperature groups were separated from day 0 samples in a time-related fashion. Mapping continued to show further separation as storage time increased. Mappings from the three temperature groups did not separate from each other on day 1, indicating that odor profiles of all fish fillets from the three temperature groups were similar. This result showed a good correlation with sensory analysis, which classified all of the fillets from the three temperature groups as grade A fillets on day 1. Samples stored at 12.8 °C for 3 days (grade C products by sensory analysis) showed mappings that were separating from the other temperature groups and day 0 controls (grade A or B). Mappings for fillets stored at 7.2 °C (grade C) started to separate from day 0 controls by day 5. Comparison of the results from AromaScan and sensory analysis reveals that AromaScan can identify odor differences between grade C fillets and grade A and B fillets.

AromaScan could not differentiate odor differences for mahi-mahi fillets stored at 1.7 °C for any storage day (Figure 5). Sensory analysis of mahi-mahi indicated that fillets stored at 1.7 °C were grade A products during the 5 days of storage. Mappings of fillets stored at 7.2 °C started to separate from day 0 controls on day 5 when

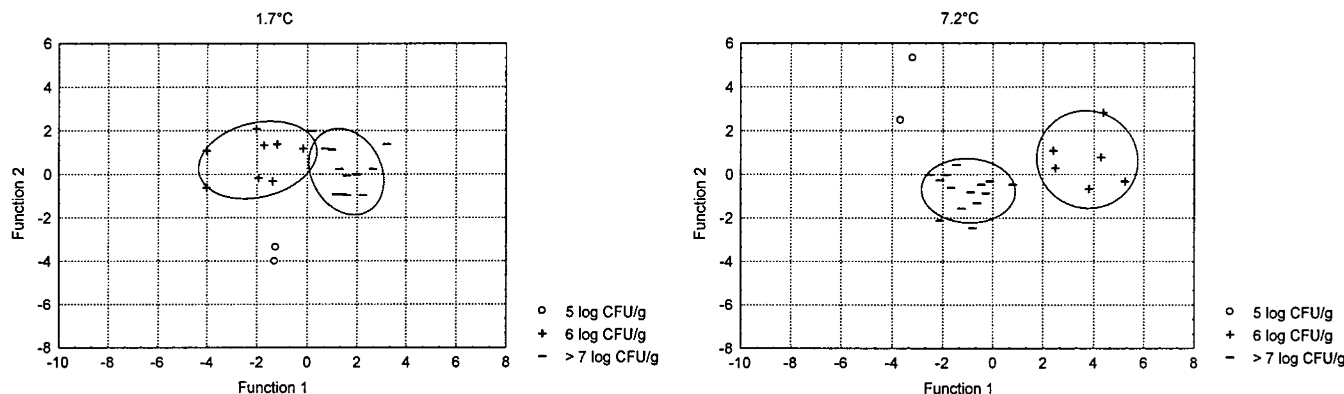

Figure 6. Correlation of the AromaScan analysis results with bacterial counts in mahi-mahi fillets stored at different temperatures.

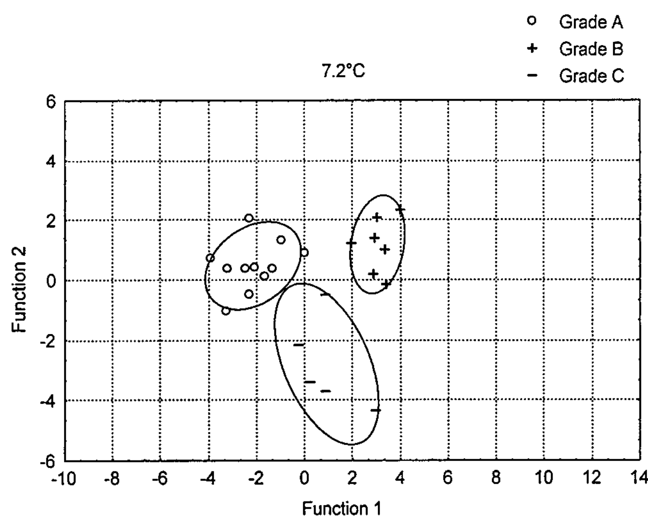

Figure 7. Correlation of the AromaScan analysis results with sensory grades in mahi-mahi fillets stored at 7.2 °C.

fillets were rated as grade C products, whereas the fillets stored at 12.8 °C started to separate from day 0 controls on day 3 when fillets were rated as grade C products. These results indicated that AromaScan can differentiate odor differences between grade C and grade A products.

Multiple discriminant analysis was used to show significant differences between samples by the spatial separation between clusters. In discriminant analysis, X and Y functions account for differences between different samples. Results from the multiple discriminant analysis showed that clusters for mahi-mahi fillets stored at different temperatures with different microbial loads separated from each other in the canonical discriminant graphs (Figure 6). Clusters with closer bacterial numbers were closer to each other on the graphs. High correlation was also found between AromaScan and sensory analysis. AromaScan can be used to predict the grade of mahi-mahi fillets stored at 7.2 °C (Figure 7). Clusters for mahi-mahi fillets with different sensory grades separated from each other in the canonical discriminant graphs. This indicated that odor profiles for mahi-mahi fillets stored at 7.2 °C with different sensory grades were different from each other. Thus, AromaScan is capable of detecting differences in odor profiles for mahi-mahi under different stages of decomposition and can be used for quality and freshness evaluation.

Conclusion. Storage temperature and microbial counts played important roles in the spoilage of mahi-mahi fillets. Changes in fish quality correlated with increases in microbiological measurements. Histamine level has little value in monitoring the loss of freshness for mahi-mahi fillets during storage at different temperatures. It cannot be used as an objective indicator of gross spoilage of mahi-mahi. Bacterial levels is a useful and objective indicator of gross spoilage of mahi-mahi and is imperative for safety considerations. Monitoring the content of biogenic amines in high-risk fish species remains important for assurance of seafood quality and safety. Biogenic amines may be used as indicators for mahi-mahi quality and safety. Human sensory study provides a useful means to monitor both changes in freshness and the onset of spoilage. AromaScan has the capacity to identify the changes in odor profiles for mahi-mahi fillets during storage. Changes in quality as detected by AromaScan correlated with microbiological measurements as well as with sensory analysis. The loss of freshness and decreased quality in mahi-mahi fillets can be objectively determined by AromaScan analysis. Thus, AromaScan analysis may provide a viable, quantitative approach to determine fish freshness, which could be used for quality control and inspection purposes. It can do this objectively and in a short analysis time (12 min). Histamine in methanol extracts of mahi-mahi can be directly analyzed by CE without cleanup and derivatization. Data obtained by CE from tested maih-mahi samples showed exceptionally high correlation with those analyzed by GC method. This sensitive method can thus be used for rapid screening and determination of histamine content in seafood samples. The GC procedure for simultaneous analysis of five putrefactive amines provides a useful tool for assessing these chemical indicators in spoiled seafoods.

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