

Determination of Quality Attributes of Blue Crab (*Callinectes sapidus*) Meat by Electronic Nose and Draeger-Tube Analysis

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ABSTRACT. In this study, five groups of sequentially spoiled crabmeat were evaluated by a trained sensory panel, and these results were compared with the findings from a Cyranose 320 Electronic Nose and Draeger gas

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Luis F. Martinez and Dr. Murat Balaban from the University of Florida are recognized for recommending the compressed air method. The idea of using compressed breathing air that cycles into sample containers was first developed at the University of Florida. A similar setup was used for this study. Funding for this research was provided by the Virginia Sea Grant Program, the World Food Logistics Organization, the International Association of Refrigerated Warehouses (WFLO/IARW), and the National Fisheries Institute (NFI) Scholarship Program.

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analyzer. Using the electronic nose with filtered compressed breathing air yielded the best results. Although this approach resulted in 100 % separation of the known groups, only 30% of the coded unknown samples were correctly identified. All 5 groups of samples analyzed using Draeger-Tubes were found to be significantly different at $\alpha=0.05$ using a Tukey-Kramer ANOVA statistical procedure. The coded unknown samples were correctly identified at a rate of 83%. The simplicity and precision of this latter procedure may present opportunities for use of Draeger-Tubes by crab processing industries and other food processing industries as an objective method for quality control.

KEYWORDS. Electronic nose, blue crab, Draeger-tube, quality, ammonia, sensory

INTRODUCTION

The blue crab industry represents a major resource for the American seafood industry. The blue crab (*Callinectes sapidus*) is fished commercially from Maryland to south of Galveston, Texas; however the highest population of the species resides in the Chesapeake Bay and its tributaries. From 1968 to 2004 the annual harvest was 73 million pounds in the Chesapeake Bay alone (Bonzek et al., 2005). In recent years the harvests have dipped into the 50 million pound range. However, in 2004, the harvest was approximately 60 million pounds. The 2004 harvest translated to approximately 55 million dollars in revenue. The industry is important to the economy of the Chesapeake Bay region, and care needs to be taken to ensure that over-crabbing, habitat destruction, and other adverse environmental conditions do not lead to extinction of the blue crab.

The spoilage of crustaceans involves metabolic activities of microorganisms and enzymes. All of these processes are interrelated and occur simultaneously or at different stages of spoilage. The mechanism for the chemical breakdown of crabmeat is not as well established as for some other types of seafood, such as fish. However, it is believed the chemical breakdown is relatively similar to that of other shellfish, but not fish, as crabmeat has a higher free amino acid composition compared to fish. High levels of taurine, proline, glycine, alanine, and arginine are found in crustaceans (Martin et al., 1982). Other nitrogenous compounds, such as peptides and other nonprotein nitrogen components found in shellfish,

form typical spoilage metabolites such as indole, ammonia, putrescine, histamine, and cadaverine (Eskin et al., 1971). An increase in tissue ammonia levels during spoilage is attributed to several enzymatic processes: deamination of free amino acids, degradation of nucleotides, and oxidation of amines (Gill, 1990). Since ammonia and other volatile amines have been implicated in seafood spoilage, it is believed that being able to measure their levels relatively quickly and easily would be of great advantage in determining quality levels.

Amines are typically produced in shellfish by the bacterial deamination or decarboxylation of free amino acids. Deamination of amino acids results in the formation of ammonia and organic acids, while decarboxylation leads to the production of amines and carbon dioxide. Breakdown of trimethylamine oxide (TMAO) may also result in the formation of trimethylamine (TMA) and dimethylamine (DMA) in certain seafood products (Martin et al., 1982). These compounds are part of a total volatile base (TVB) analysis, and methods have been derived to determine TMA and DMA concentrations separately (Jones et al., 1998; Yerlikaya and Gökođlu, 2004). In an analysis of cod fish by Gill (1990), the relationship between TMA and subjective evaluation of odor was approximately linear.

Finding an economical way to evaluate seafood quality is difficult. Quality can be evaluated using trained certified sensory experts, but these experts are expensive to train, and periodic recertification is needed if federal and state agencies are to accept them as experts. Sensory evaluation also is expensive and difficult for the industry due to frequent personnel turnover and the extensive time commitments needed to maintain certified sensory personnel.

A possible solution to this problem is to develop a method to determine quality using electronic instrumentation. Possible instrumentation that could be used to determine quality includes the electronic nose. The electronic nose has been used in a variety of applications, including food applications (Arshak et al., 2004), and is a rapid technique with analysis time often taking less than 2 minutes.

The electronic nose uses an array of sensors designed to mimic the mammalian olfactory system's response to aromas. Instead of receptor proteins, which are part of the human olfactory system, the electronic nose in most cases uses a sensor array system. The odor molecules are drawn into the electronic nose using sampling techniques such as head-space sampling, diffusion methods, bubblers, or preconcentrators (Pearce et al., 2003). The sample odors are drawn across the sensor array, which

induces a reversible physical or chemical change in the sensing material, in turn causing a change in electrical properties (Harsanyi, 2000). Each sensor in an array behaves in a similar manner to a mammalian protein receptor responding to different odors to varying degrees. The sensor signal is transduced into electrical signals, which are preprocessed and conditioned (usually a normalization technique is used) before being identified by a pattern recognition system.

Other methods for determining spoilage in seafood products include analyzing for ammonia and volatile amines. An AOAC-approved method for the colorimetric determination of ammonia in crabmeat exists (Steinbrecher, 1973), but this procedure is laborious and time-consuming. Ammonia can also be measured by diffusion, paper test strips, and ammonia selective electrodes, but these methods are often less sensitive compared with the colorimetric method and have interference issues, such as sodium interfering with an ammonia-sensitive electrode. The established methods require an aqueous extract. Volatile amines are also a good indicator of spoilage in seafood as total volatile base (TVB) analysis is often used to determine spoilage. In that regard, ammonia-sensitive Draeger-Tubes[®], used mostly for the detection of ammonia gas leaks, may work as a rapid analytical test to determine spoilage in crabmeat. The tubes have cross-sensitivities with other basic substances, such as organic amines that are likewise indicated but have differing sensitivities (Dräger-Rohrchen, 2001). Since a no-specific test has previously proved somewhat effective (TVB analysis), the cross sensitivity to other amines may be advantageous for determining spoilage in shellfish.

MATERIALS AND METHODS

Blue Crab Samples for Electronic Nose and Draeger-Tube Analysis

Experiments were conducted with body meat (i.e., regular or special) obtained from a local crab processing company (Hampton, VA, USA) and harvested from the Chesapeake Bay during the same harvest season. Eight separate batches of crabmeat were spoiled (136–181 kgs/batch) and used to train the sensory panel. One of these spoilage batches was also used to train the electronic nose and one was used to establish the standard curve for the Draeger-Tube analyses. To validate the electronic nose and Draeger-Tubes, an additional batch of crabmeat was sequentially

spoiled and used to verify the accuracy and precision of the analytical methods. The batches were separated into individual spoilage increments (18 kg/increment) and then vacuum packed in Kapak (Minneapolis, MN, USA) SealPac pouches, 0.47 liter (2.5 mil PET/LLDPE). Reference (0 day) samples were first frozen at -18°C for 12–20 hours and then stored at -80°C . The remaining samples were sequentially spoiled at ambient (21°C) and refrigerated storage conditions (4°C), frozen at -18°C for 12–20 hours, and then stored at -80°C until needed for analysis. The following samples were analyzed by the electronic nose: reference (0 day); 6 and 9 h ambient; and 7, 8, and 9 day cold samples. Draeger-Tube (Dräger-Röhrchen, Luebeck, Germany) analyses were conducted on reference samples (0 day) and samples held for 4, 5, 6, and 7 days at 4°C . Samples were thawed overnight in a refrigerator (4°C) before analysis.

Microbial and Sensory Analysis

Fresh-picked crabmeat was collected from the processing plant and held on ice at refrigeration temperatures ($< 4.4^{\circ}\text{C}$). The initial bacterial load was enumerated by mesophilic aerobic plate count using standard AOAC-approved methods as listed in the FDA Bacteriological Analytical Manual (FDA, 2001). Twenty-five gram portions of well-mixed crabmeat were blended with 225 ml sterile 0.1 % Peptone buffer in a filter stomacher bag and stomached for 2 min at 230 RPM for a 1:10 dilution. Subsequent dilutions were prepared by adding 1 ml to 9 ml of sterile Peptone broth. One ml portions were plated on 3M Petrifilm aerobic count plates and incubated at 35°C for 48 hours. Aerobic plate counts (APC) were reported as colony forming units per gram (CFU/g).

Crabmeat was divided into 5 lb. increments and spread into 10 by 16 in. lidded plastic containers for controlled decomposition at 21°C (ambient temperature) or 4°C (refrigerated storage). Crabmeat was periodically examined for sensory quality, packaged in 100–200 gram portions in Kapak 402 bags (1 pt. size, 6 1/2 in. by 8 in.), vacuum sealed, labeled, and frozen overnight at -18°C before placement at -80°C for long-term storage. Fifty grams were used for bacteriological examination, as described above, for each spoilage increment. Reference samples were immediately bagged, frozen, and stored.

Spoiled crabmeat was evaluated for odor, taste, and texture against reference samples using a 6-member trained sensory panel. The sensory panelists underwent training for 8–12 months using the Spectrum™ Descriptive Analysis Method (Meilgaard et al., 1999) to evaluate the

attributes of coded unknown blue crabmeat samples spoiled under refrigerated (4°C) and ambient conditions (21°C). A 10 cm line scale was used to evaluate the sensory attributes of the crabmeat samples with a score higher than 5.0 cm classified as a fail. Samples with scores higher than 2.5 cm but less than 5.0 cm were classified as borderline pass (mid-pass). Similarly, samples with scores lower than 7.5 cm but higher than 5.0 cm were classified as borderline fail (mid-fail). Samples were removed from -80°C storage and thawed overnight in a refrigerator (4°C). Packages were opened and the crabmeat was placed into 2 oz. lidded cups. The cups were heated for 20 seconds at 50% power in a 1000-watt microwave oven and immediately served to panelists. Reference samples representing different food intensity attributes were provided to the panelists prior to evaluating the crabmeat samples. Sensory and microbiological analyses were conducted at the Virginia Seafood Agricultural Research and Extension Center, Hampton, VA, USA.

Electronic Nose Analysis

Thawed 25-gram samples were placed into individual sealed 200-ml glass jars (Ball Corporation, Broomfield, CO, USA), which then were placed in a water bath at 40°C for 30 minutes. All samples reached equilibrium temperatures to allow headspace odors to accumulate.

Manufacturer's Recommended Method

Electronic nose analysis used individually sealed glass jars for headspace analysis. A hole was punched into the lid top to allow an entry point for the insertion of the electronic nose sniffing needle. A small piece of sticky foam (Darice Inc., Strongsville, OH, USA) was purchased from the local craft store and used as a septum. The electronic nose used for this analysis was a Cyranose320™ (Smiths Detection, Pasadena, CA, USA), equipped with 32 polymer-conducting sensors. Ten samples of known quality crabmeat were used to build the training set for each class, and 20 separate coded crabmeat samples were analyzed to validate the system. The operating conditions used were described in the manual. The settings used were as follows: the baseline purge 10 seconds, sample draw one 25 seconds, first air intake purge 10 seconds, and second sample gas purge 75 seconds. Digital filtering and normalization were used; the substrate heater was set to 45°C. Pump speed was set to high (180 cc/min). After analysis of each sample, a 1-min equilibration period allowed the sensors to equilibrate before being exposed to another sample.

Compressed Air Method

Compressed tank breathing air (Airgas Mid America, Radford, VA, USA) filtered through activated carbon and moisture traps cycled through a reference and sample container was used. One of the outlets on the reference container was connected to the purge inlet of the electronic nose to ensure the nose was receiving clean, dry air that was consistent on a day-to-day basis. The flow rate through this apparatus was 15 ml/sec. A tube connected the sample inlet of the electronic nose to the sample container. Ten samples were used to build the training set for each class, and 20 coded crabmeat samples were used to validate the system. Samples were not reanalyzed. The inside of the sample container was cleaned between samples. The settings used were as follows: the baseline purge 15 seconds, sample draw one 30 seconds, first sample gas purge 10 seconds, first air intake purge 10 seconds, and second sample gas purge 30 seconds. Digital filtering and normalization were used; the substrate heater was set to 45°C. Pump speed was set to medium (120 cc/min). After each sample analysis, a 2-min equilibration period allowed the sensors to equilibrate before being exposed to another sample.

Crab Reference Method

The same settings used for the compressed air method were used for the crab reference method. The reference (0 day) crabmeat sample was placed in the reference container connected to the purge inlet. The flow rate through the apparatus was 15 ml/sec. Ten samples were used to build the training set for each class, and 20 coded crabmeat samples were used to validate the system. Samples were not reanalyzed. Both the sample and reference containers were cleaned between samples. A 2-min equilibration period allowed the sensors to equilibrate before being exposed to another sample.

Draeger-Tube Analysis

Samples were thawed in the refrigerator (4°C) overnight. Samples were brought to 40°C in a bag using a water bath. Bags were stomached for 1 min at 230 RPM. The 25-gram samples were then transferred to a sealed glass jar. Headspace was allowed to accumulate for 10 min. Samples were then analyzed using 2–30 ppm, or 5–70 ppm ammonia-sensitive Draeger-Tubes. The Accuro® (Dräger-Röhrchen, Luebeck, Germany) gas detector pump was used to pull 100 ml of sample through the short-term

detector tubes. Ten known samples were used to construct a standard curve using 2 replications for each spoilage group. Then validation of the trial was performed using unknown samples.

Statistical Analysis

Electronic nose data was processed using the PC Nose software version 6.5 (Smiths Detection, Pasadena, CA, USA), JMP IN 5.1 (SAS Institute, Cary, NC, USA), and SAS 9.1 (SAS Institute, Cary, NC, USA). The built-in PC Nose software contains a program that automatically runs canonical and principal component analysis (PCA). In addition, the raw sensor response data from the electronic nose was processed using statistical software (JMP and SAS). Canonical discriminant analysis (CDA) and stepwise discriminant analysis (SDA) were performed using SAS products. For all three methods, the following sensors were omitted from the model for stepwise discriminant analysis: 4–6, 11, 14–16, 19, 21, 22, 24, 26, 28, 30, and 31. Stepwise variable selection was done to eliminate sensors that showed correlation with other sensors (data) in the model. Analysis of variance (ANOVA) for the Draeger-Tube results was performed using JMP.

RESULTS AND DISCUSSION

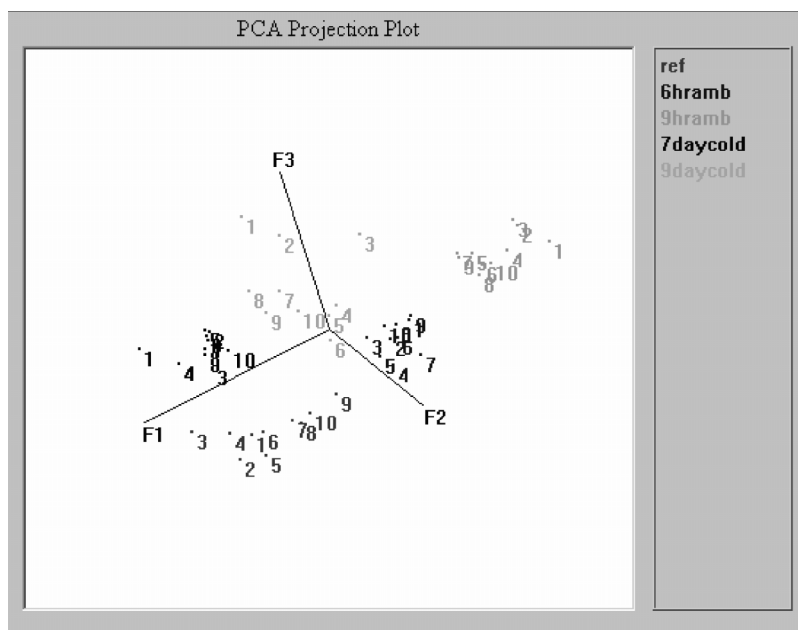
Electronic Nose Analysis

The electronic nose has been used for food quality applications (Chantarachoti et al., 2006; Hu et al., 2005; O'Connell et al., 2001). Van Deventer and Mallikarjunan (2002) found the Cyranose 320 conducting polymer electronic nose to be superior to metal oxide and quartz crystal microbalance systems, which is why this particular electronic nose was selected for this study. For the compressed air and crab reference methods, filtered, clean, dry air from the same tank and a filtering procedure were used. The manufacturer recommends using ambient air for the test; however, it is possible that results can be affected by the varying overall quality and composition of ambient air on a daily basis. It was hypothesized that using filtered, clean, dry air rather than ambient laboratory air may provide more accurate results by eliminating sensor drift, thus achieving a more stable baseline. In addition to being able to classify differences between groups through electronic nose training procedures, validation of the model is needed in order to demonstrate the accuracy

and precision of the electronic nose and Draeger-Tubes. This was accomplished by using known samples from multiple spoilage trials to train the electronic nose and to establish the standard curve for the Draeger-Tube analyses. The final 2 spoilage trials used coded samples to validate the electronic nose and Draeger-Tube analyses, respectively.

The compressed air method achieved 100% correct separation of all 5 groups of sequentially spoiled crabmeat as verified by a cross-validation technique (see Figure 1). This technique treats each individual sample as an unknown and then re-enters it into the statistical model. A cross-validation statistic of 100% shows each sample belongs to only one group. A poor cross validation statistic means that one or more samples can be included in multiple groups, thus decreasing the ability to distinguish between groups. For the compressed air method, all groups were significantly different at $\alpha = 0.05$ using CDA and SDA. In fact, all groups were significantly different ($p < 0.0001$). Separation of different known samples was excellent using the compressed air method, but identification of coded

FIGURE 1. PC nose PCA projection plot for training set data for the compressed air method.



samples was not as accurate. Only 30% of the coded samples were correctly identified using CDA and SDA models. The PCA model was even less effective at correctly identifying coded samples; only 20% of the coded samples were correctly classified using this model (see Table 1).

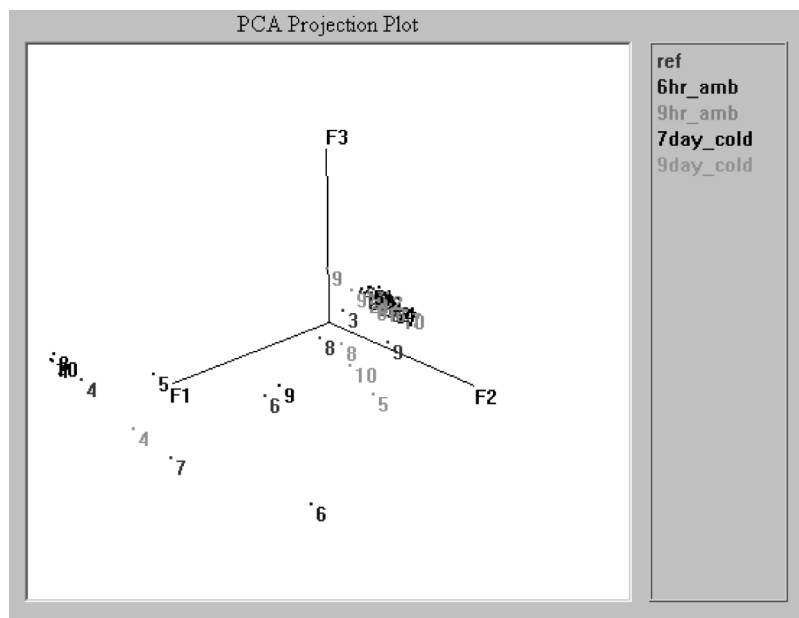
The manufacturer's recommended method correctly separated 30% of known sample groups using PCA analysis (see Figure 2). Better separation was achieved using CDA analysis (Table 1). Correct group separation was improved to 44% using CDA analysis, and 50% using SDA analysis, respectively. Classification of coded samples of crabmeat was low using the manufacturer's recommended method. SDA analysis yielded the best results, correctly identifying 25% of coded samples. PCA and CDA analysis correctly identified only 10% and 20%, respectively, of the coded crabmeat samples.

The crab reference method used reference crabmeat (high quality meat) as a baseline for comparison of spoiled samples to unspoiled samples. Only 50% of the samples were correctly separated using PCA as the statistical method. CDA was able to correctly separate 68% of the groups, while SDA was able to correctly separate 92% of the groups at $\alpha = 0.05$. CDA eliminates sensors that show correlation with other sensors from the model; in this case using this statistical method seemed to improve group separation. Correlation between electronic nose sensors was most likely high due to the samples analyzed having similar attributes. Sensory scores indicated that degrees of spoilage between samples were similar; however there is no tangible way to link the electronic nose sensor response to sensory data because the relationship of specific chemical odorants to specific sensors is proprietary. However, the crab reference method should

TABLE 1. Comparative results for electronic nose studies

Setup	Statistical Method	Group Separation (%)	Correct Identification (%)
Manufacturer's Recommended	PCA	30	10
	CDA	44	20
	SDA	50	25
Compressed Air	PCA	100	20
	CDA	100	30
	SDA	100	30
Crab Reference	PCA	50	20
	CDA	68	35
	SDA	92	20

FIGURE 2. PC nose PCA projection plot for training set data for the manufacturer's recommended method.



have accentuated differences due to spoilage, since spoilage attributes should have been the only differences between the samples. For the coded crabmeat samples, CDA classified 35% of samples correctly. The PCA and SDA results were less accurate, correctly identifying only 20% of the coded samples. It was expected that SDA analysis on the coded crabmeat samples would result in the highest correct classification method, since for the previous analyses, separation and identification of unknown samples were highest using this statistical method.

The compressed air method had the highest success rate in separating and identifying the coded samples. The manufacturer's recommended analysis procedure produced the poorest results for separation and identification of the coded crabmeat samples. These results suggest that methods using clean dry tank air will produce better group separation. However, low rates of correct classification of coded samples were also obtained using clean, dry, filtered air.

ANOVA analysis and declassification of some of the groups were also conducted. ANOVA analysis of individual sensors in the 32-sensor array was conducted to see if one sensor could be used to determine spoilage

levels. In these analyses, one sensor was not more effective than PCA, CDA, or SDA for correctly identifying spoilage levels. Another technique employed was to group together the 6 and 9 h ambient samples, the 7 and 9 day cold samples, and reference samples (0 day) forming a 3-group model. Sensory evaluations indicate that the ambient increment and cold spoiled increment had similar attributes regardless of spoilage time (see Table 2). However, combining the groups did not considerably improve the accuracy of the unknown identification results. Water may have interfered with the sensors, confounding the results. When the water-sensitive sensors, numbers 5, 6, 23, and 31 according to The Practical Guide to the Cyranose 320™ (Cyrano Sciences, 2001), were omitted from the model, group separation was still poor. Perhaps these sensors are also sensitive to the polar components present in the aroma profile of spoiling crabmeat.

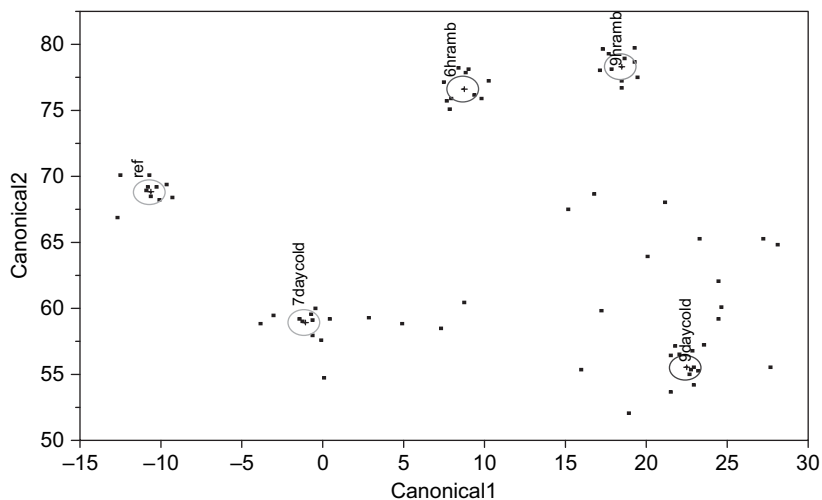
In order to gain insight into why the coded sample results differed from the training data using known samples, the coded sample results were plotted using the training data of the compressed air method (see Figure 3). Most of the coded samples were identified as low quality even though high quality (0 day) samples were present in the coded sample set. None of the 0 day samples were correctly identified. There may have been a “hold over” affect from the more spoiled samples that affected the results of the less spoiled samples, even though the sample containers were cleaned after each trial and the sensors were purged according to the prescribed settings of the Cyranose 320 manual. Since samples were of similar quality (according to microbial and sensory analysis) for both known and unknown samples, differences between the training data and the coded sample data may be due to deficiencies in the sensor purging system of the electronic nose.

The electronic nose may not have been sufficiently sensitive to distinguish between minor differences between classes of spoiled crabmeat.

TABLE 2. Aerobic plate counts and sensory results for samples used for the Cyranose 320™ analysis

Sample	APC (CFU/g)	Sensory Classification
Reference (0 day)	$9.2 \cdot 10^4$	High pass
6 hr. ambient	$4.6 \cdot 10^5$	Mid-pass
9 hr. ambient	$1.0 \cdot 10^6$	Mid-pass/low pass
7 day cold	$7.5 \cdot 10^6$	Low fail/mid-fail
9 day cold	$4.0 \cdot 10^7$	Mid-fail/high fail

FIGURE 3. Compressed air method CDA plot with coded unknown samples plotted.



The crabmeat samples were of varying degrees of spoilage, which to the electronic nose may not have been perceived as a noticeable difference. Ammonia, which is associated with crabmeat spoilage, is soluble in water. This may have led to ammonia not being at a detectable level by the electronic nose, since crabmeat also contains an appreciable amount of water. In addition, no recommended calibration procedure exists for this electronic nose. It is also possible that as the conducting polymer sensors age, sensor drift may be an issue with electronic nose devices (Hines et al., 1999). These issues have been shown to occur when the electronic nose has been used to quantitatively identify components (Harper, 2001). This study is similar to a quantification study, since as spoilage increases, the intensity of spoilage attributes (odors) increase.

Suggestions for improving the electronic nose results include using fewer sample groups, bootstrapping, and neural networks. Instead of using 5 groups for training, use a high quality group and a low quality group. Bootstrapping is an approach best utilized for validating a given model, especially when a small sample size is used. Bootstrapping creates a larger sample size by using real data to create imaginary data. For example, 30 real observations can create 1000 "bootstrap" observations. This approach is best when the error in analysis is attributed to small sample

size. The bootstrap method was used in a study of beef spoilage, and it improved the classification accuracy from 87% to 98% (Balasubramanian et al., 2004).

Artificial neural networks are mathematical creations inspired by the biological nervous system. An artificial neural network consists of a lattice of information processing elements called neurons, which are connected together in a certain way (known as the architecture). The strengths of these connections are called synaptic weights. These weights are determined either during a training (or learning) phase for supervised neural networks or by an algorithm for unsupervised neural networks (Gardner and Bartlett, 1999). Therefore, a neural network can “learn” as data is imputed in, whereas a pattern recognition technique simply looks for patterns. The use of neural networks significantly improved pattern recognition for electronic nose systems (Zhang et al., 2003) and was found to be effective for quantifying different spice mixture compositions (Zhang et al., 2005). Due to time constraints, this technique was not applied to this data set.

Draeger-Tube Analysis

The data from the sequentially spoiled samples was plotted in a standard curve, using an average of 2 replications for each group (see Figure 4). These data show an expected pattern: as the product spoils, the ppm value for ammonia increases. These data fit an exponential equation rather than a linear equation. This is not surprising since microbial growth follows an exponential equation (Marr, 2000). ANOVA analysis was conducted on the Draeger-Tube results (see Figure 5). Means were separated using a Tukey-Kramer statistical test.

Coded samples were analyzed using the Draeger-Tube method (See Table 3). Correct classification of 83% of the coded samples was achieved. Code 411 was the only sample totally misclassified—the sample was a 7-day cold sample, but was classified as a 6-day cold sample by ANOVA and the standard curve. Codes 247 and 302 were not significantly different from the 0-day cold or 4-day cold samples according to Tukey-Kramer ANOVA analysis (See Figure 6). These two samples were classified into either group. These results are not surprising, since the sensory analysis and aerobic plate counts showed that the 0-day cold and 4-day cold samples were quite similar (see Table 4). Sensory analysis classified the 0-day cold and 4-day cold samples as having attributes associated with a mid quality sample. When spoilage was estimated using the

FIGURE 4. Ammonia standard curve. Both linear and exponential equations are plotted on the same graph. The exponential equation better fits the data as is reflected in the R^2 value.

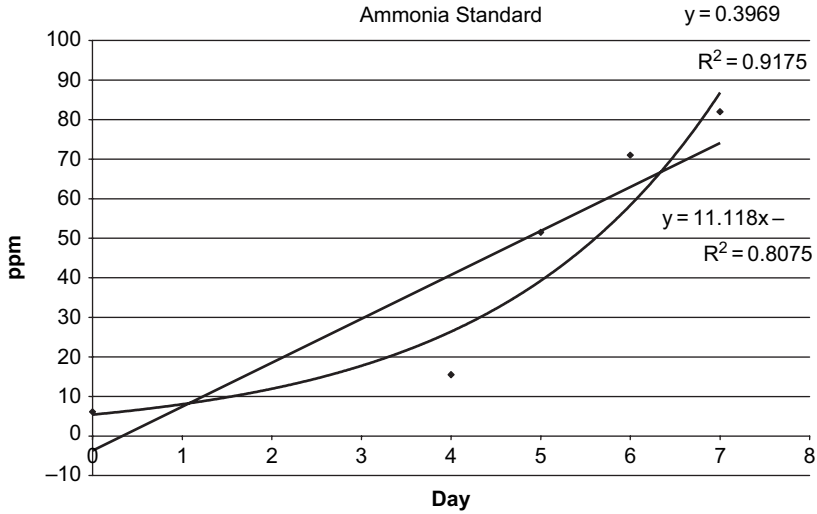


FIGURE 5. Ammonia data analyzed by the Tukey-Kramer test for One-Way ANOVA. All groups were found to be significantly different at the $\alpha = 0.05$ level.

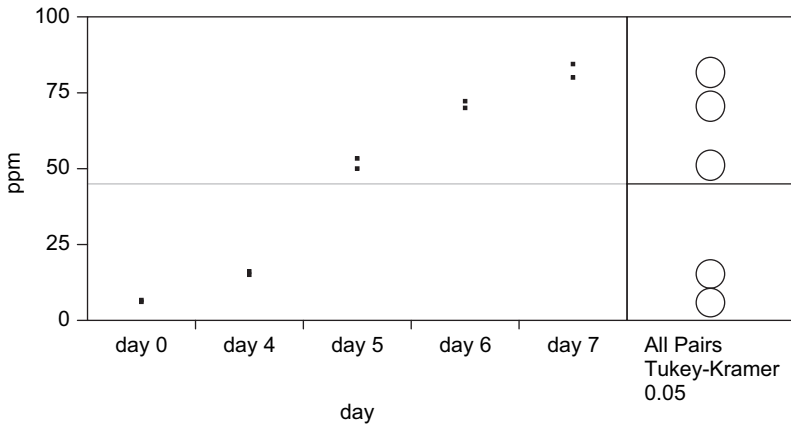
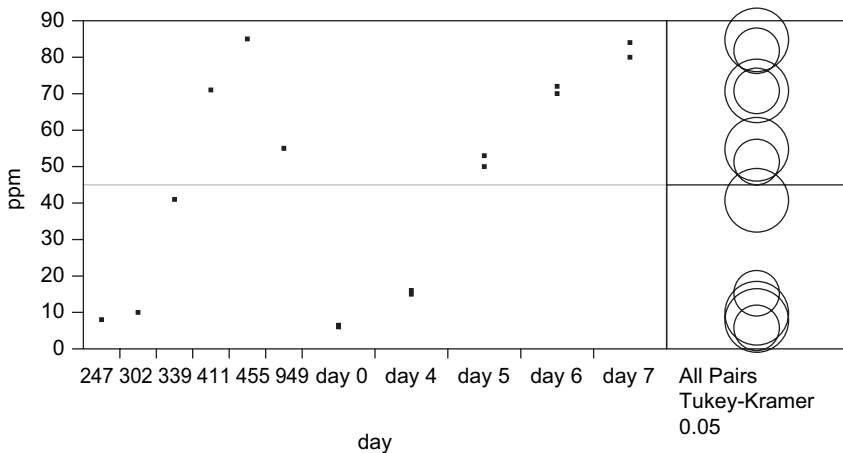


TABLE 3. Draeger coded unknown results

Code	Parts Per Million (ppm)	Estimated Day (Standard Curve)	Actual Day	ANOVA Classification (Day)
411	71	6.4	7	6
949	55	6.0	5	5
247	8	3.1	0	0/4
455	85	6.6	7	7
339	41	5.6	5	5
302	10	3.4	4	0/4

Parts per million (ppm) values were determined by Draeger-Tubes analysis and standard curve (exponential) was used to compute the estimated day. JMP IN was used for the ANOVA classification

FIGURE 6. Ammonia data analyzed by the Tukey-Kramer test for One-Way ANOVA with coded unknowns. A means comparison analysis was performed on this data and the statistical procedure identified into which spoilage day each coded unknown should be classified.



standard curve, code 247 was estimated to be a 3.1-day sample (closer to 0 day), and code 302 was estimated to be a 3.4-day sample (closer to a 4 day), thus corresponding closely with the actual spoilage increment. Most importantly, both samples were classified correctly as “pass.” What is important about this method is the sensitivity of the Draeger-Tube

TABLE 4. Aerobic plate counts (APC) and sensory results of samples used for Draeger-Tubes analysis

Sample	APC (CFU/g)	Sensory Classification
Reference (0 day)	$1.65 \cdot 10^5$	Mid pass
4 day cold	$8.5 \cdot 10^5$	Mid Pass
5 day cold	$1.6 \cdot 10^6$	Low pass
6 day cold	$3.2 \cdot 10^6$	Low pass/low fail
7 day cold	$1 \cdot 10^7$	Mid fail/high fail

method compared to the electronic nose method. This method may be sufficiently sensitive (at least for crabmeat) to separate crabmeat quality into different categories. Thus, the coded samples 247 and 302 were considered to be correctly classified.

The Draeger-Tubes rely on a simple chemical reaction. The tubes contain a pH indicator. The reaction principle is that as NH_3 (a base) is exposed to the pH indicator, a color change occurs due to a rise in pH, demonstrated by a change in color from yellow to blue inside the tube. Perhaps this reaction can still be used, but it may need to be modified to develop a dipstick test that industry could easily use. The Draeger method is simple and more rapid compared with the chemical determination of ammonia and total volatile bases. The technology may also be easily transferable to industry because many seafood processors already use Draeger-Tubes to check for ammonia refrigerant leaks.

Comparison of Draeger-Tube and Electronic Nose Methods

It is important to note that the training samples and the samples used for validation (coded unknown samples) were from 2 different batches. The sample groups (0, 4, 7 day cold, etc.) for training and validation were of the same or similar spoilage time, but they were from different crabmeat batches. Duplicate samples were run during training and validation and yielded different results using the electronic nose (refer to Figure 2). Draeger-Tube and sensory analysis were able to recognize similar spoilage groups across different batches of crabmeat; however the electronic nose often misclassified or even classified replicate samples into different spoilage groups. This suggests that the Draeger-Tube procedure was more robust than the electronic nose under conditions of use in this study.

CONCLUSIONS

The results for the coded validation samples indicate that if the Cyranose 320 electronic nose is used as a quality indicator for crabmeat, improvement in its technology is needed. The results indicate that the Draeger-Tubes show promise for determining spoilage levels for crabmeat and could be used as a quality control procedure by industry. More research is needed to determine if the method works across many batches, seasons, environments, and species.

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