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Microbiological, Sensory, and Electronic Nose Evaluation of Yellowfin Tuna under Various Storage Conditions†

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ABSTRACT

Microbiological assessment, sensory evaluation, and electronic nose (AromaScan) analysis were performed on yellowfin tuna stored at 0, 4, 10, and 22°C for 0, 1, 3, 5, and 9 days. Fish color, texture, appearance, and odor were evaluated by a trained sensory panel, while aroma-odor properties were evaluated using an AromaScan. Bacterial enumeration was performed using plate count agar containing 1.5% NaCl. Tuna fillets stored at 22°C for 3 days or longer had a bacterial load of over 10^7 CFU/g and were rated not acceptable for consumption (grade C) by the sensory panel. Tuna fillets stored at 4°C for 9 days or 10°C for over 5 days were rated as grade C products and also had a bacterial load of over 10^7 CFU/g. The change in fish quality as determined by AromaScan followed increases in microbiological counts in tuna fillets, indicating that bacterial load can serve as a useful and objective indicator of gross spoilage. Electronic nose devices can be used in conjunction with microbial counts and sensory panels to evaluate the degree of decomposition in tuna during storage.

Seafoods are highly susceptible to spoilage and deterioration due to autolysis and growth of postmortem microbial populations *(6).* Microbial activity on seafood products produces pronounced off-flavors and off-odors, leading to shorter shelf life and economic losses *(21).* It is therefore important for food processors to resolve the cause of spoilage as quickly as possible to avoid unnecessary and expensive product recalls and reoccurrence of similar problems in the future *(13, 16).*

Traditionally, seafood freshness has been assessed by sensory evaluation methods. Sensory panels are used in the seafood industry to classify products as fresh or spoiled. Although fast, simple, and, for the most part, accurate, sensory analysis is sometimes perceived to be inherently subjective. Sensory panels, no matter how skilled, are not on call during all phases of production, nor can they function efficiently for prolonged periods of time. Although chemical methods using chromatographic and chemical techniques for determining seafood freshness are precise and objective, they are time-consuming, expensive, and species-dependent. Therefore, there is a need to develop new technologies that can give a rapid and objective classification of seafood quality and freshness so that the processing industry can quickly evaluate the products and maintain quality.

Of all the senses, smell has been the most difficult to define objectively. Only in the last several years has the ability to measure and characterize smell become possible. Advances in organic chemistry, electronics, and computing have made possible the development of new digital aroma technology (electronic nose; AromaScan Inc., Hollis, N.H.) that parallels the human nose *(23).* The electronic nose employs an array of chemical sensors based on conducting polymers, metal oxides, surface acoustic wave devices, quartz crystal microbalances, or combinations of these devices *(3, 4, 7, 12, 13, 14, 28).* The potential for the electronic nose is vast. It is anticipated that there will be a considerable reduction in quality-control costs when the nose is used to replace expensive sensory panels for routine analysis of product quality.

AromaScan (AromaScan) is an electronic nose unit that mimics the human nose. As aroma-odor vapors from test samples are drawn over an array of 32 organic polymer sensors, the volatiles are adsorbed and desorbed from the polymer surface. These temporary changes in electrical resistance due to interaction with the sensor can then be expressed as an aroma histogram. The combined resistance from the 32 sensors, which represent the continuous and real-time analysis of the overall aroma-odor properties for each sample, can be condensed into a single representative data point on a two-dimensional AromaMap. The axes representing Euclidean distances between each sample can be plotted and the differences among samples determined. Multiple samples appear as populations on the AromaMap and demonstrate the reproducibility of using AromaScan for quality determination *(23).* Very little sample preparation time is needed for analysis with an AromaScan. Therefore, the analysis can be completed within a few minutes. An electronic nose, such as AromaScan, can be used in parallel with sensory panels or to complement the more

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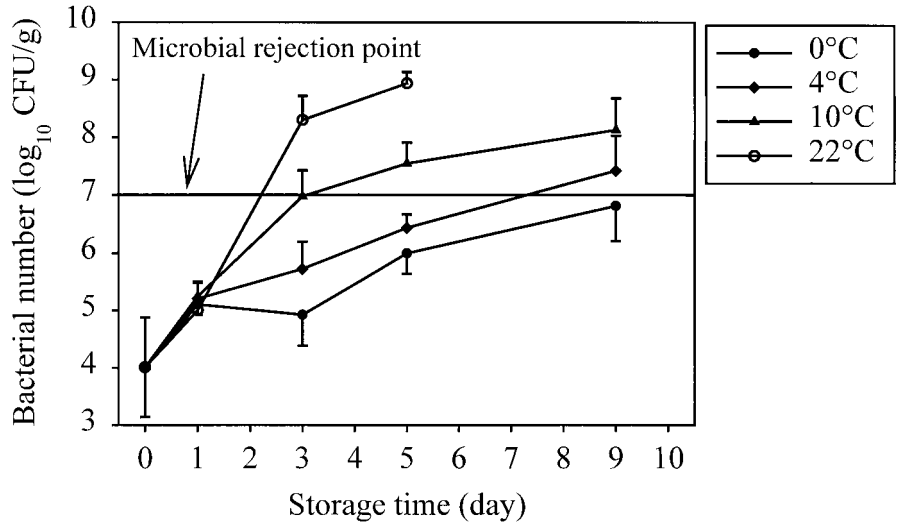

FIGURE 1. *Time-related changes in bacterial numbers on tuna fillets stored at 0, 4, 10, and 22°C.*

costly and time-consuming chemical analysis for product quality determination *(1, 3, 4, 7, 10, 16, 17, 20, 28).*

The primary advantage of using the electronic nose as a quality-assurance tool for the food industry is speed of analysis, in terms of data generation and interpretation. Rapid, meaningful data interpretation is possible with various chemometric (multivariate analysis) techniques. The artificial neural network of AromaScan is data-processing algorithms based loosely on the structure of the human brain. Quality-control models can be developed by "training" the electronic nose, then routine samples can be tested against the model, providing a "goodness of fit" approximation or a sample accept or rejected answer *(3, 4, 7, 16, 17, 20, 28).*

Although the electronic nose has been applied in grading coffee, detecting the adulteration of whiskey and wine, classifying grains and seafood, controlling beer fermentation, and differentiating the quality of aged Parmesan

TABLE 1. *Time-related changes in sensory rating by a sensory panel for tuna fillets stored at different temperatures for up to 9 days*[a]

Defects	Day	Temperature 0°C	4°C	10°C	22°C
Appearance	1	E 1.5 ± 0.1 E[b,c]	E 1.8 ± 0.2 E	E 2.0 ± 0.2 E	E 1.8 ± 0.0 E
	3	FG 3.9 ± 0.4 E	F 3.1 ± 0.2 EF	F 5.2 ± 0.4 F	F 5.8 ± 0.2 F
	5	F 3.6 ± 0.2 E	G 6.0 ± 0.6 FG	H 7.1 ± 0.4 G	G 6.5 ± 0.2 G
	9	G 5.0 ± 0.2 E	G 6.9 ± 0.4 F	G 6.2 ± 0.4 F	ND
Discoloration	1	E 1.5 ± 0.1 E	E 1.6 ± 0.1 E	E 1.8 ± 0.2 E	E 1.8 ± 0.2 E
	3	F 3.5 ± 0.2 E	F 3.1 ± 0.1 E	F 5.2 ± 0.7 F	F 5.4 ± 0.3 F
	5	F 3.2 ± 0.1 E	G 6.5 ± 0.7 F	H 8.1 ± 0.2 G	G 7.1 ± 0.3 G
	9	G 6.0 ± 0.8 E	H 7.9 ± 0.6 F	G 6.6 ± 0.4 E	ND
Texture	1	E 1.9 ± 0.1 E	E 1.7 ± 0.2 E	E 1.9 ± 0.1 E	E 2.0 ± 0.1 E
	3	F 3.8 ± 0.2 E	F 3.4 ± 0.4 E	F 5.1 ± 0.2 F	F 5.9 ± 0.2 F
	5	F 4.1 ± 0.4 E	G 5.8 ± 0.4 F	G 6.1 ± 0.4 F	F 6.3 ± 0.3 F
	9	G 5.5 ± 0.4 E	G 6.6 ± 0.4 E	G 6.1 ± 0.4 E	ND
Odor	1	E 1.6 ± 0.1 E	E 2.0 ± 0.2 E	E 1.9 ± 0.3 E	E 2.0 ± 0.3 E
	3	F 3.7 ± 0.3 E	E 2.7 ± 0.2 E	F 7.0 ± 0.5 F	F 6.3 ± 0.2 F
	5	F 3.1 ± 0.2 E	F 5.5 ± 0.4 F	F 7.2 ± 0.4 G	G 9.8 ± 0.1 H
	9	G 5.4 ± 0.7 E	G 7.2 ± 0.2 F	G 9.1 ± 0.2 G	ND
Overall mean ± SD	1	1.6 ± 0.2 A[d]	1.8 ± 0.1 A	1.9 ± 0.1 A	1.9 ± 0.2 A
	3	3.7 ± 0.2 A	3.1 ± 0.3 A	5.6 ± 0.9 B	6.5 ± 0.5 C
	5	3.5 ± 0.5 A	5.9 ± 0.4 B	7.1 ± 0.8 C	6.8 ± 2.0 C
	9	5.5 ± 0.4 B	7.2 ± 0.6 C	7.0 ± 1.4 C	ND

[a] ND, not determined; SD, standard deviation.

[b] Each rating represents the mean of 10 judgments.

[c] Within each row, means folllowed by the same letter are not significantly different from each other at $P = 0.05$. Within each column, means preceded by the same letter are not significantly different from each other at $P = 0.05$.

[d] A: sensory rating 1–4 (fresh); B: sensory rating 4–6 (initial decomposition); C: sensory rating 6–10 (advanced decomposition, rejected).

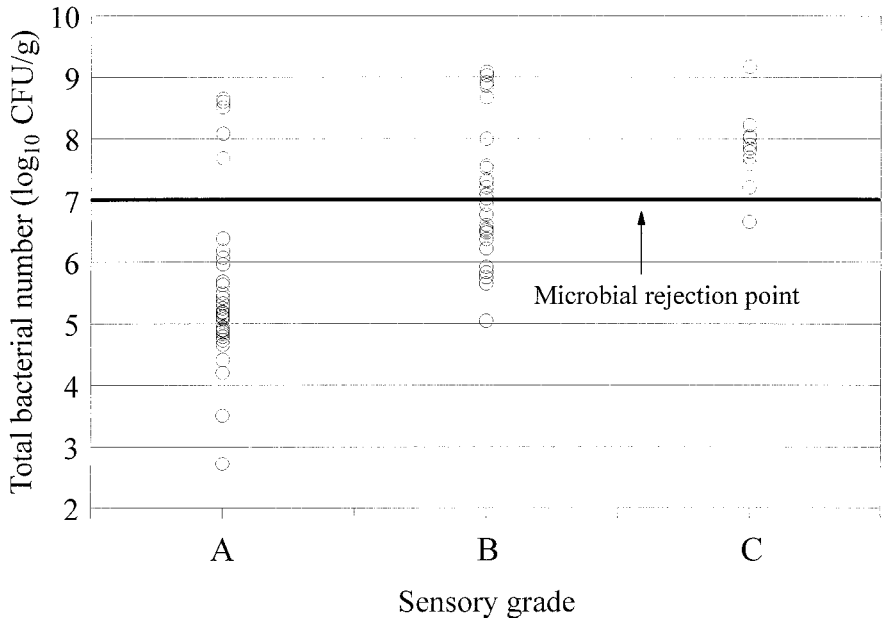

FIGURE 2. *Relationship of sensory grading with bacterial counts in tuna fillets.*

cheese *(1, 10, 14, 15, 19, 22, 23),* AromaScan has not been widely tested with seafood products. Therefore, the objectives of this study were (i) to determine the quality of tuna stored at 0, 4, 10, and 22°C for 0, 1, 3, 5, and 9 days using microbiological assessment and sensory analysis (by a trained sensory panel and an AromaScan), and (ii) to determine the applicability of these methods for quality determination of tuna.

MATERIALS AND METHODS

Tuna preparation and storage. Fresh yellowfin tuna (*Thunnus albacores*) loins of 15 to 20 kg each were purchased from a local seafood store in Gainesville, Fla., and transported in ice to the Food Science and Human Nutrition Department, University of Florida. The outer layers of the tuna loin were then carefully removed with a sterile knife. The loins were cut to prepare 120 pieces of tuna steaks (12 by 10 by 2 cm) of about 250 g each. After each steak was placed in separate sterile, labeled Zipper bags (26 by 28 cm, Tenneco Packaging, Pittsford, N.Y.), the samples were divided randomly into four groups and stored at 0, 4, 10, and 22°C for 1, 3, 5, and 9 days.

At each sampling interval, five pieces of tuna steaks were removed from each temperature group for determination of bacterial counts and sensory quality using a 10-member sensory panel and an AromaScan. Five pieces of tuna steaks were used as day 0 control to determine background bacterial loads and sensory quality, both by the sensory panel and AromaScan.

Microbiological assessment. For total microbial counts, approximately 20-g portions of fish fillets were cut from test samples at each sampling period and homogenized at high speed for 2 min in sterile blenders with nine volumes (1/9, wt/vol) of sterile Butterfield's phosphate buffer, pH 7.2 *(2).* The homogenates were serially diluted with sterile Butterfield's phosphate buffer. Then, 0.1-ml aliquots of the diluents were surface plated on quadruplicate aerobic plate count agar (Difco Laboratories, Detroit, Mich.) plates containing 1.5% NaCl. The pour plate method was also used for some homogenates with anticipated low bacterial counts. Bacterial colonies were counted after the plates were incubated at room temperature for 48 to 72 h *(5).*

Sensory evaluation of tuna fillets by panelists. A 10-point sensory scale for judging progressive changes in the quality attributes of tuna fillets was used by a 10-member panel (three females and seven males), ages 24 to 48 years, from the Department of Food Science and Human Nutrition, University of Florida. Panelists met for two training sessions and used fresh and stale samples to review the attributes and establish a fairly uniform degree of sensory evaluation. All samples were coded with random three-digit numbers. Panelists wearing gloves were requested to evaluate the quality of fish samples by touching, smelling, and visual observation of appearance. They were also instructed to change gloves between samples. Panelists described on the evaluation sheets any differences occurring between tuna fillets of various temperature groups, including appearance defects, discoloration, texture changes, and formation of odor. The degrees of abnormality (or defect) were categorized into slight, moderate, and excessive with intensity ratings of 1 to 10 for each category, where 1 to 4 = fresh, 4 to 6 = initial decomposition, and 6 to 10 = advanced decomposition, respectively, for descriptive terms. The final grading (A, B, or C) was made from the sensory evaluation

TABLE 2. F-*value from ANOVA of sensory ratings; values in parentheses indicate the level of significance*

Source	df	Appearance	Discoloration	Texture	Odor
		F-value (Pr > *F*)			
Day	3	74.57 (0.0001)	96.94 (0.0001)	64.58 (0.0001)	105.18 (0.0001)
Temperature	3	16.22 (0.0001)	25.29 (0.0001)	5.54 (0.0013)	29.50 (0.0001)
Day × temperature	8	4.56 (0.0001)	7.95 (0.0001)	2.77 (0.0072)	16.21 (0.0001)

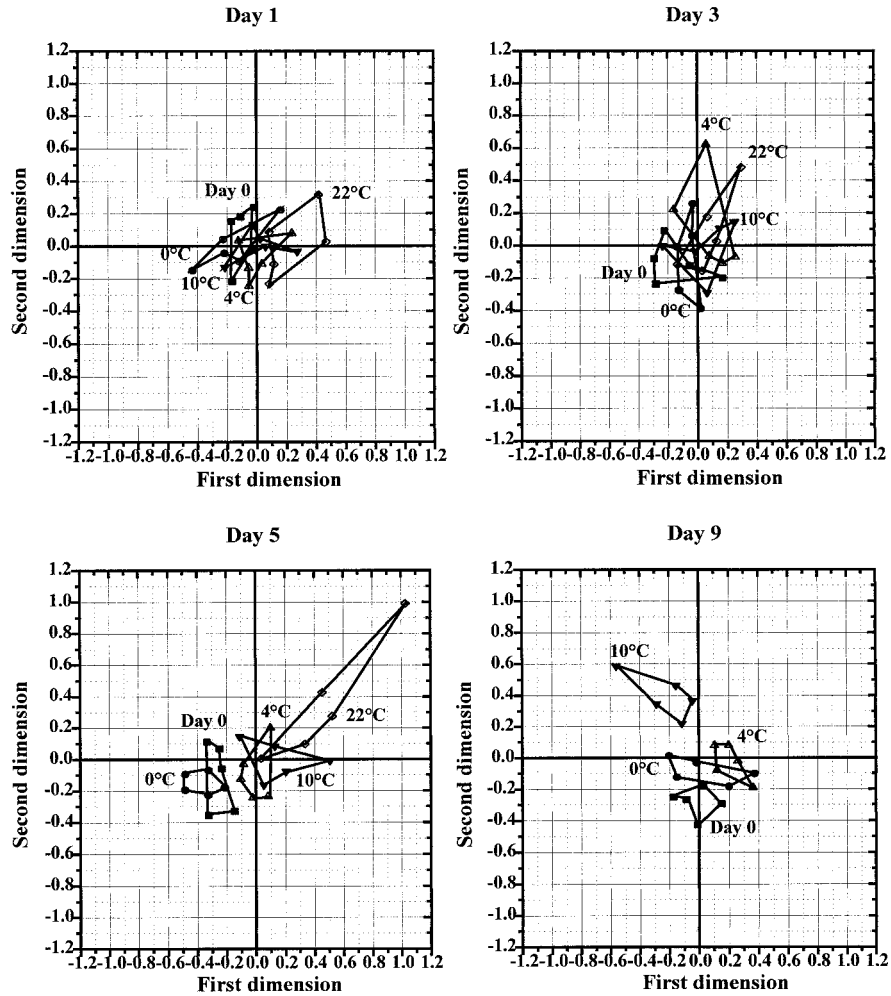

FIGURE 3. *Comparison of AromaMaps for tuna fillets stored at different temperatures on days 1, 3, 5, and 9 of storage (■, day 0; ●, 0°C; ▲, 4°C; ▼, 10°C; ◆, 22°C).*

sheets following those described by the National Marine Fisheries Service's *Fishery Products Inspection Manual (18).*

Determination of fish freshness using AromaScan. An AromaScan equipped with an array of 32 electrically conducting organic polymer sensors was used to determine the freshness of tuna fillets *(7).* Portions (10 g) of tuna samples were placed in an analysis bag, and the bag was evacuated and then filled with carbon-filtered air. The absolute humidity in the bag was 5 g/m^3, while the reference humidity was 8 g/m^3. The headspace of the sample bag was allowed to equilibrate at 35°C for 10 min prior to analysis. 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 about 15 g/m^3) for 30 s. 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% isopropanol and then allowed to react with reference air for 2.5 min before analysis of the next sample. 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.

Statistical analysis. Microsoft Excel for Windows 95 version 7.0 was used to calculate the mean and standard deviation of microbial and sensory data. The General Linear Model procedure prepared by SAS Institute *(25)* was used for analyzing the sensory data. When significance ($P < 0.05$) was determined in the model, means were separated using the least significant difference test *(25).*

Multidimensional data were generated when a sample aroma was analyzed on AromaScan's unique 32 polymer sensor array. The Sammon mapping technique was the statistical method used for AromaScan mapping. This technique reduces the multidimensional original pattern space to a two- or three-dimensional pattern configuration (AromaMap) in order to visualize an easy application to gas and odor discrimination without the loss of pattern data sets. The reduced dimensional data presented in the AromaMap allowed for visual comparison of sample differences or similarities *(24).* Multiple discriminant analysis was also used to process the AromaScan data. Multiple discriminant analysis is a statistical method that enables the reduction of multidimensional data into two or three dimensions, which can then be viewed in a single plot *(8, 11, 26, 27).* Differences between samples are shown by the spatial separation between the clusters.

RESULTS AND DISCUSSION

Microbial analysis. Bacteria grew rapidly on tuna fillets stored at 10 and 22°C (Fig. 1). By day 3, total aerobic counts on these fillets reached 10^7 to 10^8 CFU/g. Tuna fillets stored at 4°C did not have this level of bacterial load until after 9 days, while those stored at 0°C never reached this level. Spoilage characteristics, such as slime formation, occurrence of odor, and a yellow color on tuna fillets, be-

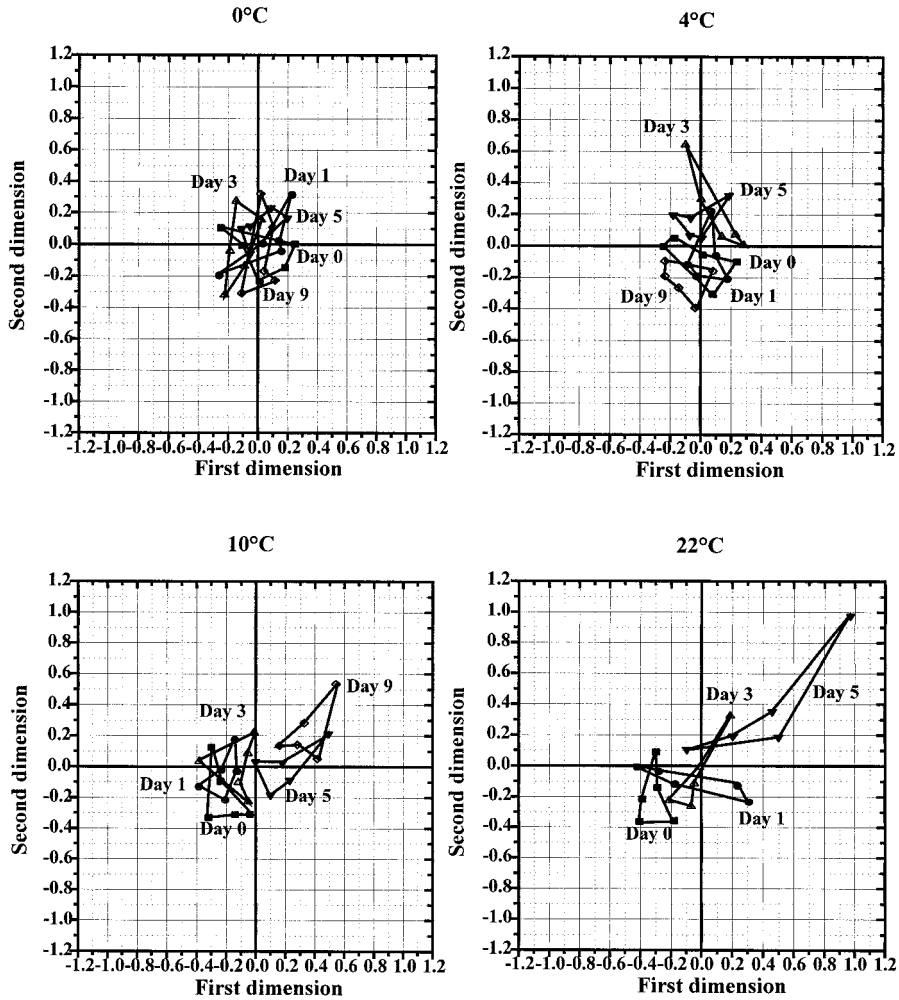

FIGURE 4. *Comparison of AromaMaps for tuna fillets stored at 0, 4, 10, and 22°C for various time periods (■, day 0; ●, day 1; ▲, day 3; ▼, day 5; ♦, day 9).*

came noticeable only when bacterial counts reached 10^7 CFU/g or higher. The International Commission on Microbiological Specifications for Foods *(9)* recommends that good-quality seafood products have three of five samples with an aerobic plate count of less than 5×10^5 and that no sample have an aerobic plate count of more than 10^7 CFU/g. Therefore, tuna fillets should be considered unacceptable and rejected from consumption when bacterial counts reach 10^7 CFU/g or higher.

Sensory evaluation of tuna fillets. Fresh tuna fillets (day 0) with an overall sensory rating of 1.3 had a firm texture with a typical red color and no off-odor (Table 1). The color and odor of the test fillets changed with increasing storage time and temperature. Day 1 fillets stored at all four temperatures had a similar physical appearance and color ($P > 0.05$) as fresh tuna (Table 1). However, by day 3, the fillets stored at 0°C exhibited a slight fish odor and a slight discoloration. Samples stored at 10 and 22°C started to produce putrefactive odors, undesirable color changes, and surface slime. Decomposition was more noticeable in fillets stored at 22°C than at 0 and 4°C. Fish fillets stored at 4°C also showed a slight fish odor and a nice red color by day 3, and they were scored by the sensory panelists as the best fillets among the four temperature groups.

Tuna fillets stored at different temperatures for 5 days showed significant differences ($P < 0.05$) in quality. Fillets stored at 10 and 22°C produced strong putrefactive odors by day 5. They were considered unpleasant and inedible and were thus rejected by panelists. Fillets stored at 4°C for 5 days showed a deterioration in sensory quality for appearance, discoloration, texture, and odor compared to those fillets stored at 0°C. However, the sensory panel still rated the product acceptable for consumption. Tuna fillets stored at 0°C were considered acceptable for consumption even after 9 days.

The increase in sensory scores followed the increase of total bacterial counts (Fig. 1 and Table 1), especially at the point of rejection. When tuna was classified as grade C fillets, their total bacterial counts reached 10^7 CFU/g or higher (Fig. 2). Thus, bacteria played an important role in tuna spoilage. Hence, storage time and temperature play important roles in affecting sensory attributes of tuna fillets. Statistical analysis results (Table 2) showed that storage time, temperature, and their combined action significantly affected the overall sensory attributes (appearance, discoloration, texture, and odor) of tuna fillets.

AromaScan analysis of tuna fillets. AromaScan analysis of tuna fillets stored for 1, 3, 5, and 9 days showed

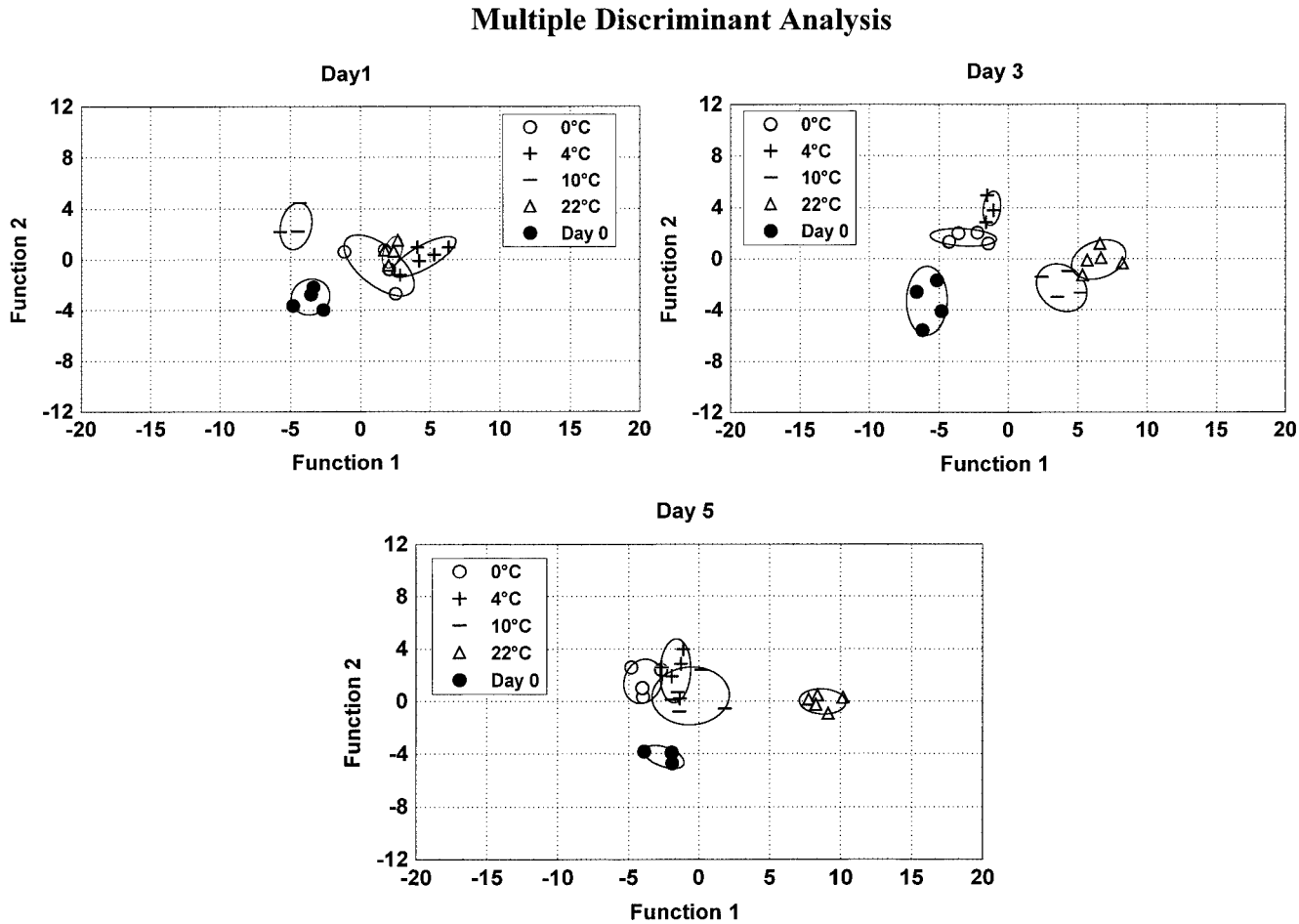

FIGURE 5. *Canonical discriminant graphs for tuna fillets stored at different temperatures for 1, 3, and 5 days. Ellipses around clusters represent 95% confidence bands. Increasing distance between clusters relates to greater dissimilarity between samples.*

that mappings of the fillets stored at 0, 4, 10, and 22°C separated from day 0 controls in a time-related fashion (Fig. 3). As storage time increased, clusters for each temperature separated further from each other. The four temperature clusters from day 1 did not separate from each other, indicating that these tuna fillets from the four groups had similar odor profiles. These findings matched well with the sensory analysis results in which all the fillets from these four temperature groups were classified as grade A products at day 1 (Table 1).

However, day 3 fillets also did not separate from each other, although sensory analysis showed differences in fillet quality among the four temperature groups (Fig. 3 and Table 1). A good agreement was found between the results from AromaScan and sensory analysis with fillets stored at the four temperatures by day 5. The mappings of fillets stored at 4, 10, and 22°C (grades B and C) started to separate from those of the 0°C group and the day 0 controls (grade A) by day 5. Comparing the results from AromaScan and sensory analysis, we found that AromaScan can identify odor differences between grade A fillets and those of grade B and C fillets. Fillets with similar sensory ratings can not be separated from each other on the mappings. A similar trend was found in AromaMap for tuna fillets stored at different temperatures for 9 days. As the difference in the sensory score grew greater, so did the cluster separation in the mappings.

A comparison of the storage time effect on the mappings of tuna fillets stored at 0°C showed that the days of storage did not cause separation of the AromaScan mappings (Fig. 4). This indicates that the quality of the tuna stored at 0°C did not change much during 9 days of storage. This low storage temperature also affected the release of volatiles from test samples for assessment by AromaScan.

The storage time had a more profound effect on mapping when tuna fillets were stored at temperatures higher than 0°C. As the storage temperature increased, samples of different storage time increasingly separated from each other, especially those at the higher storage temperatures for a longer time (Fig. 4). By a comparison of the results from AromaScan and sensory analysis, it was found that AromaScan can be used to identify odor differences between grade C fillets and grade A and B fillets. The mappings of tuna fillets stored at 10°C for 5 or 9 days that had a sensory rating of greater than 6 (grade C) were well separated from those stored for 0, 1, or 3 days and had a sensory rating of less than 6 (grades A

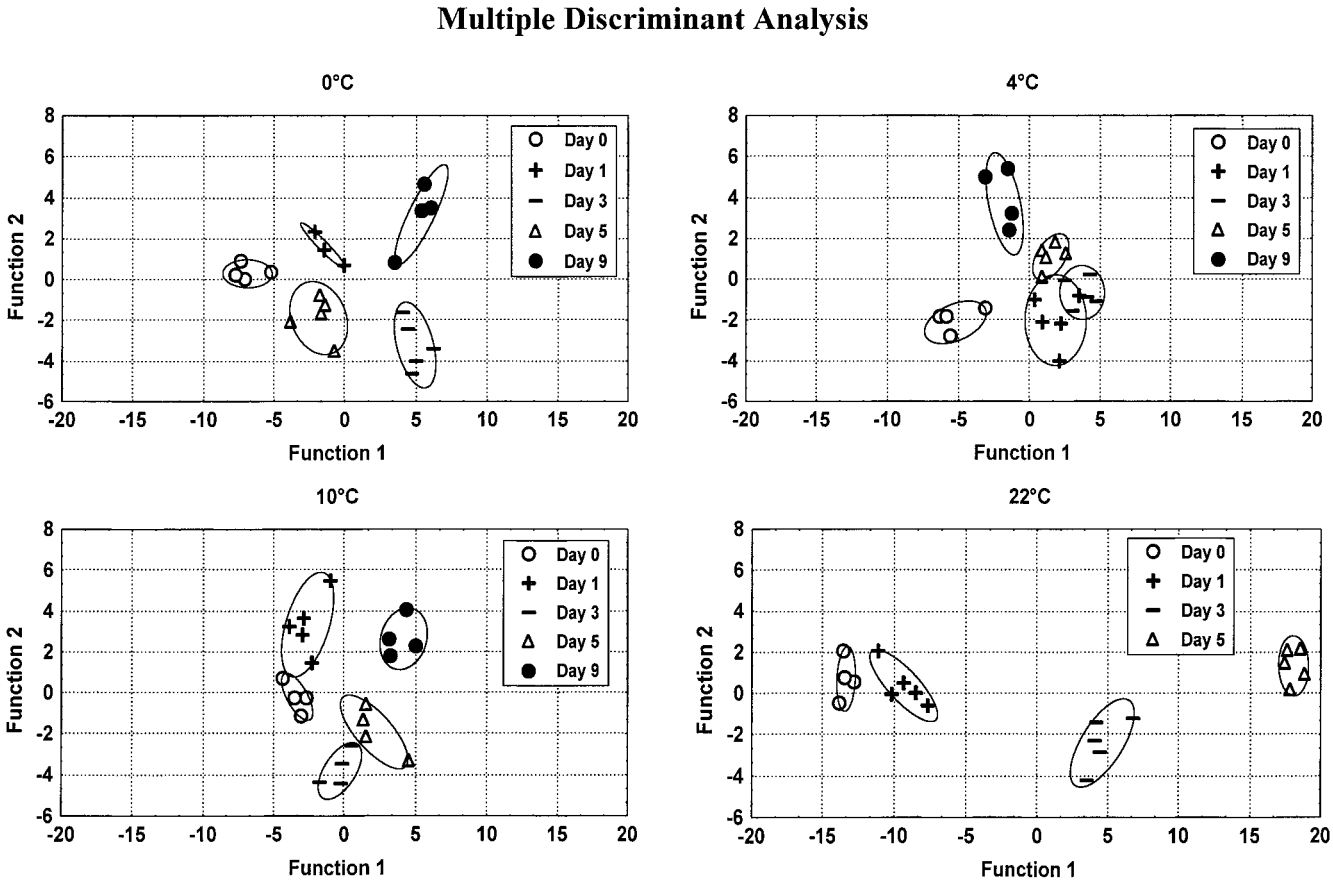

FIGURE 6. *Canonical discriminant graphs for tuna fillets stored at 0, 4, 10, and 22°C for various time periods. Ellipses around clusters represent 95% confidence bands. Increasing distance between clusters relates to greater dissimilarity between samples.*

and B). Thus, AromaScan can be used to differentiate the degree of decomposition in tuna during storage. Good agreement was also found between AromaScan results and sensory analysis of tuna fillets stored at 22°C. Tuna fillets with similar sensory ratings do not separate from each other on mappings. The larger is the differences in sensory scores, and the greater is the separation from each other on the mappings.

Multiple discriminant analysis (in Statistica software version 4.5, StatSoft) was also used to process data obtained from the AromaScan analyses, and these data were compared with the sensory and microbial results. Due to the limitations of this software, only the data from 16 sensors were used. After comparing all the data from 32 sensors, the data obtained from the sensors that showed a greater ability for odor detection were removed from analysis, because they had similar response to the odor profile of fish samples. Only data from the remaining 16 sensors showing a better detection of odor discrepancy were used. In discriminant analysis, the X and Y functions account for differences between different samples rather than replicate to replicate differences (system or method parameters). The results showed that odor profiles from tuna stored at the four temperatures could be differentiated by days and grouped into separate clusters on canonical discriminant graphs (Fig. 5).

Another set of canonical discriminant graphs (Fig. 6) also showed that the clusters for storage time at the same temperature began to separate as storage temperature increased. The clusters of tuna samples showed a temperature-related separation in the plots; the clusters of fillets stored at 22°C had more spatial separation than those stored at 0, 4, and 10°C. A good agreement was found between AromaScan and sensory analysis (odor rating) of fillets stored at 10°C. The canonical discriminant graphs showed that the clusters for day 0 and 1 fillets (with similar odor ratings of 1.3 and 1.9) were closer to each other, while the clusters for day 3 and 5 fillets (with similar odor ratings of 7.0 and 7.2) were closer to each other. However, the clusters of these fillets were further separated from that of day 9 fillets (odor rating of 9.1). This result indicates that AromaScan is capable of differentiating odor changes occurring with tuna fillets during storage.

The AromaScan also differentiated the sensory grade of tuna fillets stored at 4, 10, and 22°C (Fig. 7). A temperature-related relationship occurred with the cluster separation of the sensory grade; the clusters of tuna fillets stored at 22°C were further separated compared to those stored at 4 or 10°C. A good agreement was found between AromaScan and sensory analysis (odor rating) of fillets stored at 4°C. The cluster of grade B fillets did not com-

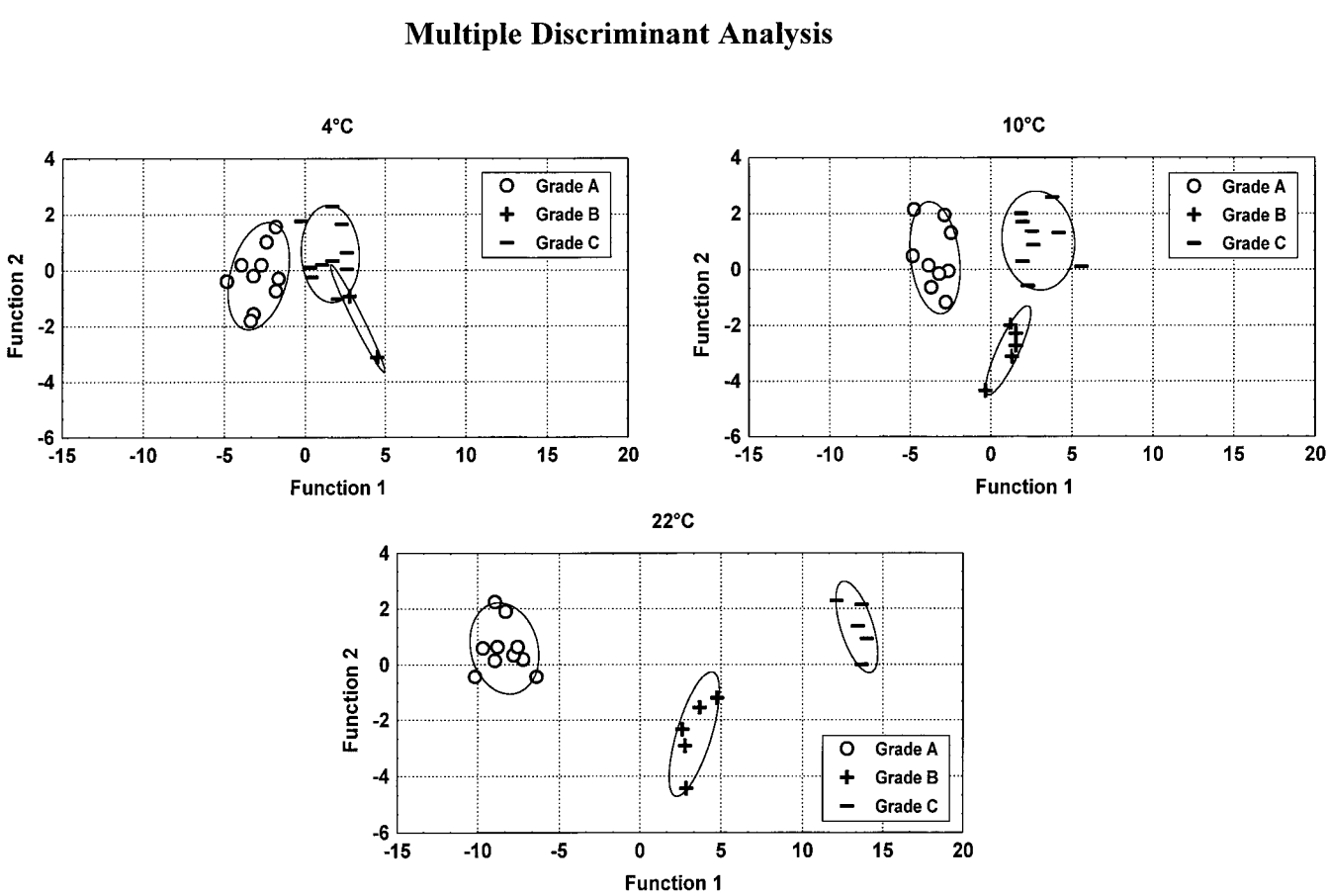

FIGURE 7. *Correlation of AromaScan analyses with sensory grading for tuna fillets stored at different temperatures. Ellipses around clusters represent 95% confidence bands. Increasing distance between clusters relates to greater dissimilarity between samples.*

pletely separate from that of grade C fillets. Sensory data (Table 1) also showed that odors of grade B fillets (5.5) did not show significant differences ($P > 0.05$) from those of grade C fillets (6.3). This result indicates that AromaScan is capable of differentiating sensory grades for tuna fillets at different storage temperatures. Research conducted by AromaScan also demonstrated that the AromaScan analyzer can clearly discriminate among four different classes (fresh, early decomposed, very decomposed, and rancid) of freshness in mahi-mahi and scallops.

The change in quality as detected by AromaScan matched that of microbiological measurements. The clusters of tuna fillets stored at different temperatures with different microbial loads separated from each other in the canonical discriminant graphs (Fig. 8). They also showed a temperature-related separation in the plots; as the temperature increased, the clusters were separated further. The clusters of fillets with similar total bacterial counts were close to each other on the graphs. Thus, AromaScan results can be used as microbial-quality indicators of stored tuna fillets.

CONCLUSION

Storage temperature and microbial counts played important roles in causing spoilage of tuna fillets. As the stored fillets showed time- and temperature-related increases in bacterial loads, they showed deterioration of quality. Therefore, bacterial level is a useful and objective indicator of gross spoilage in tuna. The change of tuna quality as detected by AromaScan matched that of microbiological measurements. AromaScan can be used to indicate the degree of decomposition in tuna during storage. Human sensory study provides a useful means to monitor both changes in freshness and onset of spoilage. AromaScan analysis may provide a viable quantitative approach for determining fish freshness that could be used for quality-control and inspection purposes. It can achieve these goals objectively and in a short analysis time (12 min). It can also be used as a quality-control instrument to assist a sensory panel in evaluating seafood products. AromaScan can be trained with representative aromas and odors from different classes of seafoods, enabling a rapid classification to be made either at the source of purchase or at any point along the supply and distribution channels.

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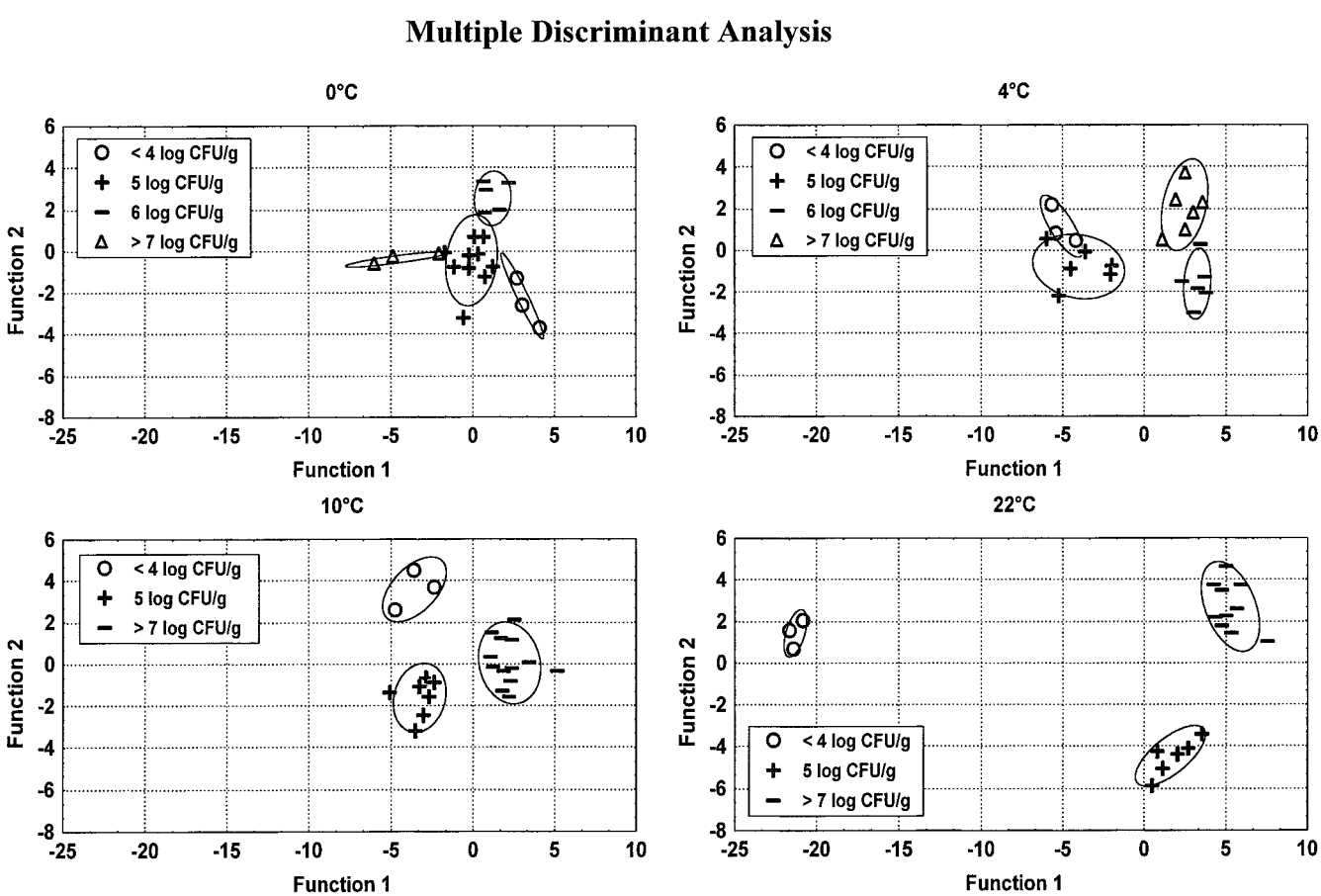

FIGURE 8. *Correlation of AromaScan analyses with total bacterial counts in tuna fillets stored at different temperatures. Ellipses around clusters represent 95% confidence bands. Increasing distance between clusters relates to greater dissimilarity between samples.*

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