Instrumental Methods for Determining Quality of Blue Crab (*Callinectes sapidus*) Meat

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By

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ABSTRACT

The purpose of this study was to find an alternative instrumental method to sensory analysis and to further investigate the aroma properties of spoiling blue crab meat. This was accomplished by use of a Cyranose 320™ Electronic Nose, Draeger-Tubes®, and solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS). These techniques were compared to the more established techniques for determining quality of blue crab meat of sensory and microbiological analysis. Three different electronic nose methods were used to evaluate five sequentially spoiled groups of crab meat. The manufacturer's recommended setup only resulted in a 30 % correct separation of the known groups, and only 10 % of the samples were correctly identified when coded unknown samples were used to validate the electronic nose training results. The compressed air method which utilized compressed tank breathing air, filtered through activated carbon and moisture traps resulted in 100 % separation of the known groups, but only correctly identified 20 % of the coded unknown samples. Draeger-Tubes® were found to be more accurate and precise compared with the electronic nose. 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 rapidness of this procedure allows it to possibly be an alternative for the crab industry as an alternative to sensory analysis.

SPME-GC-MS found trimethylamine (TMA), ammonia, and indole to best correlate with spoilage of blue crab meat. TMA was found to be sensitive to the minor changes in the early

stages (0 - 4 days of refrigerated storage) of spoilage for blue crab meat. Indole corresponded well with sensory results, which suggests that indole may be a promising indicator for detecting early, mid, and highly spoiled samples. It is feasible that these methods can be applied to other crustaceans to determine spoilage level.

ATTRIBUTION

Author Paul J. Sarnoski is the major contributor and writer of the manuscripts in chapter 3 and chapter 4 of this thesis. Co-author Dr. Michael L. Jahncke, Ph.D., Food Science, Cornell University, 1985, provided advice, supervision, funding, and laboratory support. His group obtained the blue crab meat samples, and conducted the sensory and microbiological analyses. Co-author Dr. Sean F. O'Keefe, Ph.D., Food Technology, Iowa State University, 1988, provided advice, supervision, and laboratory support. Dr. O'Keefe helped with method development for the studies described in chapter 4. Co-authors Dr. Parameswarakumar Mallikarjunan, Ph.D., Food Engineering, University of Guelph, Canada 1993, and Dr. George J. Flick, Jr., Ph.D., Food Science and Technology, Louisiana State University, 1969, provided advice and support for this project.

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CHAPTER 1

INTRODUCTION

I. Overview of the Blue Crab Industry and Harvestable Stock

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. Blue crab populations have remained fairly steady until 1999, when a significant decrease in population occurred. This may have been due a variety of issues including over-crabbing, habitat destruction, environmental conditions (e.g. winter duration), pollution of the bay and estuaries, growing development of the land in the bay and watershed etc. (Ju and others 2003; Prager and others 1990; Weis and others 1992).

In the Chesapeake Bay alone, harvest value during the 1990s averaged more than 50 million dollars per year (B-SBCA Committee, 2000). However, from 1999-2003 population growth dropped to historically low levels due to several factors such as habitat destruction, coastal development, overfishing, etc. Surveys estimated that in 1999, seventy two percent of the blue crab population was removed from the bay (B-SBCTA Committee, 2006). Since this period stringent over-crabbing regulations have been put in place. As a result crab populations in the bay have started to recover. According to the National Marine Fisheries Service (NMFS) database, blue crab landings from the Chesapeake Bay totaled 58.3 million pounds and 58.6 million pounds of blue crab in 2004 and 2005, respectively. This translated to 50 and 51 million dollars in revenue in 2004 and 2005 respectively. Although, it seems that the crab populations are on the rise, they are still significantly lower than the (1968-2004) average of 73 million pounds (Bonzek and others 2005).

The recent decline in crab landings seems to suggest that in the past a higher number of adult crabs were present in the adult stock, whereas now harvest seems to be dependent more on spawning season. The increased variability between year to year harvests also seems to suggest this trend. For this reason the blue crab stock needs to be managed closely in order to bring the stock back to normal levels. In addition the environment of the Chesapeake needs to be monitored for pollution, and unusually high numbers of microflora in the bay and its estuaries. The concentration of microflora in the environment has also been shown to cause a significant difference in microbial populations present on an aquatic organism and in its meat (Pullela and others 1998; Huang and Leung, 1993). Thus, it is feasible that higher microbial populations due to environmental conditions may lead to unusually rapid spoilage in finished crab meat products; especially if processing companies do not follow Good Manufacturing Practices (GMPs), maintain good sanitation practices, good employee hygienic practices, and maintain good temperature control of the product from harvest through production, transport, storage, and consumption by the consumer.

II. Current Status of the Crab Processing Industry

Since the mid-1990s crab meat imports have more than doubled in volume and the domestic blue crab processing industry must respond to the rapid market changes based on the increasing amounts of imported crab meat. Although mature and well established, the USA blue crab industry is faced with significant constraints on its growth due to variations in supply, unresolved quality issues, and competition from increasing amounts of imported crab meat. Constraints are related to increased market demand for picked crab meat, increased demand for basket (live) and frozen (whole crabs), and factors such as decomposition (e.g., ammonia) in

finished crab meat products in both domestic and imported products. With increased demand for crab meat, product loss needs to be minimized without sacrificing food quality.

Food quality, including food safety is of major concern to the food industry today. A number of surveys have shown that consumer awareness about quality of their food is increasing (Huss and others 2003). In addition to consumer acceptability, good food quality control and manufacturing practices such as good hygienic practices (GHP), hazard analysis critical control point (HACCP), and GMP need to be followed in order to pass government inspection. The importance of a strong food quality control system is supported by data on import detentions and rejections. The United States Food and Drug Administration (USFDA) provide reports on detentions of food imported into the United States and make this information accessible through the Internet. From a period of July 1996 to June 1997 the most prevalent reason for rejection of food products during both periods was contamination with filth from insects, rodents or birds, indicating poor hygienic practices in food processing and handling. Following this, the main reasons for import detentions included microbiological contamination, breach of low-acid canned food procedures, violation of labeling rules, excess levels of pesticide residues and decomposition (Kenny, 1998). Therefore a rapid, accurate test to determine decomposition would be beneficial not only for in house quality control procedures, but also to limit the number of detentions and rejections. Thus limiting the amount of product sent for importation only to be rejected, which saves time and money.

III. Scope of the Research

The Food and Drug Administration (FDA) deems a food as adulterated if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food

(FDA, 2004). No guidelines for instrumental methods of determining spoilage have yet to be imposed by the FDA. Although indole level is used by the FDA to validate the sensory evaluation of shrimp decomposition, a level of 25 μg indole/100g has been suggested by the FDA as the limit before shrimp was considered spoiled by sensory analysis (Shamshad and others 1990). Yet, it is possible that instrumental methods may be cost effective, independent, more precise and accurate compared with sensory experts (Metcalfe and Marshall, 2004).

Microbial analysis (e.g., aerobic plate counts [APCs]) has also been used to determine quality of food products (Phillips and Peeler, 1973; Robson and others 2007; Sullivan and others 1983). An APC count exceeding 10^7 CFU/g for most muscle foods indicates spoilage (Ayers, 1960). Microbial analyses can also help to identify points in the process where bacterial contamination has occurred, and there are rapid methods that can provide results within 24 hours. However, other authors have shown difficulty in relating APCs to food quality (Fernandes and others 1997; MacMillan and Santucci, 1990).

Sensory evaluation of seafood products by inspectors is currently the primary method to determine if products are spoiled. However, difficulties exist for inspectors in determining the relationship between fleeting ammonia odors to quality of crab meat. Additional training of regulatory personnel is required to address this issue, since this is a major area of disagreement between regulatory agencies and the industry.

Ideally, a quick chemical or instrumental test which would require only a few minutes to perform is the most desirable approach to determining food quality. Training of inspectors is still needed on how to use the chemical or instrumental methods, but if written protocols are followed, then inspectors and industry personnel should be able to evaluate the quality of food on

a reliable and consistent basis. Ideally analyses could be conducted prior to the product's departure from the plant to ensure that the product is high quality.

IV. Objective

A need in the seafood industry is to develop a reliable, impartial, and economic method to evaluate seafood quality. The quality of seafood is evaluated using certified sensory experts. However, these experts are expensive to train and periodic recertification is needed if federal and state agencies are to accept them as experts. Sensory testing is also expensive and difficult for the industry to maintain because of personnel turnover and the extensive time commitments needed to retrain and maintain certified sensory personnel.

The objective of this project was to determine key indicators of spoilage in crab meat using an electronic nose, volatile amine measurements and gas chromatography-mass spectrometry (GC-MS). The electronic nose and amine measurements are the most rapid, and most understandable to the industry. The GC-MS analyses were conducted to gain insight into which chemicals are present in high quality, mid-quality, low quality and spoiled crab meat, and to determine if they are reliable quality indicators for blue crab meat.

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CHAPTER 2

REVIEW OF LITERATURE

I. Chemical Breakdown of Crabmeat

The decomposition of seafood is more rapid than other muscle foods. This can mainly be attributed to seafood containing a higher portion of water in the edible portion than other muscle foods. A high water activity and loose connectivity between protein fibers provide a rich environment for microbial growth in seafood. This makes microbial spoilage more rapid in most seafood than other muscle foods such as beef, pork, chicken, etc.

The mechanism for the chemical breakdown of crab meat is not as well established as for some other types of seafood. However, it is believed the chemical breakdown is relatively similar to other shellfish, but not fish, as crab meat has a higher free amino acid composition compared to fish. High levels of taurine, proline, glycine, alanine, and arginine are found in crustaceans (Martin and others 1982). Other nitrogenous compounds such as peptides and other non-protein nitrogen components are found in shellfish, which form typical spoilage metabolites such as indole, ammonia, putrescine, histamine and cadaverine (Eskin and others 1971). Increases 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).

An analysis of the decomposition of raw surimi and artificial crab meat evaluated putrescine, cadaverine, and histamine as possible indicators of spoilage. On first analysis, cadaverine seemed promising as a chemical indicator for decomposition because of the steepness of the slope of the curve with increasing storage time. However, additional analysis indicated that cadaverine concentrations began to change significantly after decomposition had already set

in as judged by sensory analysis. Neither putrescine nor histamine concentrations are viable candidates as spoilage indicators because of their relative insensitivity to increasing decomposition (Hollingworth and others 1990).

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 (TAMO) may also result in the formation of trimethylamine (TMA) and dimethylamine (DMA) in seafood products (Martin and others 1982). It is believed the presence of these compounds correlate to spoilage. These compounds are part of a total volatile base (TVB) analysis, but methods have been derived to determine TMA and DMA concentrations separately. Methods used rely on a colorimetric method, gas chromatography, ion specific electrode, solid state gas sensor, high performance liquid chromatography, or enzymatic diagnostic test kit (Gill, 1990). In an analysis of cod fish by Gill, the relationship between TMA and subjective evaluation of odor was approximately linear (Gill, 1990). Many different chemical methods to evaluate seafood quality have been proposed, with varying degrees of success.

II. Total Volatile Bases (TVB), TMA & DMA, Biogenic Amines, and Ammonia

Total volatile base (TVB) analysis is a simple method to use and therefore widely used to assess freshness quality (odor/flavor) of seafood. Several different methods are used to determine TVB. All of the methods measure the total amount of nitrogenous bases, although the specific analytical method used to determine the amount of nitrogenous bases may vary.

The TVB method is used to determine if the fish is fit or unfit for human consumption. Identification of the early stages of freshness is not possible with TVB, TVB was a useful indicator of advanced decomposition for raw surimi and flaked artificial crab meat (Hollingworth and others 1990). TVB determinations are used as a standard method to determine if chilled, frozen, dried, and canned seafood is spoiled (Botta, 1995). The determination of TVB by direct distillation of fish portions is suitable as a standard method to assess the marketability of fish because it is simple, quick, and economical. The TVB concentration in unfrozen seafood consists primarily of ammonia and trimethylamine, whereas the TVB from frozen seafood consists primarily of ammonia, trimethlyamine, and dimethylamine (Shahidi and Botta, 1994). Generally, TVB concentrations are better quality indicators for fish not shellfish.

Trimethylamine and dimethylamine are normally included in TVB analyses; however, the two chemicals function as reliable indicators of seafood quality on their own. Both of these compounds are a product of trimethylamine oxide (TMAO) decomposition. TMAO is used as an osmoregulatory compound in marine animals. TMAO is degraded to TMA through bacterial degradation. TMAO is degraded in certain species during frozen storage to dimethylamine (DMA) and formaldehyde.

The methods for determination of TMA and DMA includes the Dyer method (colorimetric method), gas chromatography, ion specific electrode, solid state gas sensor, high performance liquid chromatography, and an enzymatic diagnostic test kit. A major advantage of the chromatographic method is that it provides specificity, while some of the other methods do not (Gill, 1990). TMA is primarily suitable for evaluating samples of medium to poor freshness quality as it usually does not indicate a change in freshness quality until the fish have been stored in ice for approximately six days (Howgate, 1982). This is moderately superior to that of TVB,

which typically does not change until the fish have been stored in ice for approximately 10 days (Oehlenschlager, 1992). Evidence suggests that TMA is most useful in medium to late stages of spoilage, when most spoilage is attributed to microbial growth. While DMA has shown to be a useful indicator of spoilage at the earlier stages of spoilage when enzymatic reactions are mostly the cause of spoilage.

Monitoring the levels of biogenic amines produced has been another proposed method to determining spoilage. Biogenic amines are thought to be produced by the decarboxylation of free amino acids present in the tissue of fish and shellfish by bacterial enzymes. Like both TVB and TMA, the formation of these chemicals is a result of microbial degradation and therefore, is measurable primarily during the latter stages of chilled storage (Botta, 1995). Often the biogenic amines that are monitored include histamine, putrescine, and cadaverine. Histamine is thought to be produced from the decarboxylation of histadine, putrescine from ornithine, and cadeverine from lysine in scombrotoxic species (Gill, 1990). Biogenic amines and their usefulness as an indicator of quality often depend on the species being examined and its environment. Depending on conditions in the environment, the species may need to raise the pH by producing amines. This will affect the concentration of amines, which will in turn affect the values for their degradation products. Therefore changes in the concentration of biogenic amines may not be necessarily attributed to decomposition. Methods of analyzing these compounds have been accomplished by gas chromatography of chemical derivatives, HPLC, and Technicon Autoanalyzer (Gill, 1990).

Since ammonia is the main component of TVB it may be hypothesized that ammonia content may serve as a good indicator of spoilage. In general ammonia is a superior indicator of freshness in crustaceans than in finfish (Botta, 1995). However, ammonia has also been reported

as an acceptable indicator of spoilage in cod and squid (Gill, 1990). It is believed that early ammonia evolution is attributed to an enzymatic process, although in shrimp ammonia formation is not typically linear during storage (Luzuriaga and others 1995).

Ammonia concentration is quantitatively measured using methods including diffusion, accelerated micro diffusion; enzymatic conversion of ammonia and keto-glutarate to glutamate, and a test kit in the form of a paper strip (Botta, 1995). In addition an ion selective electrode can be used to measure ammonia; however, this requires an extraction step to remove sodium ions which act as an interferite.

III. Colorimetric Determination of Ammonia in Crab Meat

The colorimetric method involves a color reaction between ammonia, thymol, and alkaline bromine water. Depending on the amount of ammonia from the decomposing samples, the samples were classified into Class 1, Class 2, and Class 3 quality.

In one study, nine collaborators representing 6 Food and Drug laboratories, 2 State laboratories, and 1 trade association laboratory participated in the study. Fresh frozen Dungeness crab was used for this study. The meat was allowed to thaw in shell at room temperature. Meat was periodically examined by organoleptic analysis, and the meat at a certain stage of decomposition was removed from the shell, ground 3 times through a meat grinder with thorough mixing, and frozen for distribution to collaborators. Collaborators were requested to analyze 6 samples: I. Class 1; II. Late Class 1; III. Early Class 2; IV. Early Class 2; V. Early Class 3; VI. Early Class 3.

Ground crab meat samples were reacted with a phophotungstic acid solution, and then filtered. The filtrate was then reacted with a NaOH solution, thymol solution, and bromine

solution. The solution was then allowed to stand for approximately 20 minutes. Figure 1 describes this reaction scheme. An extraction was done with n-butyl alcohol. The alcohol layer was dried with sodium sulfate before being analyzed. The absorbance was of the solution was then determined at 680 nm in a 1 cm cell against a blank as a reference. A standard curve using std. NH_3 solution was used for quantitation.

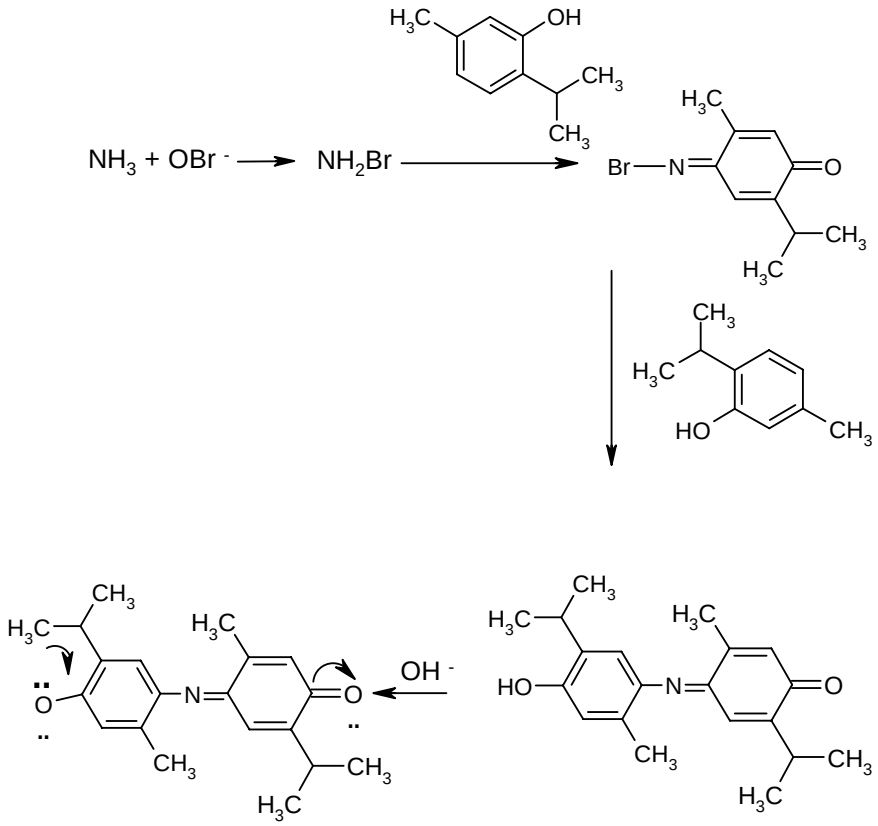

Figure 1. Reaction Scheme for the Colorimetric Determination of Ammonia.

The collaborators reported only absorbance values for the standards and samples. The Associate Referee calculated the ammonia content, expressed in µg NH_3/g crabmeat, by deriving the equation of a line from the standard absorbance values by the least squares method and by substituting sample absorbances in the equation derived (Steinbrecher, 1973). Absorbances were essentially linear over the concentration range of the standards. The 6 samples sent to

collaborators, in effect, represented 4 classes of decomposition: one sample Class 1, one sample late Class 1, duplicate samples early Class 2, and duplicate samples early Class 3. The mean for Class 1 decomposition was concluded to be around {247 μg NH_3/g}, for late Class 1 {314 μg NH_3/g}, early Class 2 {568 μg NH_3/g}, and early Class 3 {1376 μg NH_3/g} (Steinbrecher, 1973). A t-test conducted on the collaboratively submitted data, indicated that significant differences existed between respective means.

Particular interest was paid to the difference in the means between all of class 1 (class 1 and late class 1) and early class 2. Comparison of these 2 classes showed both a significant difference in means. This method was adopted by the Association of Official Analytical Chemists (AOAC) for determination of ammonia in crab meat in 1983.

IV. Review of Electronic Nose Literature

Devices designed to detect odors date back to around the 1960s. In 1961 Moncrieff designed a mechanical instrument consisting of a thermistor coated with a number of different materials to detect odors (Moncrieff, 1961). The first electronic nose can be credited to Wilkens and Hatman in 1964. This device used redox reactions at an electrode to detect odorants (Wilkens and Hatman, 1964). In 1965, Buck and others detected odorants by monitoring modulation of conductivity when exposed to odorants (Buck and others 1965). These were crude devices, and it was not until the 1980s when intelligent chemical array devices were employed. Persaud and Dodd were the first to employ this technology in 1982 when they exposed the nose to known chemical odorants (Persaud and Dodd, 1982). The Hitachi Research Laboratory in Japan was also working on a similar device during the same time (Ikegami and Kaneyasu, 1985). The term electronic nose appeared during the 1980s but it was not defined

until 1994 in an article by Gardner and Bartlett (1994). They defined an electronic nose as an instrument, comprised of an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognizing simple or complex odors (Gardner and Bartlett, 1994). Alpha M.O.S. developed an electronic nose called Kronos™ which employed fingerprint mass spectrometry as means of detecting odorants (M.O.S., 2006). This instrument did not employ a detector array. Thus the term "electronic nose" is not limited to volatile gas sensitive instruments that employ a sensor array system.

The electronic nose is designed to mimic the mammalian olfactory system by using an array of sensors designed to simulate mammalian olfactory responses to aromas. Instead of receptor proteins, similar to the human olfactory system, the electronic nose has a sensor array system. The odor molecules are drawn into the electronic nose using sampling techniques such as headspace sampling, diffusion methods, bubblers or pre-concentrators (Pearce and others 2003). The sample odors are drawn across the sensor array which induces a reversible physical or chemical change in the sensing material, 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 identification by a pattern recognition system. The general differences between the mammalian olfactory system and an electronic nose system are shown in Figure 2.

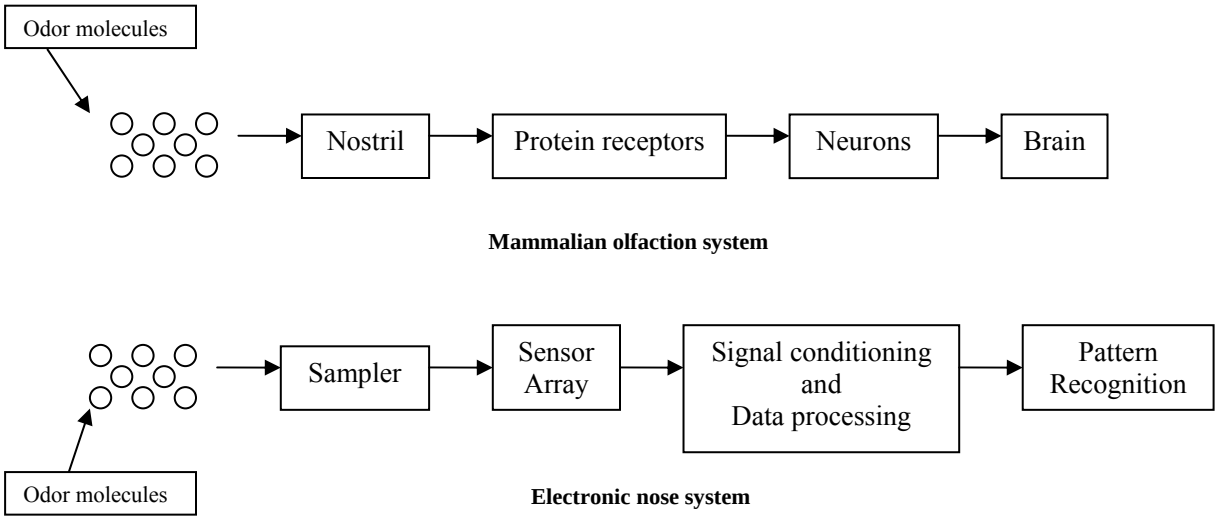

Figure 2. Comparison of the Mammalian Olfactory System and an Electronic Nose System.

The sensor array system is composed of different sensor types such as conducting polymer, metal oxide, quartz crystal microbalance, metal-oxide-semiconductor field-effect transistor sensors (MOSFET) and surface acoustic wave (SAW) sensor devices. Table 1 summarizes the different sensor types and some of their advantages and disadvantages. The sensors are designed to have a broad partial sensitivity similar to receptor proteins of the human nose. Broad partial sensitivity allows response to a range or class of gases rather than to a specific one. This is the opposite of an ideal gas sensor, which responds to only one gas, and provides a unique output. However, the goal is to identify an odor containing hundreds of chemicals. Therefore the use of multisensor arrays is common. Specific sensors in the array are designed for specificity of certain types of chemicals. When these sensors are combined to form an array the number of detected compounds is vast. This potentially allows the electronic nose to be a powerful tool in a wide range of applications.

Table 1. Summary of Commonly used Electronic Nose Sensors

Active material	Sensor type	Measure	Advantages	Disadvantages
Conducting polymer	chemoresistor	conductivity	Operate at ambient, inexpense, diverse range of coatings	Sensitive to temperature and humidity
Metal Oxide	chemoresistor	conductivity	Fast response and recovery time, inexpensive	High operating temperatures, sulfur poisoning problem, limited range of coatings
Lithium niobate, polymeric, liquid crystal, lipid layer, etc.	surface acoustic wave device (SAW)	piezoelectricity	Diverse range of coatings, high sensitivity, good response times	Complex surface circuitry
Quartz crystal with membrane coating (usually a type of polymer)	quartz crystal microbalance (QCM)	piezoelectricity	Diverse range of coatings, good reproducibility	Poor signal to nose ratio, complex circuitry
Catalytic gate (usually a catalytic metal)	MOSFET	threshold voltage change	Small, inexpensive sensors	Baseline drift, need controlled environment

Trained sensory panels are often used to evaluate the odor of a food. However, these panels are costly, experts can only work for relatively short periods of time due to a variety of issues such as physical and mental health issues, and fatigue (Pearce and others 2003). Thus, instrumental methods such as gas chromatography with various detector types have been employed to aid human panels to asses the quality of products through odor evaluation and identification and in addition to obtain more consistent results. These techniques are lab-oriented and are not portable.

Electronic noses have been used for a wide variety of applications and show varying success rates. There are many different kinds of electronic noses on the market, employing different types of technology, including sensor technology, number of sensors, sensitivity of the instrument to the specific analyte, sample type and conditions, signal processing techniques, statistical manipulations, etc. Depending on the application, one electronic nose may work well for one type of analysis, but another type of electronic nose may work very poorly for the same analysis. Laboratory-made electronic noses have also been employed. In many cases, the purpose of the electronic nose analysis is to determine whether the electronic nose will work well for the application, before any useful information about the samples can be obtained, especially when nothing is known about the specificity of specific sensors in the array, since often the manufacture will not release this information. Table 2 contains a list of applications for which the electronic nose has been used. The most commonly reported application is to classify the smell of various beverages or foodstuffs. In some cases the sample is graded, and in other tests the electronic nose simply tests for freshness (i.e. fresh or spoiled). In general, best results are obtained for electronic noses when a few simple odors are analyzed such as methanol, ethanol, and propanol (Slater and others 1993). Consequently the electronic nose performs better in freshness studies where it differentiates between two groups (Gardner and Bartlett, 1999b). This is logical because as the number of groups increase the statistical methods become more confounded, and separation becomes more difficult.

Table 2. Applications of the Electronic Nose

Manufacturer	Sensor type	Applications	website/reference
Airsense	metal oxide	food evaluation, flavor and fragrance testing	www.airsense.com
Alpha M.O.S.	conducting polymer, metal oxide, quartz microbalance, surface acoustic wave devices	quality control, analysis of different food types, dairy products	www.alpha-mos.com Collier (2003) McKellar (2005)
AromaScan PLC	conducting polymer	chemical quality control, off-flavor detection, microbial quality	Du (2001) Osborn (2001)
Cyrano Science Inc.	conducting polymer	chemical analysis, food quality, freshness, spoilage, contamination detection	www.smithsdetection.com Van Deventer (2002) Chantarachoti, Oliveira, and others (2006)
FreshSense	no information	evaluation of aquatic food products	Jonsdotter (2004) Zeng (2005)
HKR-Sensorsystems	quartz crystal microbalance	retained solvents, flavor fade	Van Deventer (2002) Williams (2006)
Laboratory-made	wide range of sensor types	sample classification, changes in flavor with aging, quality based discrimination of wine	Brudzewski (2004) Monge (2004) Garcia (2006)

Many of the electronic nose studies dealing with seafood focused on freshness (i.e. fresh or not fresh). This makes the multivariate statistics used simple, leading to improved results. As the number of groups analyzed increases, the amount of multivariate space decreases, making separation of the groups additionally difficult. If only two groups are analyzed, there is more canonical space, and achieving group separation is less difficult. In addition if an identification

of unknown samples is performed, the electronic nose chooses between only two groups for classification, thus decreasing possible error.

In a study by Chantarachoti and others (2006), spoiled Alaskan pink salmon was investigated using an electronic nose, sensory analysis, and microbial analyses. The Cyranose 320™ (Cyrano Sciences, Inc., Pasadena, Calif., U.S.A.) electronic nose differentiated between two classes of spoiled salmon. The correct classification rate using stepwise discriminant analysis was 85 % and 92 % for electronic nose analysis of belly cavity volatiles for fish held at 14 ºC and slush ice, respectively (2006). However, when the electronic nose was asked to differentiate between different days (e.g. day 1, 2, 3) of belly cavity volatiles correctly, for the same conditions the correct classification rates went down to 73 % and 77 %, respectively (2006). The electronic nose was used to differentiate between different days of gill odors, but this approach yielded even lower correct classification rates compared with the belly cavity volatiles.

In a study by O'Connell and others (2001) samples of hake were examined for their freshness using an electronic nose system with tin dioxide sensors. Two different patterns were obtained for the hake, an associated rotten, and an associated non-rotten pattern. These patterns were independent of storage conditions: for example, the pattern of a rotten sample was the same independently if the piece was stored several days in a refrigerator or if it was kept out at ambient conditions for one day (O'Connell, 2001). These two groups were separated by the electronic nose system. The interesting part of this study was that the pattern obtained for samples stored in a refrigerator for 2 days was similar to the pattern obtained for a sample left out of the refrigerator one day immediately after receiving it (O'Connell, 2001). This suggests

that either the volatiles produced by the microorganisms under both conditions were similar, or the samples were yet to show effects of spoilage since they were still relatively fresh.

In addition, an Electronic Nose model 4000 (EEV Inc. UK) with 12 conducting-polymer sensors differentiated between two groups of oysters (*Crassostrea virginica*) stored at 1.8 ºC and 7.0 ºC, respectively. The electronic nose device obtained a 100 % correct classification rate for the days of storage (0, 1, 3, 6, 9, 13 days) for the two groups (Tokuşoğlu and Balaban, 2004). No further steps were taken in this study and unknown samples were not used to validate the statistical model. In a similar study, a Fresh Sense (Icelandic Fisheries Laboratories and Element Sensor Systems, Artorg 1, 550 Saudarkrokur, Iceland) electronic nose was used to predict the outcome of total volatile base (TVB) analysis for capelin (*Mallotus villosus*). The electronic nose separated the groups that agreed with sensory classification (e.g. fresh, flat, sweet, stale, putrid), but had some issues correlating with TVB analysis. The NH_3 sensor of the FreshSense electronic nose was shown to best correlate with the TVB analysis (Olafsdottir and others 2000). The NH_3 sensor is selective to volatile amines; therefore it should correlate with the TVB analysis.

In order ensure that the electronic nose is working correctly, it is important to validate the training set using unknown samples. In a study of retained solvents on printed packaging using three different electronic nose systems, the Cyranose 320 (Pasadena, CA, U.S.A) and the Fox 3000 (Alpha M.O.S., Toulouse, France) systems showed the most discriminatory power by discriminating correctly all classes of samples, and correctly identify 100 % of unknown samples (Van Deventer, 2002). The HKR Sensorsystems QMB6 (Munich, Germany) was not able to discriminate between all printed packaging films, and therefore lacked the discriminatory power of the Cyranose and Fox systems. In a study of oysters, the Cyranose 320 differentiated between

oyster qualities of varying storage days (100 % separation). However, validation of the model showed that unknown oyster qualities could be identified with 93 % accuracy by the Cyranose 320 (Hu, 2004). A VOCChek (Warren, NJ, U.S.A.) electronic nose, which is a quartz microbalance system, used on the same samples correctly identified the oyster samples at a classification rate of only 22 %. The packaging films were significantly different in their composition (OPP, polyester, LDPE, and coated paper), so it is feasible that they would be easier to identify by electronic nose systems than oysters.

Electronic nose output can be convoluted, even if established methods such as PCA, DFA, etc. are used. To interpret electronic nose data, new statistical methods have been developed, such as neural networks, bootstrapping, and other statistical methods designed to make more sense of the data. Artificial neural networks are mathematical creations that have been inspired by the biological nervous system. An artificial neural network consists of a lattice of information processing elements called *neurones* which are connected together in a certain way (known as its architecture). The strengths of these connections are called synamptic 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, 1999a). Therefore a neural network, can "learn" as data is imputed in, whereas a pattern recognition technique simply looks for patterns.

Some examples of neural networks used to improve electronic nose data are described below. A neural network using multilayer perceptron (MLP) trained back-propogation was shown to improve quantitative analysis (Haykin, 1999). A time delay neural network (TDNN), a dynamic network with embedded local memory, has been shown to improve an electronic nose's pattern recognition ability (Zhang and others 2003). A 12 conducting polymer e-Nose 4000

(Neotronics, Ganesville, Ga., U.S.A.) was used to quantify a spice mixture of known composition with the implementation of neural networks. The neural networks used for this analysis were multilayer perceptron (MLP), MLP using principal component analysis as a preprocessor (PCA-MLP), and the time-delay neural network (TDNN). All three neural network models predicted the testing mixtures' compositions with a mean square error equal or less than 0.0051 (in a fraction domain where sum of fractions = 1) (Zhang and others 2005).

Two other as well established methods to improve, or optimize electronic nose data are bootstrapping and response surface methodology (RSM). Consequently, both of these procedures were used in conjunction with the Cyranose 320. Bootstrapping is a concept 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 using this method 30 real observations can create 1000 "bootstrap" observations. This approach is best used when the error of the analysis is attributed to small sample size. The bootstrap method was used in a study of beef spoilage and improved the classification accuracy to 98 % (Balasubramanian and others 2004). Response surface methodology is a process optimization tool, which characterizes the relationship between the response, and the set of quantitative factors of interest. The response is described, over the applicable range of the factors in interest, by fitting a model referred to as *response surface*. The response is graphed as a surface in 3D space, and can be used to determine important characteristics such as optimum operating conditions. This technique was used to optimize pump speed, incubation time, and incubation temperature for an analysis of grape aroma, using the Cyranose 320 (Athamneh, 2006).

V. Blue Crab (*Callinectes sapidus*) composition and odor

Blue crabs contain approximately 80 percent water (Table 3). Other major constituents of blue crab are protein and fat. Blue crab is also rich in the elements calcium, phosphorous, magnesium, sodium, potassium, manganese, zinc, and iron.

Table 3 Proximate composition of blue crab by various authors.

Moisture (%)	Protein (%)	Fat (%)	Reference
81.20	16.10	1.0	Farragut (1965)
77.4 – 86.7	11.9 – 19.2	0.4 – 1.5	Wheaton and Lawson (1985)
78.60	11.60*	0.75*	Akpan (1997)
81.58 – 83.1	14.71 – 15	0.64 -0.79	Gökoðlu and Yerlikaya (2003)

* Dry matter measurements were converted to wet matter measurements.

Minor differences between moisture, protein, and fat are shown in the in the proximate composition of claw and body meat, but the difference is small. The largest difference in blue crab between crab and claw meat is fat composition. The percentage of fat in claw and body meat is 0.64 % and 0.79 % respectively (Gökoðlu and Yerlikaya, 2003).

The mineral composition of blue crab was found to change depending on cooking procedure. In a study by Lopez and others (1981), the content of calcium, copper, iron, magnesium, phosphorous, potassium, sodium, and zinc was found to change depending upon cooking procedure. In this study, boiling, steaming, and pasteurization using deionized water were examined, and the cooking procedures were found to have an effect on mineral composition of the resulting cooked food product.

The edible portion of crab is mostly water and protein. Blue crab does contain some fat, mostly polyunsaturated fatty acids. The major fatty acids of blue crab are shown in Figure 3.

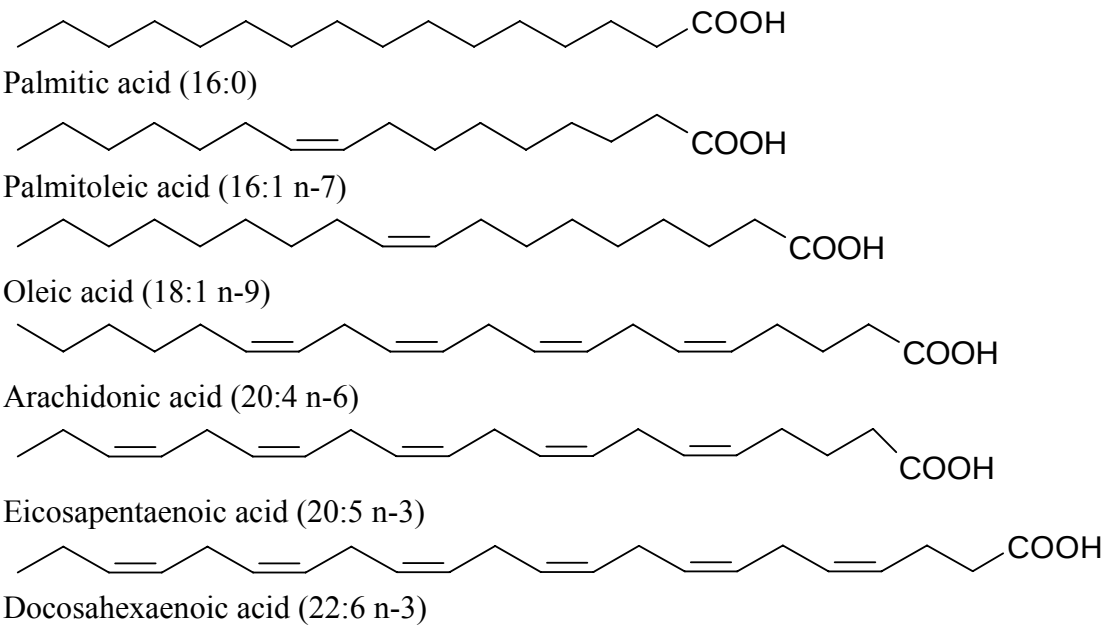

Figure 3. Main fatty acids present in blue crab.

Blue crabs contain three types of fatty acids, saturated, monounsaturated, and polyunsaturated. Palmitic acid has no double bonds and therefore is resistant to oxidation. The monounsaturated oleic acid with one double bond in the cis configuration in the Δ9 position (relative to the carboxyl group) is designated as 18:1 n-9. The "n" symbol indicates the number of carbons from the terminal end of the 18-carbon chain, a nomenclature favored by biochemists. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are polyunsaturated acids which are characterized by having two or more cis double bonds separated by a single methylene group, or a 1,4-diene structure. Both are omega three fatty acids. In addition, EPA and DHA are important in modulating the biosynthesis of oxygenated eiconsanoids, which have hormone-like functions and play an important role in inflammation processes (Fujimoto and others 1990).

The major fatty acids in blue crab in claw meat, body meat, and the hepatopancreas were reported in a study by Çelik and others (2004) and are shown in Table 4. The full fatty acid profile was reported in this study, but is omitted here.

Table 4 Major fatty acids in blue crab meat (% of total fatty acids)**.**

Fatty acid	Claw meat	Body meat	Hepatopancreas
14:0	1.13	2.03	21.6
16:0	13.5	15.0	3.71
16:1 n-7	4.07	5.59	1.60
18:1 n-9	12.9	13.7	6.85
18:2 n-9	6.51	5.30	2.63
20:4 n-6	7.48	5.02	8.22
20:5 n-3	10.6	8.41	7.78
22:6 n-3	5.92	6.75	5.30

For the most part, the fatty acid profile of claw meat and body meat is similar. The ratio of omega-3/omega-6 was also reported, and was found to be 2.32 and 3.18 in claw and body meat, respectively. It is unclear whether this ratio results in positive or negative heath benefits. Some articles point to the consumption of polyunsaturated fatty acids being beneficial (Dyerberg, 1986). While others suggest adverse effects, since polyunsaturated fatty acids are susceptible to oxidation because of their high number of double bonds (Hennig and others 2001).

Oxidation reactions of lipids fall into the category of homolytic radical reactions (also known as free radical reactions). These reactions are initiated by light, heat, metals, or by other free radicals and peroxides. Since these constituents are also present in the crab matrix, it is feasible that a large amount of oxidation can occur during storage. This can lead to volatile off-flavors and odors.

Spoilage studies of crab have shown varied results in regard to indices that define levels of spoilage. Analytical techniques applied to this type of analysis include microbial, rough chemical methods (such as total volatile bases and total volatile acids), and sensory methods. One of the drawbacks for these types of analysis is the storage methods often differ. How the

product was packed (i.e. polymer packaging), storage temperature, storage time, etc. all can affect the study. A few crab storage studies are summarized below.

Shelf life was studied in three species of blue crab *Carcinus maenas, Necora puber*, and *Cancer pagurus* by determination of aerobic plate counts (Robson and others 2007). It was found that storage conditions (i.e. on ice at 4 ºC, at 4 ºC, simulated supermarket conditions, and 20 ºC) impacted shelf life. Crabs stored on ice at 4 ºC, and crab stored at 4 ºC with no ice differed in shelf life. Live crabs stored on ice died within 24 hrs., most likely due to thermal shock and their early death was responsible for their rapid increase in bacterial numbers compared to crabs stored at 4 ºC (Robson and others 2007).

In addition whether the crab is packaged as meat or whole seems to make a difference on spoilage rate. During a 10 month frozen storage study of whole blue crabs packaged in polyethylene polyamide pouches at (-18 ºC), TVB and TVA values increased from 6-25 mg/100g for TVB values and 0.38-1.53 mg/100g for TVA values (Yerlikaya and Gökoðlu, 2004). Sensory results confirmed that the product was acceptable by consumers. Consequently, total volatile base nitrogen concentration of fresh crab meat was 26.2 mg per 100 g in a study by Gates and others (1996). A similar frozen storage study was performed by Gates and Parker (1993). In this study, minced meat from claws and picking room by-products were pasteurized and stored in low-density polyethylene tubes at -20 ºC for 10 months. After 10 months of storage, sensory scores indicated that the stored samples were not acceptable. However the same samples stored in aluminum cans were acceptable according to sensory results. Oxygen permeability of the polyethylene tubes was concluded as the most probable explanation for development of off-odors and flavors (Gates and Parker, 1993).

The volatile chemical profile of crab meat aroma has not been studied extensively. One of the reasons for this is because extraction of these types of compounds is difficult and aroma compound levels are often in the parts per million (ppm) to parts per billion (ppb) range. Obviously, humans have low detection thresholds for these compounds since sensory analysis is used to grade quality levels of seafood. Some analytical instrumentation can now detect the chemical compounds present in the aroma profile. The advent of sensitive detectors, such as mass spectrometry detectors has led to the detection of compounds in the *picogram* range.

Dynamic headspace sampling is a purge and trap system that can be used to concentrate volatile compounds. This technique combined with a gas chromatograph/mass spectrometer to identified fifty three compounds in boiled and pasteurized blue crab meat including four aldehydes, four ketones, three alcohols, thirty aromatics, two furans, two sulfur containing compounds, two terpenes, three alkanes, and three miscellaneous compounds in a study by Matiella and Hsieh (1990). Compound identification was performed by retention index and mass spectrometry. Most compounds were of higher concentration in the boiled crabmeat samples. For the boiled crab samples, crabs were whole and processing was done in the laboratory. The pasteurized samples were purchased from the store.

Chung and Cadwallader conducted extensive research on the topic of blue crab volatiles. The technique that they used to concentrate the volatiles in three studies was simultaneous steam distillation-solvent extraction (SDE). Detection of the aroma compounds was by either gas chromatography/mass spectrometry, gas chromatography/Fourier transform infrared spectroscopy, gas chromatography/flame ionization detection (FID), or gas chromatography/olfactometry. In a study of blue crab meat and processing by-products, 77 compounds were identified in crab meat, and 88 compounds were identified in the by-products

(Chung and Cadwallader, 1993). There were 58 compounds found in both crab meat and by-product. Compound identification was on the basis of retention index (RI) and mass and infrared spectra of unknowns with those of authentic standard compounds under identical experimental conditions. Samples were collected over three days. The average concentration range for each compound and relative standard deviations (RSD) of the three collection days were compared. Most compounds had RSD values close to 50 %. However, several had values greater than 100 %. Such high values indicate large natural fluctuations in concentrations of those compounds or deficiencies in the volatile extraction procedure (Chung and Cadwallader, 1993).

Aroma extract dilution analysis and gas chromatography was used to investigate blue crab meat aroma extracts (Chung and Cadwallader, 1994). This study evaluated:

1) Evaluation of atmospheric (A-SDE) and vacuum simultaneous steam distillation- solvent extraction (V-SDE)
2) Determine if aroma notes could be detected by gas chromatography/olfactometry (GC/O) at lower levels than other forms of detection

V-SDE recovered more aroma notes. In addition V-SDE had more reproducible results, and was less prone to artifact formation since the extraction was performed in an enclosed environment under vacuum. In addition the compounds 2-acetyl-1-pyrroline, pyrrolidine, and 3-(methylthio)propanal were identified for the first time in blue crabs.

Lastly, cooked blue crab claw meat aroma was compared with lump meat aroma using sensory evaluation, and aroma extract dilution analysis. Sensory evaluation of claw and lump crab meat by sniffing indicated no aroma difference ($p > 0.05$): however, differences ($p < 0.05$) were found between the taste and aromatics of aqueous extracts of both meats (Chung and others 1995). The extracts were subjected to GC/MS-FID/O with 11 odor notes being found. Out of the 11 aroma notes, the compounds trimethylamine, 2,3-butanedione, pyrrolidine, 2-acetyl-1-pyrroline, and 3-(methylthio)-propanal were positively identified. This article provides evidence

that greater sensitivity can be achieved using aqueous extracts of crab meat products than by routine sensory analysis.

Table 5 Aroma Compounds identified in four different studies of crab meat products

Chemical Compounds	Matiella & Hsieh (1990)	Chung & Cadwallader (1993)	Chung & Cadwallader (1994)	Chung & Cadwallader (1995)
Aldehydes				
2-butenal		x		
hexanal	x	x		
2-methyl-2-pentenal		x		
heptenal	x	x		
(*E*)-2-hexenal		x		
(*Z*)-4-heptenal		x	x	
octanal		x		
nonanal		x		
benzaldehyde		x		
3-methylbutanal	x			
pentanal	x			
(*E*)-4-decanal			x	
Alkanes				
decane	x	x		
dodecane		x		
tridecane		x		
tetradecane		x		
pentadecane		x		
hexadecane		x		
heptadecane		x		
octadecane		x		
nonane	x			
undecane	x			
Aromatics				
p-xylene	x	x		
m-xylene		x		
styrene	x	x		
1,2,4-trimethylbenzene		x		
1,2,3-trimethylbenzene		x		
naphthalene	x	x		
phenol		x		
benzene	x			
toluene	x			
Alcohols				
2-butanol		x		
1-penten-3-ol		x		
3-methyl-1-butanol		x		

Chemical Compounds	Matiella & Hsieh (1990)	Chung & Cadwallader (1993)	Chung & Cadwallader (1994)	Chung & Cadwallader (1995)
1-pentanol		x		
1-hexanol		x		
1-octen-3-ol		x		
1-heptanol		x		
2-ethyl-1-hexanol		x		
1-octanol		x		
1-nonanol		x		
2-undecanol		x		
1-dodecanol		x		
1-tetradecanol		x		
1-hexadecanol		x		
1-butanol	x			
3-hexanol	x			
2-hexanol	x			
Ketones				
2-propanone		x		
2,3-butanedione		x	x	x
4-methyl-2-pentanone		x		
3-hexanone	x	x		
2,3-pentanedione		x		
2-hexanone	x	x		
(E)-3-penten-2-one		x		
2-heptanone		x		
3-hydorxy-2-butanone		x		
3-octanone		x		
2-nonanone		x		
2-cyclohexen-1-one		x		
2-decanone		x		
2-undecanone		x		
acetophenone		x		
2-tridecanone		x		
2-pentanone	x			
Pyrazines				
pyrazine		x		
2-methylpyrazine		x		
2,5-dimethylpyrazine		x		
2,6-dimethylpyrazine		x		
Sulfur containing compounds				
dimethyl disulfide	x	x		
2-methylthiophene	x	x		
2,4,5-trimethylthiazole		x		
dimethyltrisulfide		x		

Chemical Compounds	Matiella & Hsieh (1990)	Chung & Cadwallader (1993)	Chung & Cadwallader (1994)	Chung & Cadwallader (1995)
3-(methylthio)-propanal			x	x
Terpenes				
limonene	x	x		
linalool		x		
menthol		x		
α-terpineol		x		
Miscellaneous compounds				
trimethylamine		x	x	x
chloroform		x		
2-pentylfuran		x		
N,N-dimethylformamide		x		
1H-indole		x		
pyrrole	x			
pyrrolidine			x	x
2-acetyl-1-pyrroline			x	x

Possible explanations of how aroma compounds were generated exist for some compounds, but not most. In an article by Baek and Cadwallader (1997) possible routes of formation of the volatile compounds were discussed. The reaction of 12- or 15-lipoxygenases and hydroperoxide lyase on eicosapentaenoic acid is thought to be responsible for the formation of volatile 6-, 8-, and 9-carbon carbonyls and alcohols. Polyunsaturated fatty acids present in shellfish are also susceptible to autoxidation. After lipid autoxidation, fresh aromas are replaced by stale, painty, and oxidized type aromas. Hexanal, 2,4-heptadienal, 3,5-octadien-2-one, and 2,4-decadienal contribute to stale aromas, and some of these compounds have been found to be aroma products of crustaceans. (E,Z)-2,4-Heptadienal and (E,Z)-3,5,-octadien-2-one were found to be formed via autoxidation of eicosapentaenoic acid (Josephson, 1991). Pyrazines and sulfur-containing compounds are thought to be thermally generated through the cooking process because of carbonyl-amino reactions (Lindsay, 1990).

Specific high impact aroma compounds were also examined individually for their possible route of formation. Trimethylamine, is thought to be the product of microbial reduction of trimethylamine oxide (Lindsay, 1990). 2,3-Butanedione is believed to be generated via the Maillard reaction (Hurrell, 1982). 2-Acetyl-1-pyrroline can be formed via Maillard reaction of the glucose-proline reaction (Roberts and Acree, 1994). It was also proposed that 2-acetyl-1-pyrroline was formed from the reaction of 2-oxopropanal (a sugar degradation product) and 1-pyrroline (degradation product of proline) (Schieberle, 1995). 3-(Methylthio)-propanal is formed via Strecker degradation of methionine (deMan, 1999).

In addition, some of the compounds identified in the previously mentioned aroma studies are associated with environmental contamination. Compounds mainly associated with seafood contamination are polychlorinated biphenyls (PCBs) (Storelli and others 2003). In addition polyaromatic hydrocarbons (PAHs), chlordane, hexachlorobenzene, hexachlorocyclohexane, along with other metal contaminants were found in seafood from the Quincy Bay in Massachusetts (Cooper and others 1991). Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) were found at trace levels in 147 food samples including 10 blue crab, 8 lobster, and 10 crawfish samples (Hayward and others 2001). However, not all of the compounds presence in the previously mentioned aroma studies can be explained. More research should be focused on correct identification of aroma compounds associated with crab, and identification of the starting materials from which the aroma is formed. Though previous research has investigated some of the aroma components in crab, these types of studies have failed to look into how the aroma components may change as crab meat spoils. This study will investigate changes in aroma profiles due to crab meat spoilage.

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CHAPTER 3

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

(Manuscript to be submitted to Journal of Aquatic Food Product Technology)

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

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ABSTRACT

Rapid, reliable, objective methods for determining the quality of food products are needed; an electronic nose or a rapid gas analyzer such as Draeger-Tubes® can possibly accomplish this. In this study, five groups of sequentially spoiled crab meat were evaluated by a trained sensory panel, and these results were compared with the findings from a Cyranose 320™ Electronic Nose. Crab meat samples were evaluated using three different analytical methods. The first method (i.e. manufacturer's recommended method) was conducted according to the instructions described in the Cyranose 320^{TM} manual, which used ambient room air to purge the samples. This method resulted in a 30 % correct separation of the known groups, but only 10 % of the samples were correctly identified when coded unknown samples were used to validate the electronic nose training results. The second method used compressed tank breathing air, filtered through activated carbon and moisture traps. This second method resulted in 100 % separation of the known groups, but only correctly identified 20 % of the coded unknown samples. The third method used compressed tank breathing air, activated carbon and moisture traps, and a high quality crab meat sample as a reference sample. This third method correctly separated 50 % of the known groups, but only identified 20 % of the coded unknown samples. Statistical

procedures used to evaluate the data obtained from the electronic nose analyses included principal component analysis (PCA), canonical discriminate analysis (CDA), and stepwise discriminant analysis. Draeger-Tubes® were more accurate and precise compared with the electronic nose. 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 rapidness of this procedure allows it to be usable for the crab industry and potentially other food processing industries as a quality control method.

KEYWORDS: electronic nose, blue crab, Draeger, quality, ammonia, sensory

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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 which cycles into sample containers was first developed at 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 and the International Association of Refrigerated Warehouses (WFLO/IARW) and by the National Fisheries Institute (NFI) Scholarship Program.

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 1968-2004 the annual harvest was 73 million pounds in the Chesapeake Bay alone (Bonzek and others 2005). In recent years the harvests have dipped into the 50 million pound range. However, in 2004 the harvest was approximately 60 million pounds, which suggests blue crab populations may again be on the rise. 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 insure over-crabbing, habitat destruction, and 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 crab meat 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 other shellfish, but not fish, as crab meat has a higher free amino acid composition compared to fish. High levels of taurine, proline, glycine, alanine, and arginine are found in crustacean (Martin and others 1982). Other nitrogenous compounds such as peptides and other non-protein nitrogen components are found in shellfish, form typical spoilage metabolites such as indole, ammonia, putrescine, histamine and cadaverine (Eskin and others 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 quickly and easily.

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 (TAMO) may also result in the formation of trimethylamine (TMA) and dimethylamine (DMA) in seafood products (Martin and others 1982). TMA most often occurs in refrigerated storage conditions and has been shown to also occur during frozen storage, while DMA occurs only during frozen storage conditions. These compounds are part of a total volatile base (TVB) analysis, but methods have been derived to determine TMA and DMA concentrations separately (Yerlikaya and Gökoðlu, 2004; Jones and others 1998). In an analysis of cod fish by Gill, the relationship between TMA and subjective evaluation of odor was approximately linear (Gill, 1990). Many different chemical methods to evaluate seafood quality have been proposed, with varying degrees of success. However, no rapid, simple method to determine TMA or DMA exists.

Finding an economical way to evaluate seafood quality is a difficulty that exists for the seafood industry. 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 can be expensive and difficult for the industry to conduct due to frequent personnel turnover and the extensive time commitments needed to maintain certified sensory personnel.

The proposed 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 and others 2004). The electronic nose is a rapid technique with analysis time often taking less than two minutes.

The electronic nose is designed to mimic the mammalian olfactory system by using an array of sensors designed to simulate mammalian olfactory responses to aromas. Instead of receptor proteins, which are part of the human olfactory system, the electronic nose in most cases consists of a sensor array system. The odor molecules are drawn into the electronic nose using sampling techniques such as headspace sampling, diffusion methods, bubblers or pre-concentrators (Pearce and others 2003). The sample odors are drawn across the sensor array which induces a reversible physical or chemical change in the sensing material, 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 identification by a pattern recognition system. Since sensory evaluation is the most established method for determining spoilage of seafood one could hypothesize that an instrument which functions on similar principal would work well for spoilage analysis.

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 crab meat exists (Steinbrecher, 1973). However, 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. For this reason it is also important to have a rapid test for detecting volatile amines. In that regard, Draeger-Tubes®, which have been mostly used for the detection of ammonia gas leaks, may work as a rapid analytical test to determine spoilage in crab meat. These tubes can be used to detect low levels of ammonia, in the ppm range. These tubes also show response to other amines, not only ammonia. 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 non-specific test has previously proved somewhat effective (TVB analysis), the cross sensitivity to other amines may be advantageous for determining spoilage in shellfish. Many food processing companies may already have Dräger-Tubes® since refrigerated storage units often use ammonia as the coolant.

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) harvested from the Chesapeake Bay. The samples collected from 8 different spoilage trials were vacuum packed in Kapak SealPak pouches, 0.47 liter (2.5 mil PET/LLDPE) KAPAK (Minneapolis, MN). 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 as described in the subsequent section at ambient and refrigerated storage conditions, 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, 6

and 9 hour ambient, and 7, 8, and 9 day cold samples. Draeger-Tube® (Dräger-Röhrchen, Luebeck, Germany) analyses were conducted on reference samples 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

Crab meat, fresh picked, was collected from processing plant and held on ice at refrigeration temperatures (<4.4 °C) until division and analysis. This was completed within 1 hour of receipt. Initial bacterial load was enumerated by mesophilic aerobic plate count using standard AOAC approved methods as listed in the FDA *Bacteriological Analytical Manual* online (FDA, 2001). Twenty-five gram portions of well mixed crab meat were blended with 225 ml. sterile 0.1 % Peptone Buffer in a filter stomacher bag and stomached for 2 minutes 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).

Crab meat 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 (cold storage). Crab meat was periodically examined for sensory quality, packaged in 100-200 gram portions in Kapak 402 bags (1 pt. size, 6 ½ in. X 8 in.), vacuum sealed, labeled, and frozen overnight before placement in -80 °C freezer for long term storage. Fifty grams was used for bacteriological examination, as described above, at each increment of spoilage. Reference samples were immediately bagged, frozen, and stored.

Spoiled crab meat was evaluated for sensory attributes of odor, taste and texture, against reference samples using a 6 member trained sensory panel. The sensory panelists underwent training for 8-12 months describing the attributes of coded unknown blue crab meat samples spoiled under refrigerated (4 °C) and ambient conditions (21 °C). Samples were removed from -80 °C storage and thawed overnight in a refrigerator (4 °C). Packages were opened and the crab meat 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 crab meat samples. Sensory and microbiological analyses were conducted at the Virginia Seafood Agricultural Research and Extension Center, Hampton, Virginia.

Electronic Nose Analysis

Thawed 25 gram samples were placed into individual sealed 200 ml. glass jars (Ball Corporation, Broomfield, CO) 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 utilized individually sealed glass jars for the 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) was purchased from the local crafts store and used as a septum. The electronic nose used for this analysis was a Cyranose320™ (Smiths Detection, Pasadena, CA), equipped with 32 polymer-conducting sensors. Ten samples of known quality crab meat were used to build the training set

for each class, and 20 separate crab meat samples were analyzed blind to validate the system. The operating conditions were in agreement to the settings described in the manual. The settings used were as follows: the baseline purge 10 seconds, sample draw one 25 seconds, 1st air intake purge 10 seconds, and 2nd 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 minute equilibration time allowed the sensors to equilibrate before being exposed to another sample.

Compressed Air Method

This method used compressed tank breathing air (Airgas Mid America, Radford, VA), filtered through activated carbon and moisture traps cycled through a reference and sample container. One of the outlets on the reference container was connected to the purge inlet of the electronic nose to insure the nose was receiving clean, dry air, which 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 unknown crab meat samples were used to validate the system. Samples were not re-analyzed. 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, 1st sample gas purge 10 seconds, 1st air intake purge 10 seconds, and 2nd 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 minute equilibration time 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 this method. The reference (0 day) crab meat 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 unknown crab meat samples were used to validate the system. Samples were not re-analyzed. Both the sample and reference containers were cleaned between samples. A 2 minute equilibration time allowed the sensors to equilibrate before being exposed to another sample.

Draeger-Tube® Analysis

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

Statistical Analysis

Electronic Nose data was processed using the PC Nose software version 6.5 (Smiths Detection, Pasadena, CA), JMP IN 5.1 (SAS Institute, Cary, NC), and SAS 9.1 (SAS Institute, Cary, NC). 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 and stepwise discriminant analysis 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 previously been used for food quality applications (Chantarachoti and others 2006; Hu and others 2005; O'Connell, 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. 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. For the compressed air and crab reference methods filtered clean, dry air from the same tank and filtering procedure was used. The manufacture recommends using ambient air for the test; however, it is possible that results can be affected because the overall quality and composition of ambient air can vary on a daily basis. 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. Validation of each method was performed using 20 coded crab meat samples of unknown quality.

The compressed air method achieved 100 % correct separation of all 5 groups of sequentially spoiled crab meat (Figure 1), as verified by a cross-validation technique. 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 canonical discriminate analysis (CDA), and stepwise discriminate analysis. 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 unknown samples were not as accurate. Only 30 % of the unknowns were correctly identified using CDA and stepwise discriminate analysis models. The PCA model was even less effective at correctly identifying coded samples; only 20 % of the unknowns were correctly classified using this model. The results of coded unknown sample analysis are shown in Table 1.

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

The crab reference method utilized reference crab meat (high quality meat) as a baseline for comparison of spoiled samples to unspoiled samples. Only 50 % of the samples were

correctly separated utilizing PCA as the statistical method (Figure 3). CDA was able to correctly separate 68 % of the groups, while stepwise CDA was able to correctly separate 92 % of the groups at $\alpha = 0.05$. Therefore eliminating some of the correlation among sensors seems to improve separation. Using this method it is not surprising that correlation between sensors is high, since many attributes between the reference and sequentially spoiled samples are similar, such as humidity, sweetness, and bitterness. However, the crab reference method should accentuate the differences due to spoilage since those attributes should be the only differences between the samples. For the coded unknown crab meat samples CDA classified 35 % of samples correctly. The PCA and stepwise CDA results were less accurate correctly identifying only 20 % of the unknown samples. It was expected that stepwise CDA analysis on the unknown coded crab meat samples would result in the highest correct classification method, since for the previous analyses separation and identification of unknown samples was highest using this statistical method.

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

ANOVA analysis and declassification of some of the groups was also performed on the data. ANOVA analysis of individual sensors of the 32 sensor array was examined to see if one sensor could be used to determine spoilage levels. It is possible that one appropriate sensor may correlate with a particular spoilage attribute, but, for these analyses one sensor was not more

effective than PCA, CDA, and stepwise CDA for correctly identifying spoilage levels. The 6 and 9 hour ambient, and 7 and 9 day cold samples were grouped together, resulting in a 3 group model. Sensory evaluation indicates that the ambient and cold spoiled increments have similar attributes regardless of spoilage time (Table 2). However, combining the groups did not considerably improve the accuracy of the unknown identification results. It is possible that water may have interfered with the sensors resulting in confounded results. However, when the water sensitive sensors, number 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 the same sensors that are sensitive to water are also sensitive to the polar components present in the aroma profile of spoiling crab meat. This could possibly be investigated by analyzing known chemical odorants.

In order to gain insight why the coded unknown results differed from the training data using known samples, the unknown coded sample results were plotted using the training data from the Compressed Air Method (Figure 4). Although, the reference (0 day) samples were part of the coded unknown sample set; none were correctly classified as a reference sample by the electronic nose (Table 1). This was puzzling, as the samples used were of the same quality, thus difference between the training data and the coded unknown sample data should be due to deficiencies in the electronic nose system.

Perhaps the electronic nose was not sufficiently sensitive to distinguish between minor differences between classes of spoiled crab meat. The samples tested were all crab meat samples of varying degrees of spoilage. Perhaps if the samples comprised different types of crustacea, the results would have improved. Ammonia which has been associated with crab meat spoilage can solubilize in water. This may have led to it not being at an appreciable level to be detected

by the electronic nose. In addition, no recommended calibration procedure exists for this electronic nose. It is also possible that as the conducting polymer sensors age, sensor drift maybe an issue with electronic nose devices (Hines and others 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 increase the intensity of spoilage attributes (odors) increase.

Suggestions for improving the electronic nose results include using fewer sample groups, bootstrapping, and neural networks. Rather than using 5 groups for training, perhaps use only a high quality group and a low quality group. Bootstrapping is a concept 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 improved the classification accuracy from 87 % to 98 % (Balasubramanian and others 2004).

The technique that shows the most promise for these types of data is neural networks. 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 *neurones* which are connected together in a certain way (known as its 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 was found to significantly improve pattern recognition for electronic

nose systems (Zhang and others 2003), and was found to be effective for quantifying different spice mixture compositions using and electronic nose system (Zhang and others 2005). However, due to time constraints this technique was not applied to this data set.

Draeger-Tube® Analysis

The data obtained from analyzing the sequentially spoiled samples was plotted in a standard curve, using an average of 2 replications for each group (Figure 5). These data shows an expected pattern; as the product spoils, the ppm value for ammonia increases. This data fits an exponentially equation rather than a linear equation. This is not surprising since microbial growth has been found to obey an exponential equation (Marr, 2000). In addition ANOVA analysis was carried out on the Draeger-Tube® results (Figure 6). All groups were significantly different at $\alpha = 0.05$ using a Tukey-Kramer HSD procedure. This is a more conservative statistical test than Fisher's LSD (student's t-test). For the comparison wise test (Tukey-Kramer) the type I error rate is smaller, leading to a more accurate test.

Coded unknown samples were also analyzed by this method (Table 3). Correct classification of 83 % of the coded unknowns was achieved. Code 411 was the only sample to be totally misclassified, the sample was a 7 day cold sample, and was classified as 6 day cold by ANOVA and the standard curve. Codes 247 and 302 were not found to be significantly different from the 0 day cold or 4 day cold samples according to Tukey-Kramer ANOVA analysis (Figure 7). Therefore these two samples could be classified into either group. However, these results are not surprising, since the sensory analysis and aerobic plate counts, showed that the 0 day cold and 4 day cold samples are quite similar (Table 4). Sensory analysis classified the 0 day cold and 4 day cold samples as having attributes associated with a high quality sample. When

spoilage was estimated using the 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. Thus, the coded samples 247 and 302 were considered to be correctly classified.

The Draeger-Tubes® rely on a simple reaction that may be modified so that it can be more easily understood by the food processing industries. The tubes contain a pH indicator. The reaction principle is as NH_3 (a base) is exposed to the pH indicator, a color change occurs due to a rise in pH, as demonstrated by a change in color from yellow to blue by the pH sensitive material inside the tube. Perhaps this reaction can still be used, but may need to be modified to develop a dipstick test that industry could easily use. Still the Draeger method is simpler and more rapid compared with determination of ammonia and total volatile bases, and the technology is transferable to industry. Although some issues with vacuum, and uniformity of the evolution of the gas up the detector tube exist with this method. It is possible to improve the method.

CONCLUSIONS

The results for the coded unknown validation samples indicate that if the Cyranose 320™ electronic nose is used as a quality indicator for crab meat improvement in its technology is needed. The results presented here indicate that the Draeger-Tubes® show promise for determining spoilage in crab meat, and could possibly be used as a quality control procedure by industry. However, more research needs to be conducted to determine if the method is applicable across batch, environment and species.

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Table 1. Crab Reference Method Identification Results. PC Nose Classification was accomplished using built in PC Nose software. Discriminant analysis was performed using SAS products.

Code	PC nose classification	DFA classification - stepwise	DFA classification	Actual Classification
636	Reference	Reference	reference	9 hr ambient
936	6 hr ambient	6 hr ambient	Reference*	Reference
603	Reference	Reference	reference	8 day cold
916	Reference	Reference	reference	9 hr ambient
334	6 hr ambient	6 hr ambient	reference	9 hr ambient
491	7 day cold	7 day cold	6 hr ambient	9 hr ambient
737	7 day cold	7 day cold	6 hr ambient	Reference
161	6 hr ambient*	6 hr ambient*	6 hr ambient*	6 hr ambient
700	6 hr ambient	6 hr ambient	6 hr ambient	8 day cold
877	6 hr ambient*	7 day cold	6 hr ambient*	6 hr ambient
226	7 day cold	7 day cold	9 day cold*	9 day cold
099	7 day cold	9 day cold *	9 day cold*	9 day cold
102	7 day cold *	7 day cold *	7 day cold*	8 day cold
088	7 day cold	7 day cold	7 day cold	9 hr ambient
457	7 day cold	7 day cold	6 hr ambient	Reference
964	7 day cold *	7 day cold *	7 day cold*	8 day cold
211	7 day cold	7 day cold	7 day cold	Reference
901	7 day cold	7 day cold	7 day cold	9 hr ambient
321	7 day cold	7 day cold	9 day cold	6 hr ambient
241	7 day cold	7 day cold	7 day cold	6 hr ambient

* Denotes correct identification

Table 2. Aerobic Plate Counts and Sensory Results for Samples used for Cyranose 320™ Analysis.

Sample	APC (cfu/g)	Sensory Classification
Reference (0 day)	$9.2 \bullet 10^4$	High Pass
6 hr. ambient	$4.6 \bullet 10^5$	Mid-Pass
9 hr. ambient	$1.0 \bullet 10^6$	Mid-Pass/Low Pass
7 day cold	$7.5 \bullet 10^6$	Low Fail/Mid-Fail
9 day cold	$4.0 \bullet 10^7$	Mid-Fail/High Fail

Table 3. Dräger Coded Unknown Results. 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.

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

Table 4. Aerobic Plate Counts (APC) and Sensory Results of Samples used for Draeger-Tubes® Analysis.

Sample	APC (cfu/g)	Sensory Result
Reference (0 day)	$1.65 \cdot 10^5$	High Pass
4 day cold	$8.5 \cdot 10^5$	High 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

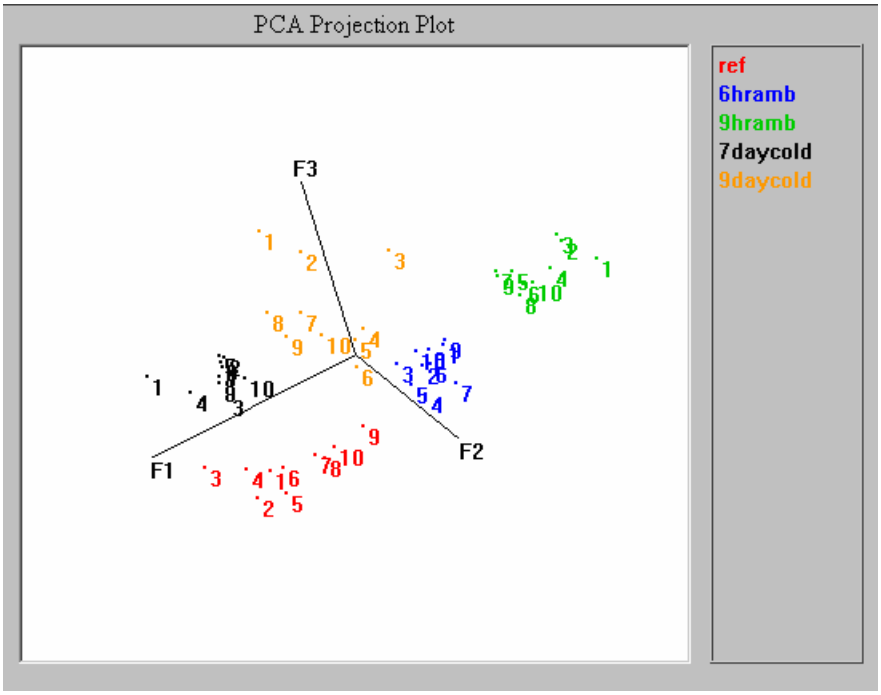

Figure 1. PC Nose PCA Projection Plot for Training Set Data for Compressed Air Method.

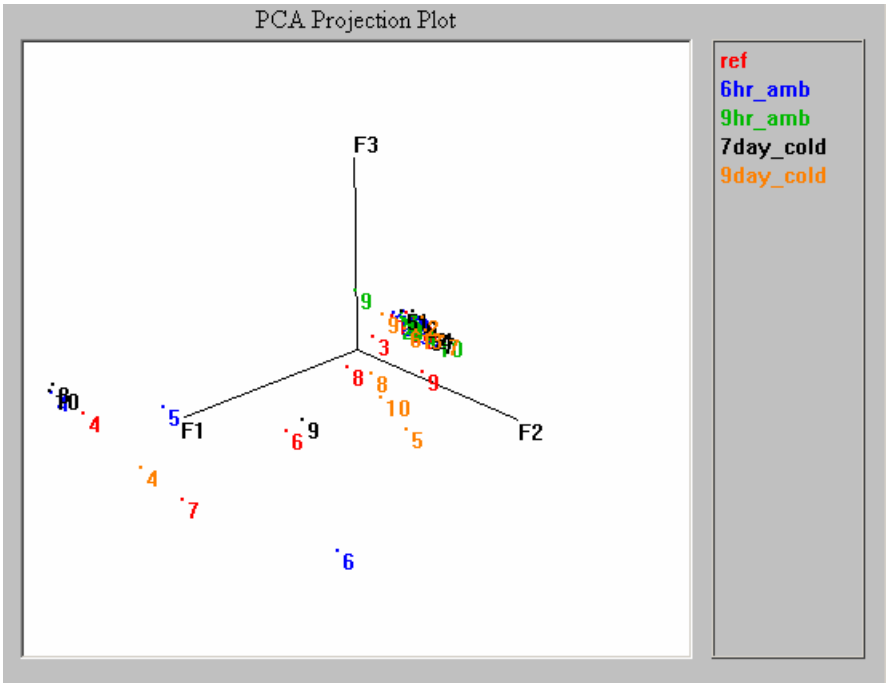

Figure 2. PC Nose PCA Projection Plot for Training Set Data for the Manufacturer's Recommended Method.

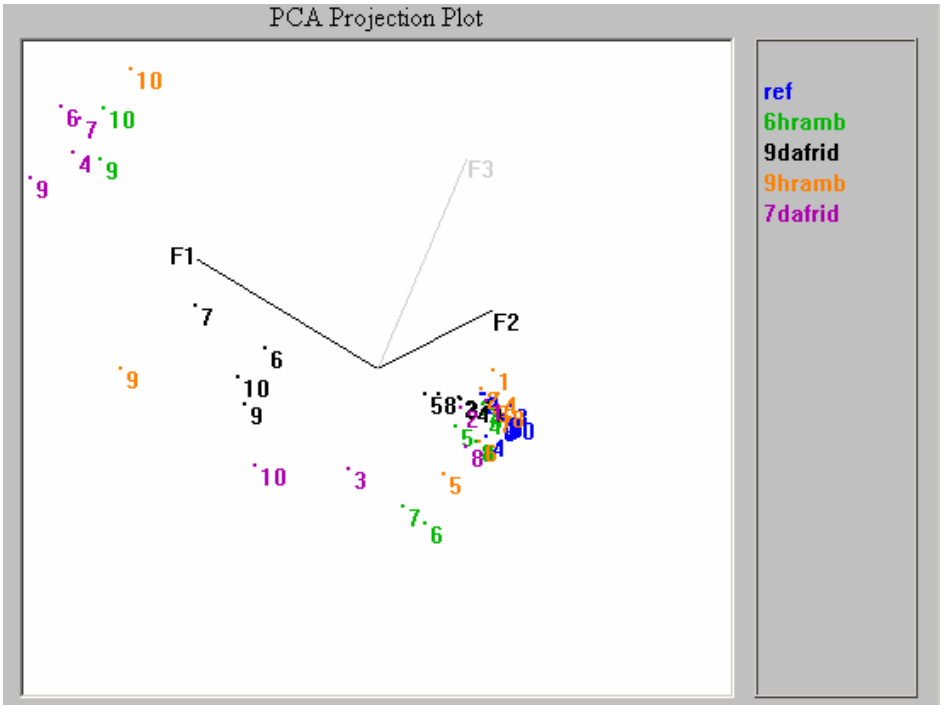

Figure 3. PC Nose PCA Projection Plot for Training Set Data for the Crab Reference Method.

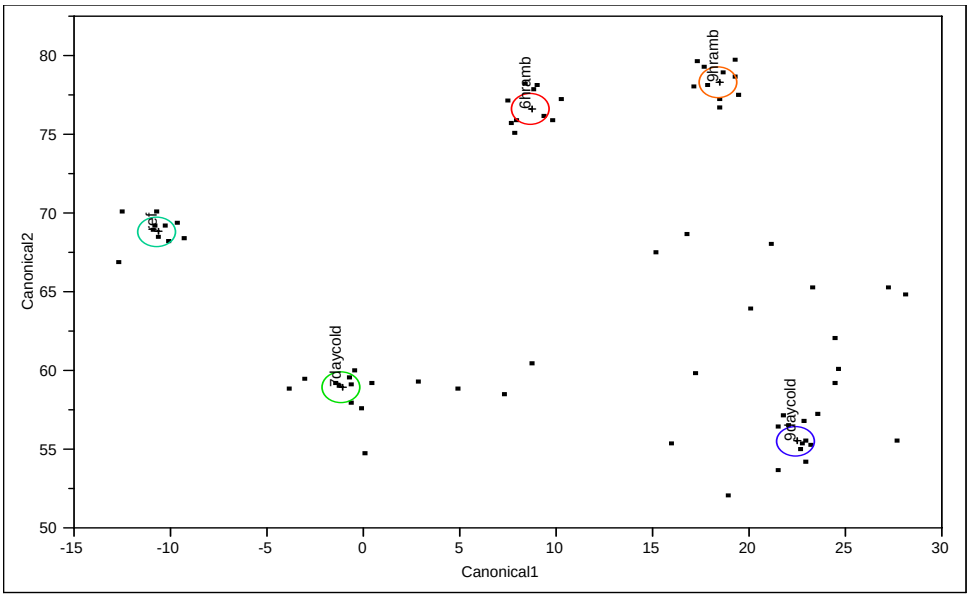

Figure 4. Compressed Air Method CDA Plot with Coded Unknown Samples Plotted.

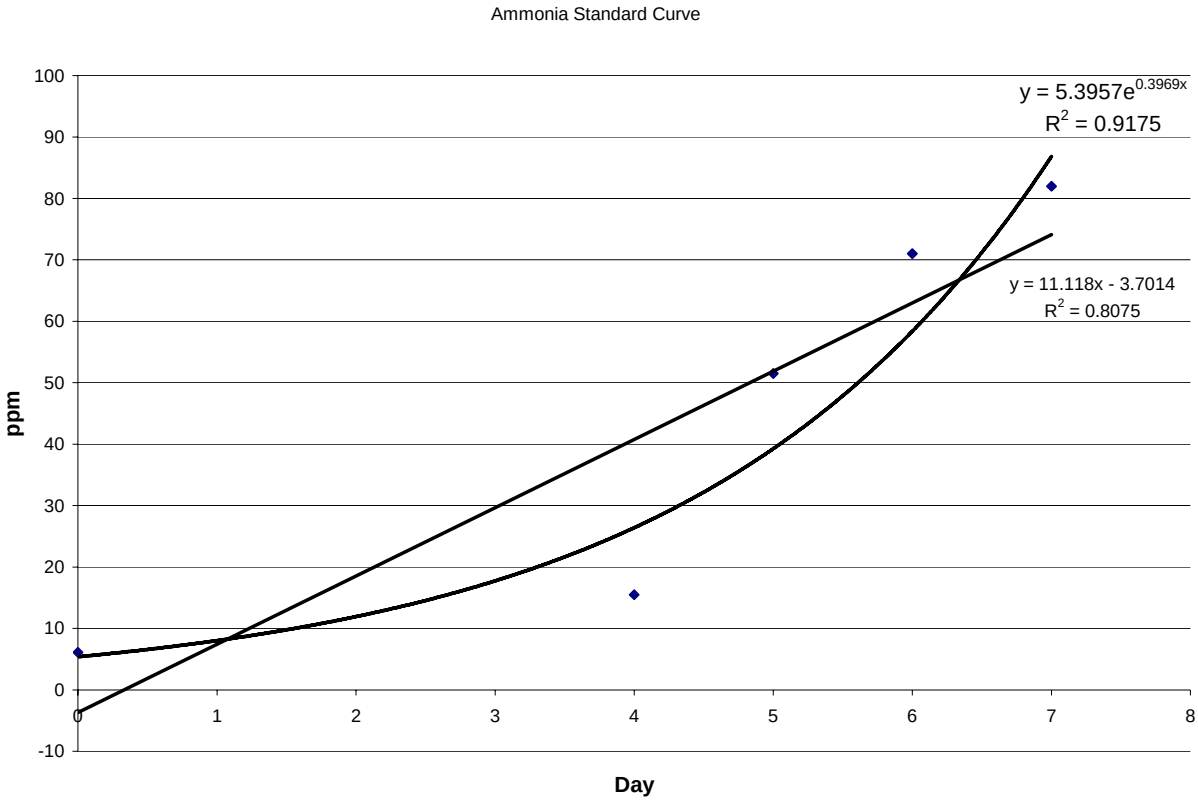

Figure 5. Ammonia Standard Curve. Both a linear and exponential equation are plotted on the same graph. The exponential equation better fits the data as is reflected in the R^2 value.

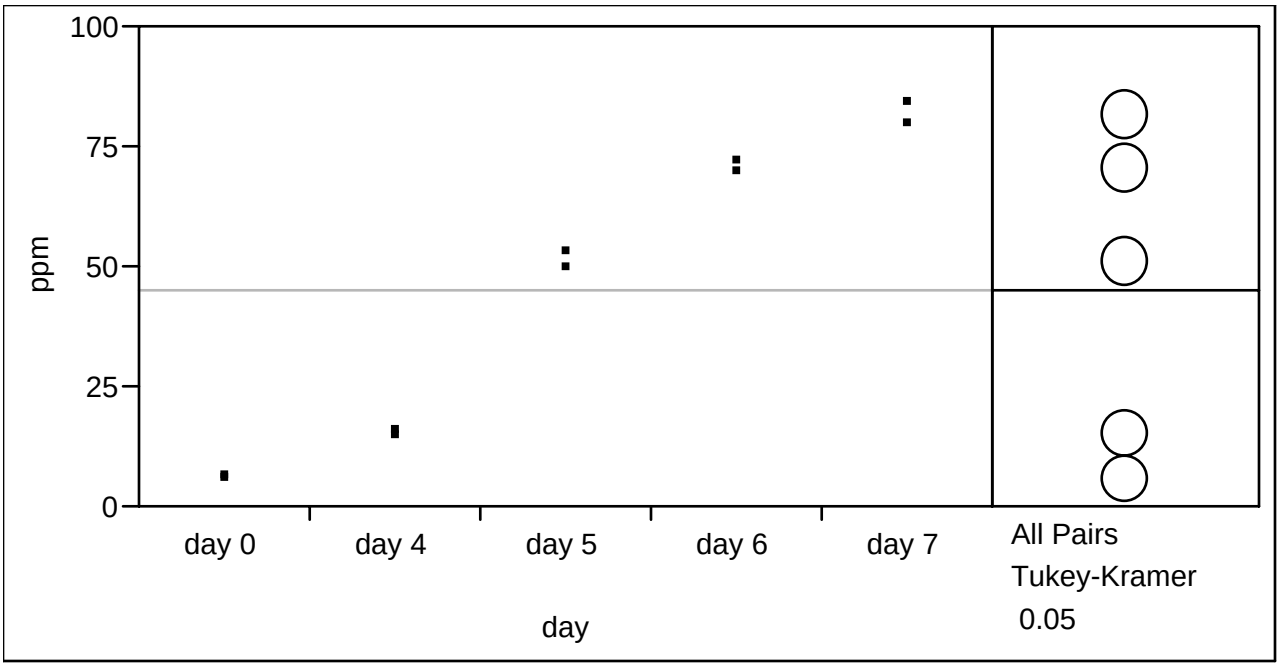

Figure 6. Ammonia Data Analyzed by the Tukey-Kramer Test for One-Way ANOVA. All groups were found to be significantly different at the $\alpha = 0.05$ level.

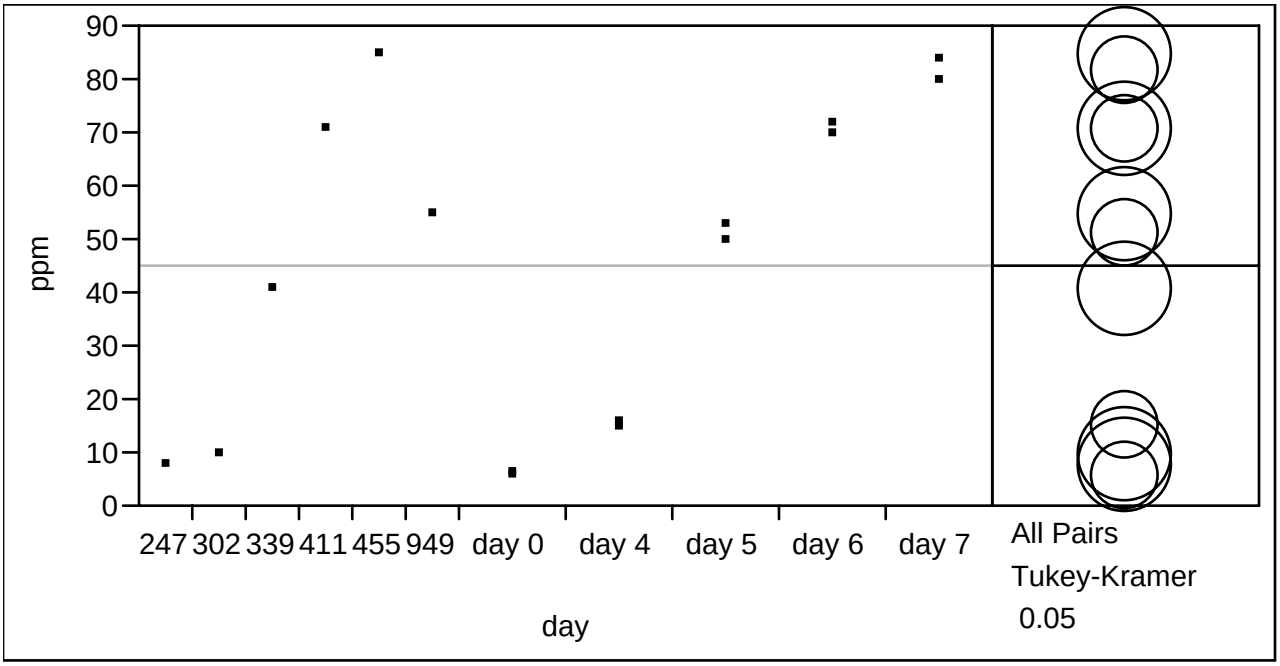

Figure 7. Ammonia Data Analyzed by the Tukey-Kramer Test for One-Way ANOVA with Coded Unknowns. A means comparison analysis was performed on this data and the statistical procedure identified into which spoilage day each coded unknown should be classified.

CHAPTER 4

Analysis of Volatile Spoilage Indicators of Blue Crab (*Callinectes sapidus*) Meat by Gas Chromatography-Mass Spectrometry

(Manuscript to be submitted to Journal of Agriculture and Food Chemistry)

Analysis of Volatile Spoilage Indicators of Blue Crab (*Callinectes sapidus)* Meat by Gas Chromatography-Mass Spectrometry

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ABSTRACT: Several instrumental methods were utilized to evaluate the aroma profile of spoiling blue crab (*Callinectes sapidus*) meat, solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) showed the most promise. The chemicals that were found to best correlate with spoilage were trimethylamine (TMA), ammonia, and indole. In addition, chemicals previously not identified in the aroma profile of crab meat were detected. Scan mode of the mass spectrometer was used to determine what compounds were extracted from the volatile profile of spoiling blue crab by the SPME fiber. Selected ion monitoring (SIM) mode was used for quantitative purposes, allowed for improved resolution, and was vital for obtaining a low level of detection. SIM mode allowed the spoilage related compounds to be detected in one run, and ambient sampling of the crab meat samples. TMA was found to be sensitive to the minor changes in the early stages of spoilage. Indole corresponded well with sensory results, which suggests that indole may be a promising indicator for detecting early, mid, and highly spoiled samples. It is feasible that this method can be applied to other crustaceans to determine spoilage level.

KEYWORDS: **Mass spectrometry, blue crab, solid phase microextraction, aroma, spoilage**

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INTRODUCTION

In general, seafood is extremely perishable, requiring more care than other muscle foods such as beef, pork, chicken, etc. That fish generally spoil faster than other muscle foods is a phenomenon identified in the early part of the 20th century. During this period, most catches were landed at ports far removed from the point of harvest. This fact, coupled with poor handling practices on board ships and on shore, invariably led to deterioration of large portions of fish and shellfish before they reached the consumer (*1*). Fish and shellfish muscles also contain less connective tissue compared with mammals and, furthermore, the cross-links formed by their collagens (the major constituent of connective tissue) are not as extensive (*2*). These factors contribute to an enhanced rate of fish and shellfish softening (*3*). The proximate composition of blue crab, which consists of approximately 80 % water contributes to its rapid deterioration at refrigerated storage (*4, 5*). A high water activity and loose connectivity between protein fibers, provides a rich environment for microbial growth, and as a result, rapid spoilage in seafood.

The mechanism for chemical breakdown of crab meat is not as well established as for other seafood such as fin fish; however it is thought to be relatively similar to other shellfish. Crab meat has a higher composition of free amino acids compared with fin fish, and thus the mechanism of decomposition is somewhat different. Blue crab does contain very minor amounts of carbohydrates, and is rich in calcium, phosphorous, magnesium, sodium, potassium, manganese, zinc, and iron (*6*).

Spoilage of crustaceans is caused by (1) biological reactions such as oxidation of lipids, reactions due to activities of the endogenous enzymes, and (2) the metabolic activities of microorganisms. In the post-mortem animal, enzymes from spoilage microorganisms primarily

metabolize the shellfish muscle, producing a wide variety of volatile compounds causing off-flavors and odors.

Trimethylamine oxide (TMAO) is an osmoregulatory compound found in a large number of marine fish and shellfish. It is broken down to trimethylamine (TMA) by either endogenous enzymes or exogenous trimethylamine oxidase (*8*). TMA is a volatile amine responsible for an unpleasant fishy odor. In the fish family Gadidae during frozen storage TMAO is degraded to dimethylamine (DMA) and formaldehyde in equimolar quantities (*9*). TMA is related to bacterial spoilage in marine species during iced or refrigerated storage and can be used as a quality index (*10*). TMA reacts with lipids in the fish muscle to produce the characteristic "fishy" odor associated with low-quality fish. When the oxygen supply is depleted, many of the spoilage bacteria utilize TMAO as a terminal hydrogen acceptor, thus allowing them to grow under anoxic conditions (*1*). This could be one of the reasons why microbial spoilage is such an issue in crustaceans. Other compounds produced as a result of microbial activity are hydrogen sulfide, dimethyl sulfide, and methyl mercaptan from sulfur-containing amino acids: various amines and ammonia from amino acids; lower fatty acids from sugars such as glucose and ribose; carbonyl compounds from lipids; and indole, skatole, putrescine, and cadaverine from proteins (*11-13*). However, the aforementioned compounds are not simply a product of microbial degradation, endogenous enzymes and nonenzymatic reactions (namely lipid oxidation) also play a role. Loss of freshness, which often precedes microbial spoilage, primarily involves autolytic reaction controlled by endogenous enzymes present in the muscle tissue as well as those leaking from the gut (*1*). Carbonyl compounds are formed from polyunsaturated lipids, oxidation of the lipids lead to the formation of hydroperoxides which then form short chain aldehydes, ketones, alcohols and other oxygenated compounds (*14*). These

reactions are initiated by light, heat, metals, or by other free radicals and peroxides. Since these constituents are also present in the crab matrix, it is feasible that oxidation can occur during storage leading to the development of volatile off-flavors and odors.

Analytical techniques used to evaluate spoilage include microbial, chemical methods (such as total volatile bases and total volatile acids), and sensory analysis (*15-17*). One of the drawbacks for these analyses is that how the product was packed (i.e. polymer packaging), storage temperature, storage time, etc. all can affect the results.

There are several methods that have been used to evaluate the volatile chemical profile of foods. The volatile chemical profile associated with crab meat has not been extensively studied. One reason is that extraction of these compounds is difficult and laborious and aroma compound levels are at low concentrations. One method used is static headspace analysis. A few milliliters of vapor above the food is removed into a gas-tight syringe and directly injected into a GC. However, since the volatiles of interest are not sufficiently concentrated detection limit issues arise.

Dynamic headspace sampling is a purge and trap system used to concentrate volatile compounds. The sample is purged with an inert gas, such as nitrogen or helium, which strips aroma constituents from the sample. The volatiles in the purge gas are then trapped, or removed from the gas stream. Matiella and Hsieh (*18*) used this technique to identify fifty-three compounds in blue crab meat. Simultaneous distillation/solvent extraction (SDE) is one of the oldest and most popular methods for aroma analysis. The SDE method was created in 1964 when Likens and Nickerson designed an original device for the analysis of hop oil (*20*). Since then, SDE has been mainly used for analysis of flavors and fragrances, and pollutants (*21*). Simultaneous distillation/solvent extraction was used by Chung and Cadwallader (*22*) to identify

seventy-seven compounds in blue crab meat. The aroma profile is influenced by volatility of the aroma compounds, solubility during solvent extraction of the distillate and, finally, volatility again during the concentration of the solvent extract. The aroma isolate contains nearly all the volatiles in a food, but their proportions may only poorly represent the true profile in a food (*19*). The popularity of this method is due to the fact that medium to high boilers are recovered well and a liquid isolate is obtained.

Solid phase microextraction (SPME) is a relatively new technique used to isolate food aromas. Pawliszyn's group (*23*) was given credit as the first to develop this method for environmental analysis. Since then the technique has been widely used for environmental analyses (*24, 25*), and for volatiles in food (*26-28*). For SPME, an inert fiber is coated with a liquid adsorbent (for which there are several choices). The absorbent is placed in the headspace of a sample and allowed to adsorb volatiles for a period of time. The fiber is then thermally desorbed in the injection port of a GC, and the released volatiles are analyzed. The main advantage of SPME is that there are no solvents for contamination; it is simple, sensitive and rapid. In addition, SPME can be automated, when used in conjunction with an autosampler, making it ideal for rapid, routine analysis (*29*).

MATERIALS AND METHODS

Blue Crab Samples for GC-MS Analyses

Experiments were conducted with body meat (i.e. regular or special) obtained from a local crab processing company (Hampton, VA) harvested from the Chesapeake Bay. The samples were vacuum packed in Kapak SealPak pouches, 0.47 liter (2.5 mil PET/LLDPE)

KAPAK (Minneapolis, MN). 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 as described in the subsequent section at refrigerated storage conditions, frozen at -18 °C for 12-20 hours and then stored at -80 °C until needed for analysis. The following groups were analyzed by GC-MS: reference, 4, 5, 6, and 7 day cold samples.

Samples were thawed overnight in a refrigerator (4 °C) before analysis. Samples were brought to 40 °C in a bag using a water bath. Bags were stomached for 1 minute at 230 RPM (Seward, Stomacher® 400 Circulator, Worthing, UK). Twenty grams were removed from the bag and put into a 40 ml. headspace vial, fitted with a Teflon-lined septum. Headspace was allowed to accumulate for 5 minutes before piercing the septum with the SPME fiber.

Volatile Compound Analysis

A 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex™ fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace sampling. This fiber is recommended by Supelco Inc. for amine analysis. The fiber was conditioned prior to use in the gas chromatograph injection port at 270 °C for 0.5 hours as recommended by the producer. Volatiles were sampled for 15 minutes at ambient (21 °C) or with the vial submerged in boiling water (99.7 °C). After adsorption, the fiber was then immediately inserted into the injection port of the gas chromatograph and the run was started manually. The fiber was left in the injection port for the 4 minutes of the run and then removed. A Shimazdu GC-17A gas chromatograph (Shimazdu Scientific Instruments, Inc., Columbia, MD) coupled to a QP5050A MSD quadrupole mass spectrometer (Shimazdu Scientific Instruments, Inc., Columbia, MD) was used for the analysis. The GC-17A was equipped with a DB5-MS (60 m length x 0.25 mm i.d. x 1 μm film

thickness, J & W Scientific, Folsom, CA) column. Operating conditions for the GC were: splitless mode using a narrow bore SPME insert, helium flow 0.7 ml. $minutes^{-1}$, linear velocity 22 cm $seconds^{-1}$ initial oven temperature 50 ºC (5 minute hold), then 10 ºC min to 300 ºC (10 minute hold), injector 270 ºC, interface 230 ºC. The mass spectrometer was operated both in scan and selected ion monitoring (SIM) modes, GC conditions were the same for both analyses. For scan mode, the scan range in mass-to-charge ratios (m/z) was 15-450 m/z, scan speed was set to 1000, threshold was set to 1000, scan interval 0.5 seconds, solvent cut time 5 minutes, MS start time 5.2 minutes. For SIM mode, the following (m/z) were selected: 15, 30, 42, 58, 59, 62, 63, 89, 90, and 117. The following SIM settings were used: scan interval 0.5 seconds, solvent cut time 4.5 minutes, MS start time 4.6 minutes. All sample groups were run in triplicate. Volatile compounds were identified by comparison of their retention indices and mass spectra with authentic standards for ammonia, trimethylamine, and indole (Sigma-Aldrich, Saint Louis, MO) all which were analytical grade or, only by Wiley Registry of Mass Spectral Data 7^{th} Edition.

Quantitative Analysis

Quantitative values for ammonia, trimethylamine (TMA), and indole in blue crab meat was determined with a standard curve using analytical grade standards (Sigma-Aldrich, Saint Louis, MO). An aqueous solution of the standards was made. These standards by comparison of retention index also functioned as positive identification of the aforementioned compounds in blue crab meat.

Microbial and Sensory Analysis

Crab meat, fresh picked, was collected from processing plant and held on ice at refrigeration temperatures (<4.4 °C) until division and analysis. This was completed within 1 hour of receipt. Initial bacterial load was enumerated by mesophilic aerobic plate count using standard AOAC approved methods as listed in the FDA *Bacteriological Analytical Manual* online (*30*). Twenty-five gram portions of well mixed crab meat were blended with 225 ml. sterile 0.1 % Peptone Buffer in a filter stomacher bag and stomached for 2 minutes 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).

Crab meat was divided into 5 lb. increments and spread into 10 by 16 in. lidded plastic containers for controlled decomposition at 4 °C (cold storage). Crab meat was periodically examined for sensory quality, packaged in 100-200 gram portions in Kapak 402 bags (1 pt. size, 6 ½ in. X 8 in.), vacuum sealed, labeled, and frozen overnight before placement in -80 °C freezer for long term storage. Fifty grams was used for bacteriological examination, as described above, at each increment of spoilage. Reference samples were immediately bagged, frozen, and stored.

Spoiled crab meat was evaluated for sensory attributes of odor, taste and texture, against reference samples using a 6 member trained sensory panel. The sensory panelists underwent training for 8-12 months describing the attributes of coded unknown blue crab meat samples spoiled under refrigerated (4 °C) conditions. Samples were removed from -80 °C storage and thawed overnight in a refrigerator (4 °C). Packages were opened and the crab meat 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 crab meat samples. Sensory and microbiological analyses were conducted at the Virginia Seafood Agricultural Research and Extension Center, Hampton, Virginia.

Statistical Analysis

JMP IN 5.1 (SAS Institute, Cary, NC) was used for all statistical analysis of the GC-MS data. Analysis of variance (ANOVA) was performed on the group means of sequentially spoiled crab meat. Differences were considered significant when means of compared sets differed at the $p < 0.05$ level of significance using a Fisher's LSD procedure.

RESULTS AND DISCUSSION

Several instrumentation methods were evaluated to determine the most effective analytical method to identify volatiles that can be used to evaluate crab meat quality. In that regard, SPME-GC-MS showed the most promise in identifying aroma volatiles indicative of spoilage in crab meat. The aroma profile of blue crab meat during spoilage was investigated using SPME and gas chromatography-mass spectrometry utilizing both scan and SIM modes. The three chemicals that were shown to correspond best with spoilage were ammonia, trimethylamine, and indole (Table 1). Ammonia is used in sensory analysis as an indicator of spoilage in fishery products (*31*). Ammonia was an effective method to determine fish quality by using an ammonia ion-selective electrode (ISE) (*32*). However this technique measured "apparent ammonia" which included other amines, not solely ammonia. The technique used in this study is more selective. In scan mode, the method could not determine if ammonia was

present, since ammonia co-eluted with water. The base peak of the mass spectrum for water was m/z 18, however, the m/z 17 also showed strong intensity. It was assumed that other substances were also present in the water peak due to unusual mass-to-charge ratios present in the mass spectrum. The base peak at m/z 18 indicated that the peak's main constituent was water. Using SIM mode, ammonia was distinguished from the water using m/z 15. The base peak of ammonia is m/z 17, but water also fragments giving a large relative intensity at m/z 17. Thus, it is difficult to distinguish what is attributed to the water, and what is attributed to the ammonia. The m/z 15 is a minor intensity for ammonia, but since water does not interfere with m/z 15, it was used to calculate the amount of ammonia present in the headspace.

The concentration of TMA in fish tissue is an accepted measure of spoilage in marine species (*33*). It is a volatile substance; characterized by a fishy/ammonia odor (*34*). Trimethylamine was separated from water (eluting just after water) and was positively identified using SIM or scan mode (Figure 1 & 2). When the sample is heated separation of the TMA peak is difficult since an appreciable amount of water is partitioned into the headspace during heating. The SPME fiber does not appreciably absorb water (*24*), but since crab meat contains roughly 80 % moisture, it was the major volatile present in the headspace and was also absorbed by the fiber. Using SIM mode without m/z ratios that do not fragment into water, a narrow, Gaussian peak without a leading edge or tailing was obtained (Figure 2).

Indole has also been shown to be an indicator of spoilage in shrimp (*35*). Indole was found utilizing SIM mode, and in scan mode when the sample was heated, since if the sample was not heated, insufficient concentrations of indole was volatilized into the headspace for absorption by the SPME fiber. Using SIM mode increases mass spectrometer sensitivity because only a few m/z are examined instead of a range of m/z. Utilizing SIM allowed for the spoilage

indicating chemicals to be detected in one chromatographic run. In addition the samples did not have to be heated, and artifact formation was less likely.

A surprising finding was the presence of butylated hydroxytoluene (BHT) in the samples that were heated. At first the presence was puzzling since the crab meat samples were not treated with an antioxidant dip before storage. The only reasonable explanation for the presence of BHT is the possible migration from the packaging material into the crab meat. The antioxidant BHT has been found to migrate from high density polyethylene and low density polyethylene films into food (*36-38*). It is a smaller, more volatile molecule than most other antioxidants. Therefore it is feasible that by heating to the sample to around 100 ºC caused it to partition into the gas phase in appreciable amounts for adsorption by the SPME fiber and detection by GC-MS.

The peaks from retention time 12 minutes to 35 minutes presence are mainly of unknown origin. It should be noted that these compounds are most likely present in a considerably smaller concentrations compared with the spoilage indicating compounds. Due to the specificity of the SPME fiber for medium length hydrocarbons; they appear in the results for the heated samples. In a study by Baek and Cadwallader (*34*) possible routes of formation of the volatile compounds were discussed. The reaction of 12- or 15-lipoxygenases and hydroperoxide lyase on eicosapentaenoic acid (EPA) is thought to be responsible for the formation of volatile 6-, 8-, and 9-carbon carbonyls and alcohols such as 1-octen-3-ol (Table 2). Through hydroperoxide decomposition as a result of autoxidation methyl linoleate will decompose to pentanal (Table 2) as one of its minor products (*39*). Polyunsaturated fatty acids present in shellfish are also susceptible to autoxidation. After lipid autoxidation, fresh aromas are replaced by stale, painty, and oxidized type aromas.

It was thought that some of the compounds identified in these aroma studies such as hexadecanal, 1,13-tetradecadiene, octadecanal, etc. may be associated with seafood contamination. In a study of petroleum pollution's effect on seafood mostly aromatic hydrocarbons were found to be present in the water soluble fraction since *n*-alkanes above C_{10} are essentially not soluble in seawater, and hence are not accumulated (*40*). Compounds mainly associated with seafood contamination are mainly polychlorinated biphenyls (PCBs) (*41*). In addition polyaromatic hydrocarbons (PAHs), chlordane, hexachlorobenzene, hexachlorocyclohexane, along with other metal contaminants were found in seafood from Quincy Bay in Massachusetts (*42*). Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) were found at trace levels in 147 food samples including 10 blue crab, 8 lobster, and 10 crawfish samples (*43*). Styrene (Table 2) was found to be one of the major aromatics to accumulate in salmon muscle due to tainting by petroleum hydrocarbons in Atlantic salmon (*Salmo salar)* (*44*), which was also found in this study. Other aromatic hydrocarbons were also found in this study, such as phenol, and 1,3-dichloro-benzene. It is possible these compounds were a result of environmental contamination.

Dimethyl sulfide was most likely a result of the breakdown of dimethylsulfoniopropionate (DMSP) found in costal waters from marine phytoplankton and seaweeds. There is evidence to suggest the breakdown of DMSP is due to enzymatic or microbial action (*45*). In addition, dimethyl sulfide could be the result of microbial breakdown of sulfur-containing amino acids. The area count for dimethyl sulfide was at its highest for day 6 samples and then decreased for day 7 samples (Figure 8). However for the other spoilage indicators, a predicted increase in area count was seen as storage days increased. A reasonable explanation is that the microorganisms must have been most active at this point, and then by day

7 became less active in the crab meat matrix, or that the enzyme responsible for the breakdown reached its maximum activity on day 6. In addition when examined by ANOVA analysis, days 4, 5, and 7 were not significantly different for dimethyl sulfide. However, on day 6 a significantly larger amount of dimethyl sulfide was present compared with other spoilage times.

Summary

Three chemicals found to correspond best with spoilage (Table 1) were examined statistically. Trimethylamine increased linearly (Figure 3). However, trimethylamine is associated with microbial spoilage, and microbial growth increases exponentially (*46*), but the day 7 samples may not have contained higher microbial numbers compared with other samples. The ANOVA analysis indicated that TMA is a good indicator of spoilage in the earlier stages, since adequate group separation of a day 0 and day 4 samples was achieved. Aerobic plate counts and sensory analysis show little difference between a day 0 and day 4 sample (Table 3). This suggests TMA may be a better indicator of the earlier stages of spoilage than the established methods for determining spoilage (sensory and APCs). However, in the later stages of spoilage, days 5, 6, and 7 group separation according to TMA was poor (Figure 4). This reflects that TMA may not be a good indicator of spoilage, as spoilage becomes more prevalent.

Indole followed an exponential equation (Figure 5). Indole changed little in the early stages of spoilage. No significant difference was seen in indole amount for a day 0 and day 4 samples, or day 5 and day 6 samples (Figure 6). However, a significant difference was seen between these samples and a day 7 sample. This suggests that indole may be a promising indicator for detecting early, mid, and highly spoiled samples. Excellent agreement of sensory analysis and APC group classification with the ANOVA indole results was seen (Table 3 &

Figure 6). For this reason, indole amount may be the best chemical indicator for use as an alternative to sensory analysis.

Ammonia followed an exponential equation. In scan mode ammonia could not be separated from water. Using SIM mode allowed for detection of m/z 15, which is attributed to fragmentation of the ammonia molecule, but since adequate column separation was not achieved for ammonia, it is possible that other chemicals may have contributed to this m/z, as ammonia was not retained on the column and was detected immediately after the void volume. Headspace quantitative analysis of ammonia is difficult since it is very volatile and fleeting. Solid-phase microextraction fibers have been found to have a definite linear range and competition between volatiles for binding sites can introduce errors (*47*), which can help explain the poor reproducibility (Figure 7). Ammonia was at a lower concentration compared with other volatiles in the headspace, thus it may have been out-competed. A general trend of increasing ammonia amount as a result of increased spoilage was seen, although not statistically significant (Figure 7). Perhaps if more replications are conducted, statistical significance could be achieved. Ammonia amount did not correlate with sensory and APCs as well as indole, but did mirror an exponential curve indicative of microbial spoilage. Thus, it is possible ammonia may actually be a better chemical indicator of microbial growth in seafood than TMA.

CONCLUSIONS

The goal of these analyses was to develop a method to identify chemical indicators of spoilage that could be used as an alternative to sensory analysis. Some promising indicators of spoilage have been established. However, if these indicators will work across many batches of

samples and across species is still in question. Therefore, further research to answer some of these questions is needed in this area.

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Table 1. Approximate Quantitation Amounts in parts per million (ppm). The values were computed by use of a standard curve using analytical grade standards of the chemicals below.

Sample	Ammonia	TMA	Indole
0 day cold	5	7	2
4 day cold	8	22	2
5 day cold	8	30	6
6 day cold	12	31	7
7 day cold	20	41	23

Table 2. Comparison of Aroma Compounds identified in different studies of crab meat products. In this study presence was determined by mass spectrum library match, unless otherwise indicated.

Chemical Compounds	Present Study	Matiella & Hsieh (*18*)	Chung & Cadwallader (*22*)	Chung & Cadwallader (*48*)	Chung & Cadwallader (*49*)
Aldehydes					
3-methyl-butanal	x	x			
pentanal	x	x			
hexadecanal	x				
octadecanal	x				
tetradecanal	x				
Alkanes/Alkenes					
tridecane			x		
tetradecane			x		
hexadecane	x		x		
dodecene	x				
tridecene	x				
tetradecene	x				
1,13-tetradecadiene	x				
Aromatics					
styrene	x	x	x		
phenol	x		x		
butylated hydroxytoluene	x				
1,3-dichloro-benzene	x				
Alcohols					
ethanol	x				
3-methyl-1-butanol	x		x		
1-hexanol	x		x		
1-octen-3-ol	x		x		
Ketones					
2-propanone	x		x		
2,3-butanedione			x	x	x
2-pentanone	x	x			

Chemical Compounds	Present Study	Matiella & Hsieh (*18*)	Chung & Cadwallader (*22*)	Chung & Cadwallader (*48*)	Chung & Cadwallader (*49*)
2-heptanone	x		x		
2-nonanone	x		x		
2-undecanone	x		x		
3-methyl-2-pentanone	x				
2-pentanone	x	x			
Miscellaneous compounds					
dimethyl disulfide	x	x	x		
limonene	x	x	x		
trimethylamine*	x		x	x	x
indole*	x		x		

* Indicates that presence was also confirmed by running an analytical standard.

Table 3. Aerobic Plate Counts and Sensory Results.

Sample	APC (cfu/g)	Sensory Result
Reference (0 day)	$1.65 \cdot 10^5$	High Pass
4 day cold	$8.5 \cdot 10^5$	High 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

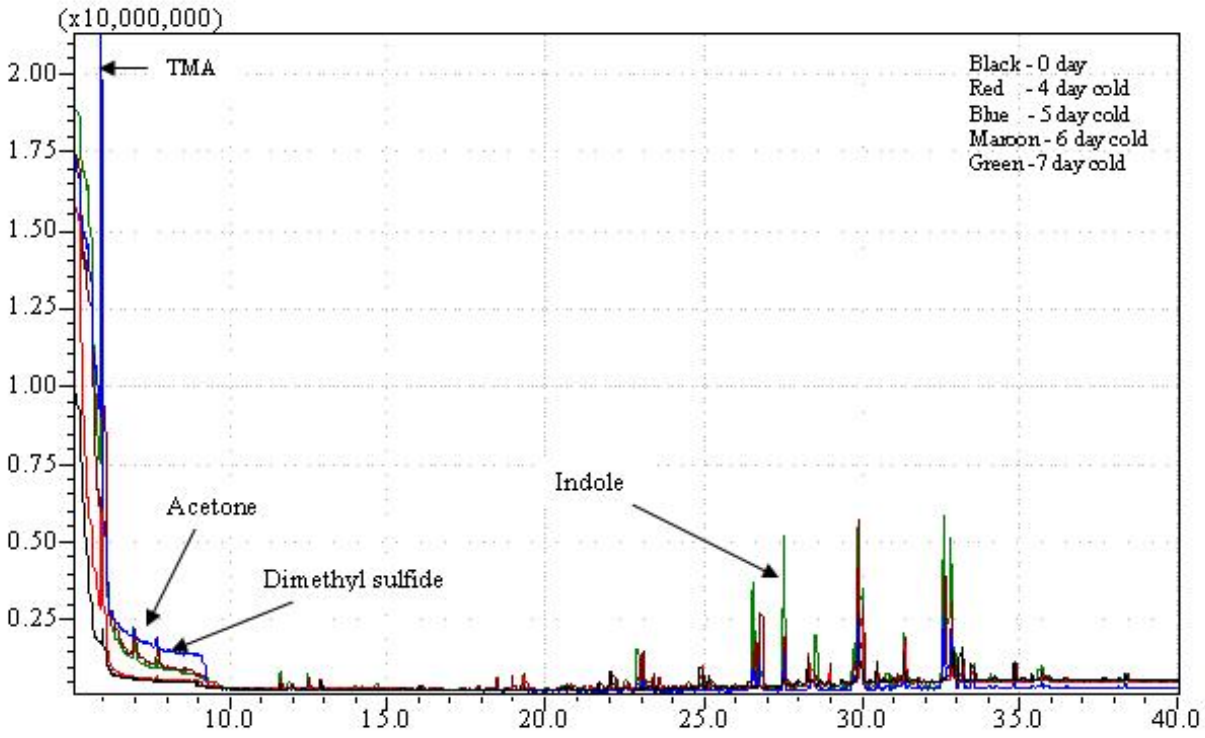


Figure 1 Scan mode chromatogram (sampled at 99.7 ºC).

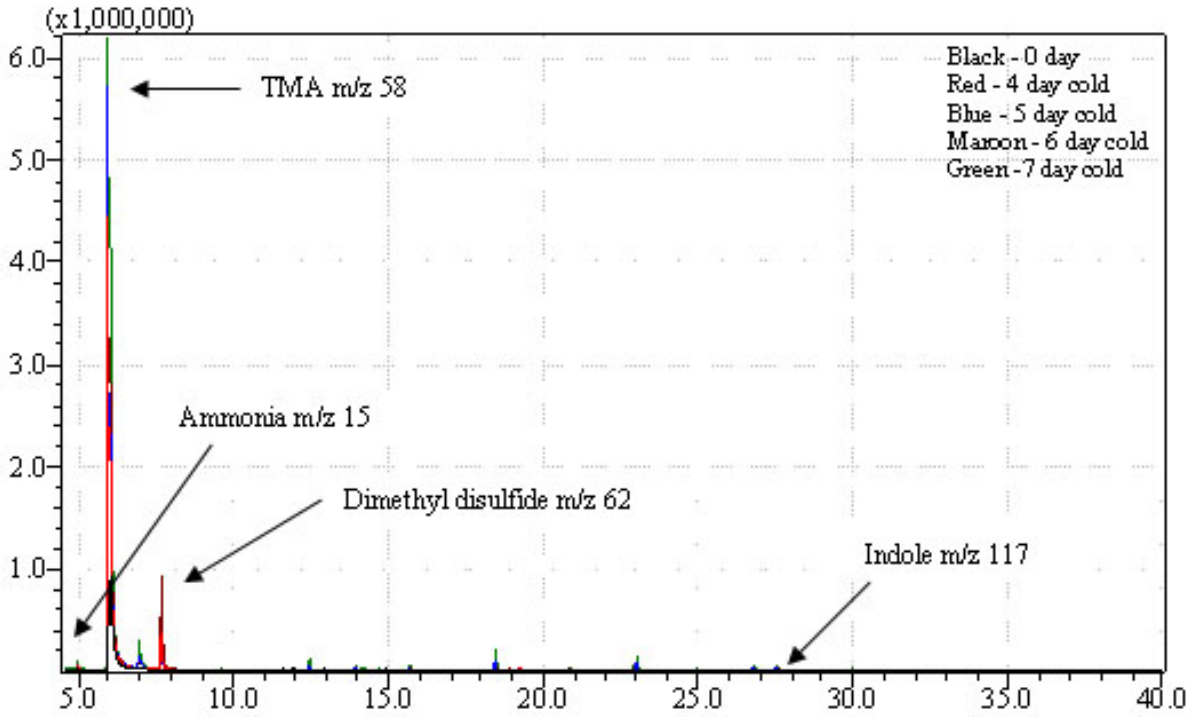


Figure 2 SIM mode chromatogram (Ambient 21 ºC).

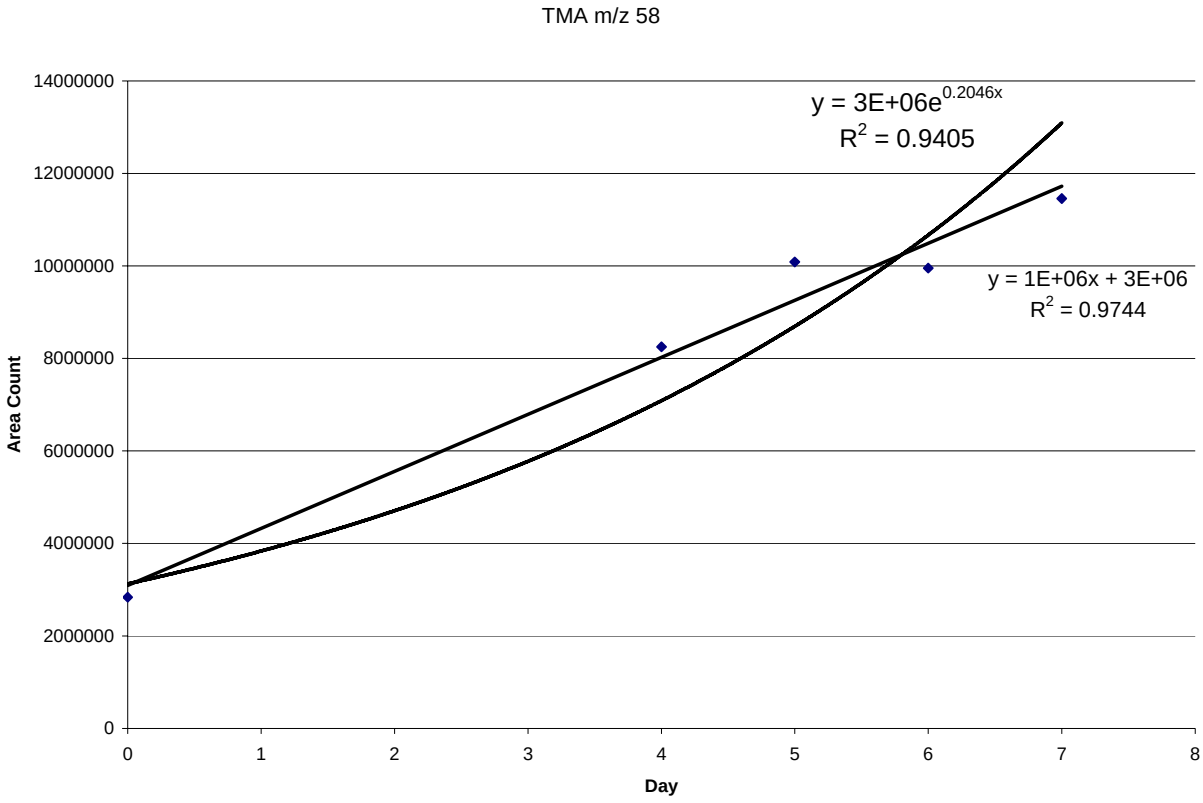


Figure 3 Trimethylamine amount as a function of Area Count. Each point is an average of three trials.

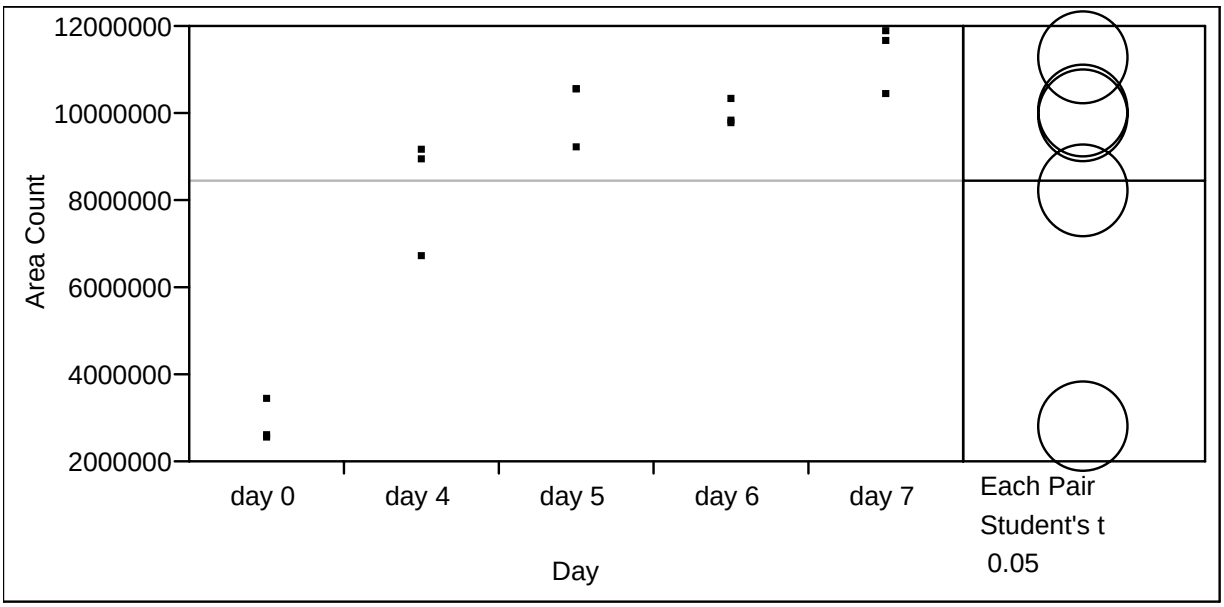


Figure 4 Trimethylamine ANOVA Analysis. Groups not connected by interval were found to be significantly different at $\alpha = 0.05$

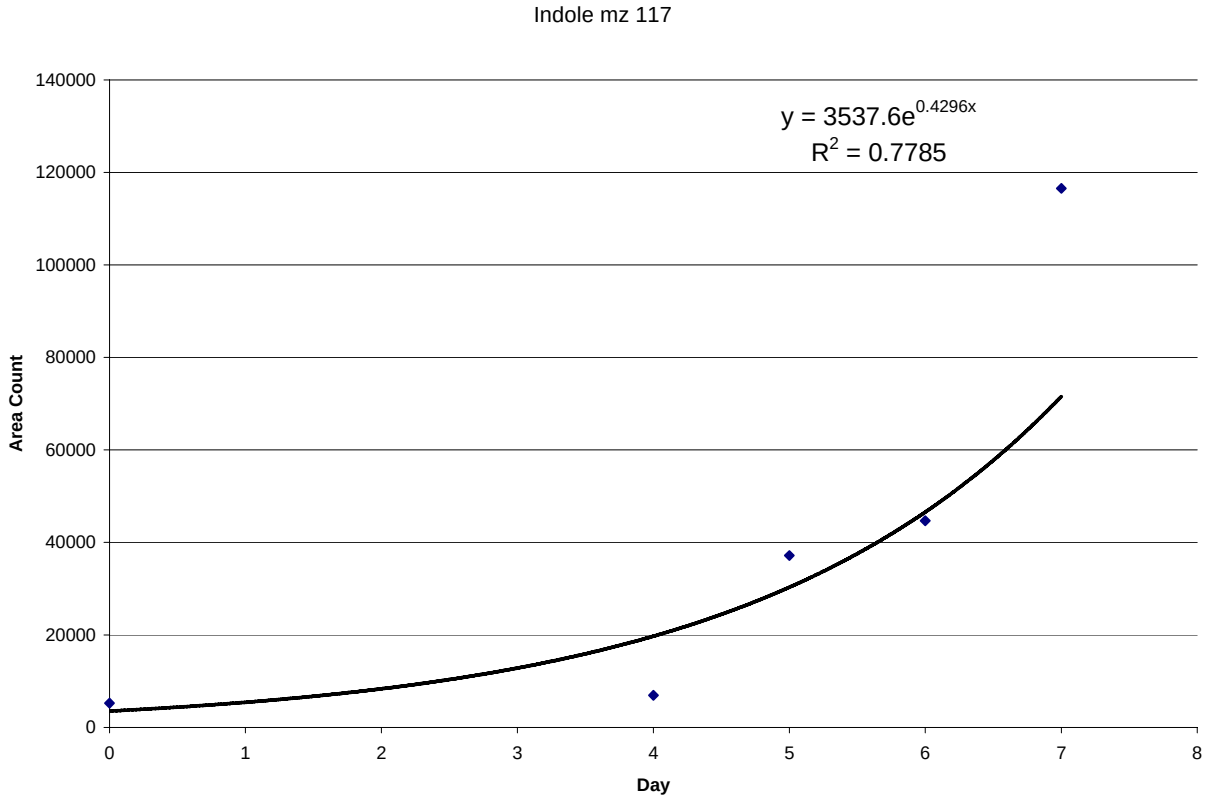


Figure 5 Indole amount as a function of Area Count. Each point is an average of three trials.

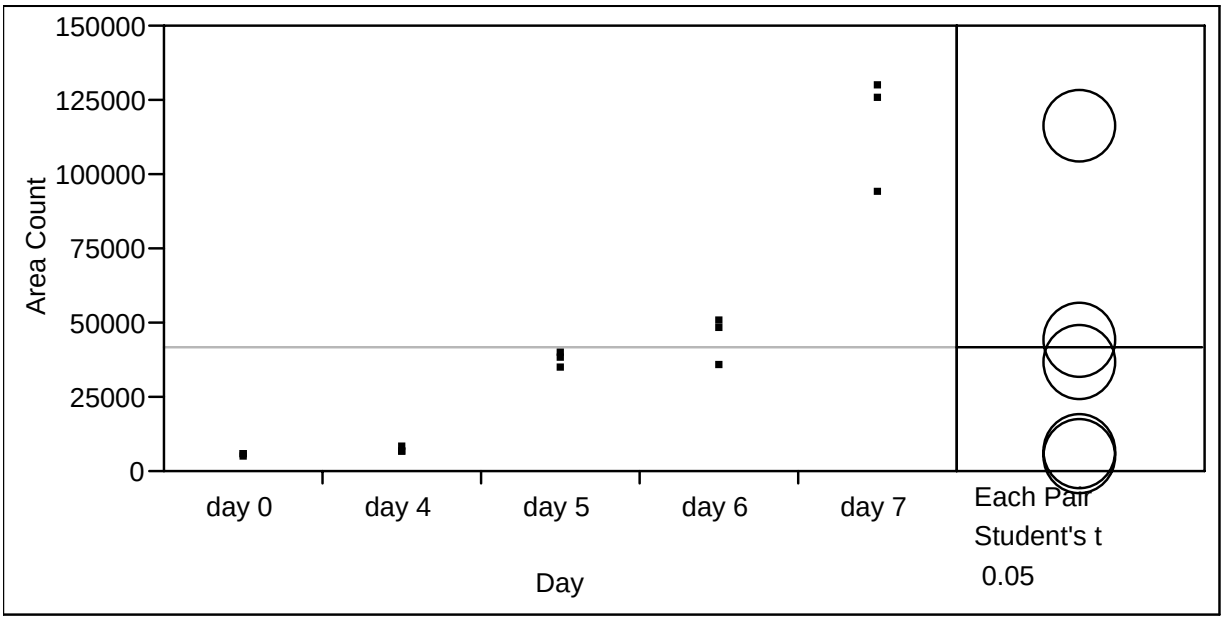


Figure 6 Indole ANOVA Analysis. Groups not connected by interval were found to be significantly different at $\alpha = 0.05$

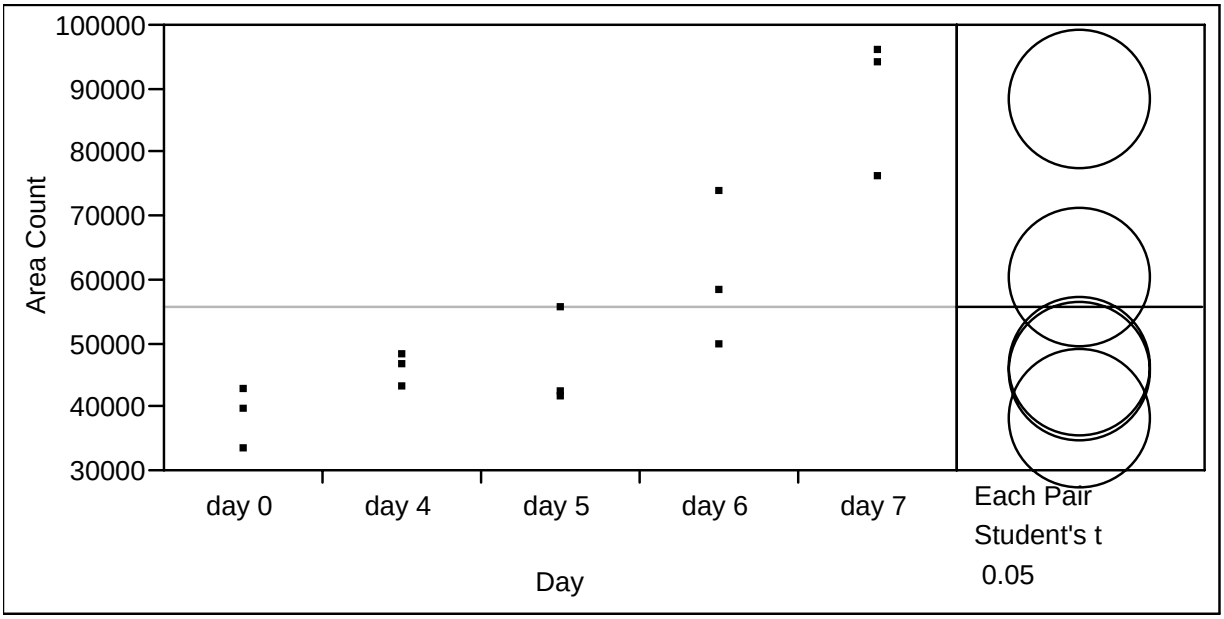


Figure 7 Ammonia ANOVA Analysis. Groups not connected by interval were found to be significantly different at $\alpha = 0.05$

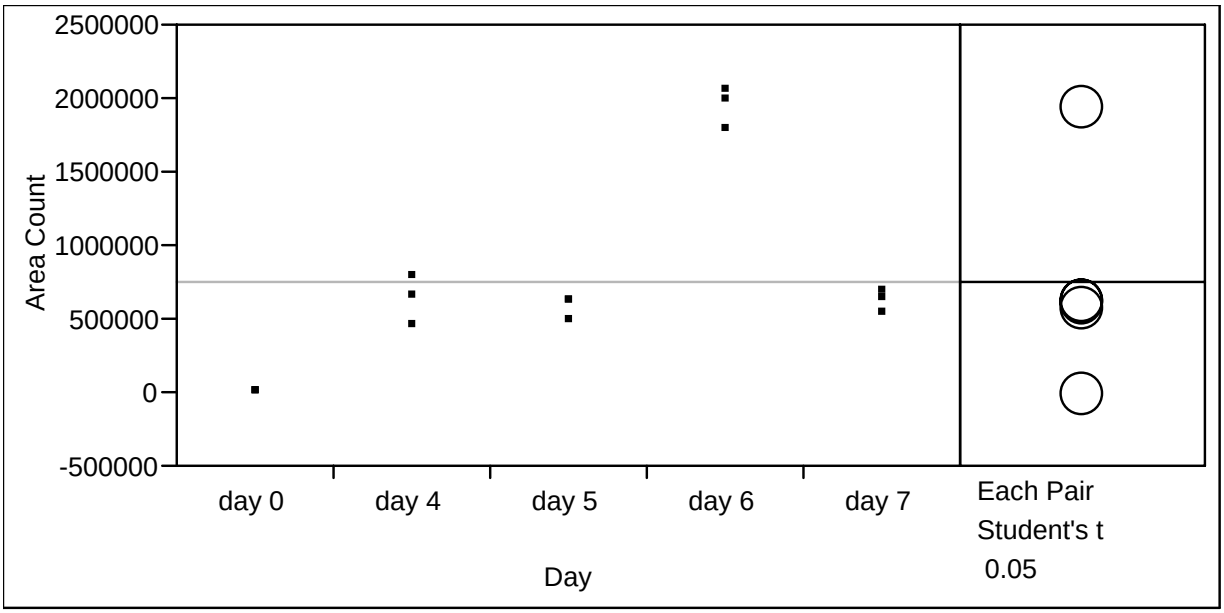


Figure 8 Dimethyl sulfide ANOVA Analysis. Groups not connected by interval were found to be significantly different at $\alpha = 0.05$

APPENDIX

Electronic Nose and Draeger-Tube® Studies

Table 1. Compressed Air Identification Results. PC Nose Classification was done using built in PC Nose software. Discriminant analysis was performed using SAS products.

Code	PC nose classification	DFA classification	DFA stepwise	Actual Classification
357	Unknown	7 day cold	7 day cold	Reference
128	Unknown	7 day cold	7 day cold	6 hr ambient
235	Unknown	7 day cold	9 hr ambient	6 hr ambient
521	Unknown	7 day cold	7 day cold	6 hr ambient
381	7 day cold	7 day cold	9 hr ambient	Reference
058	9 hr ambient	7 day cold*	9 hr ambient	8 day cold
548	9 day cold*	9 day cold*	9 day cold*	9 day cold
747	9 hr ambient	9 day cold	6 hr ambient	Reference
023	9 hr ambient	9 day cold	9 hr ambient	Reference
172	9 day cold*	9 day cold*	9 day cold*	9 day cold
801	9 day cold	9 hr ambient	9 hr ambient	6 hr ambient
809	9 hr ambient*	9 hr ambient*	9 hr ambient*	9 hr ambient
482	9 hr ambient	9 hr ambient	9 hr ambient	6 hr ambient
333	9 hr ambient	9 hr ambient	9 hr ambient	8 day cold
916	9 day cold	9 day cold	9 hr ambient*	9 hr ambient
222	9 hr ambient*	9 hr ambient*	9 hr ambient*	9 hr ambient
308	9 hr ambient	9 hr ambient	9 hr ambient	6 hr ambient
576	9 day cold	9 hr ambient*	9 hr ambient*	9 hr ambient
756	9 day cold	9 hr ambient	9 hr ambient	6 hr ambient
295	9 day cold	9 hr ambient	9 hr ambient	6 hr ambient

* Denotes correct identification

Table 2. Manufacturer's Recommended Method Identification Results. PC Nose Classification was done using built in PC Nose software. Discriminant analysis was performed using SAS products.

Code	PC nose classification	DFA classification - stepwise	DFA classification	Actual Classification
882	Reference	6 hr ambient*	6 hr ambient*	6 hr ambient
816	7 day cold	7 day cold	7 day cold	6 hr ambient
185	7 day cold	Reference*	Reference*	Reference
514	7 day cold	7 day cold	7 day cold	9 hr ambient
161	7 day cold	Reference*	9 day cold	Reference
165	Reference	Reference	Reference	8 day cold
765	Reference	7 day cold	7 day cold	6 hr ambient
255	9 hr ambient	Reference	Reference	9 day cold
224	9 hr ambient	7 day cold	6 hr ambient	Reference

Code	PC nose classification	DFA classification - stepwise	DFA classification	Actual Classification
149	9 hr ambient	6 hr ambient	6 hr ambient	8 day cold
500	9 hr ambient*	6 hr ambient	6 hr ambient	9 hr ambient
217	9 hr ambient	Reference	Reference	9 day cold
219	7 day cold	7 day cold	Reference	9 hr ambient
209	7 day cold	7 day cold	9 day cold	6 hr ambient
491	6 hr ambient	6 hr ambient	6 hr ambient	9 hr ambient
944	9 day cold	6 hr ambient*	6 hr ambient*	6 hr ambient
617	7 day cold	6 hr ambient	6 hr ambient	9 hr ambient
100	9 hr ambient	Reference*	Reference*	Reference
109	7 day cold	7 day cold	7 day cold	6 hr ambient
059	9 hr ambient*	6 hr ambient	9 day cold	9 hr ambient

* Denotes correct identification

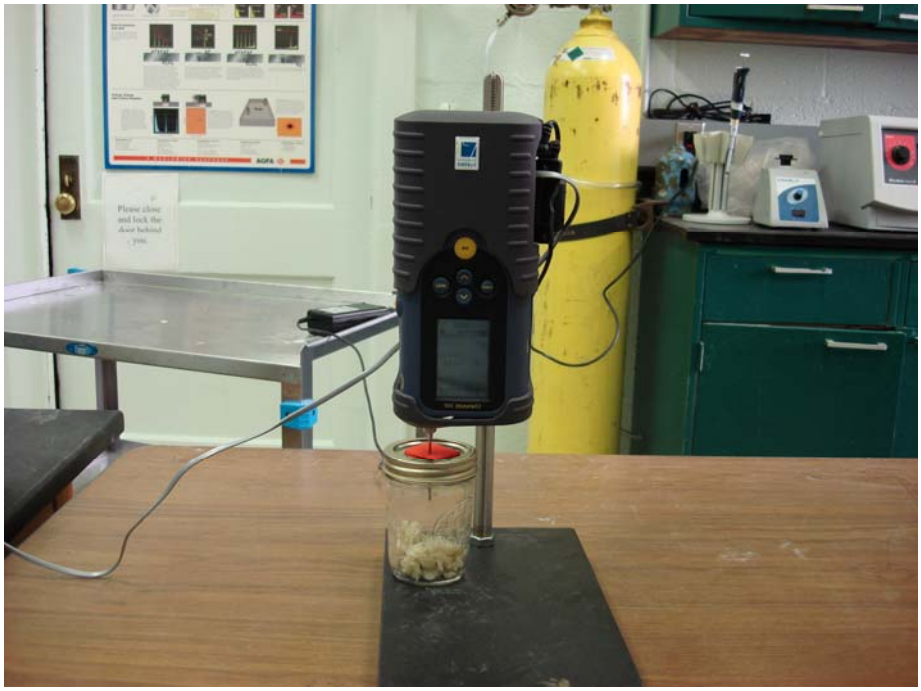


Figure 1. Manufacturer's Recommended Method Setup

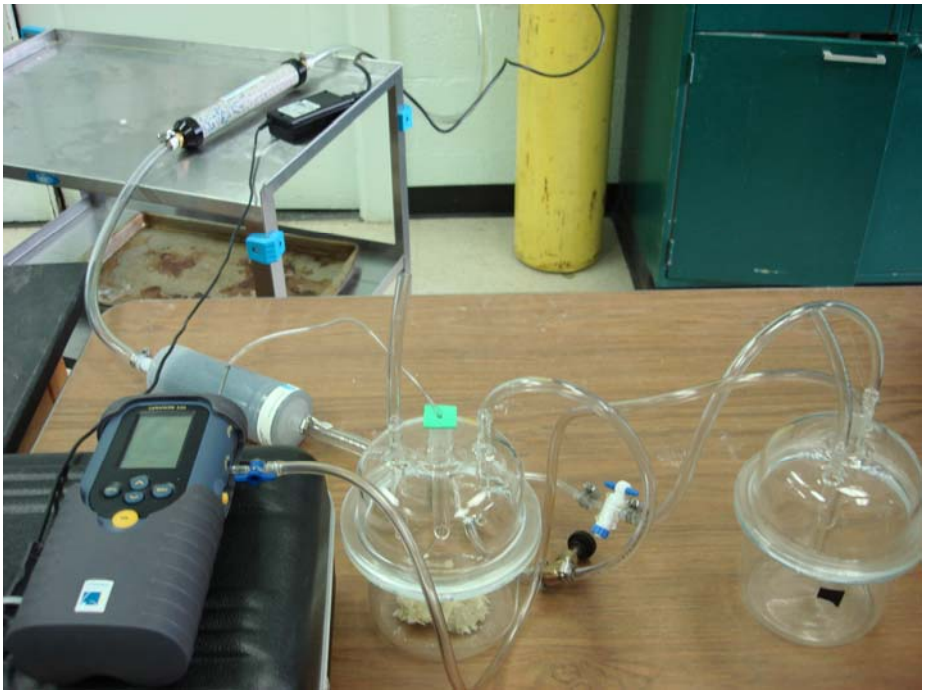


Figure 2. Compressed Air Method Setup

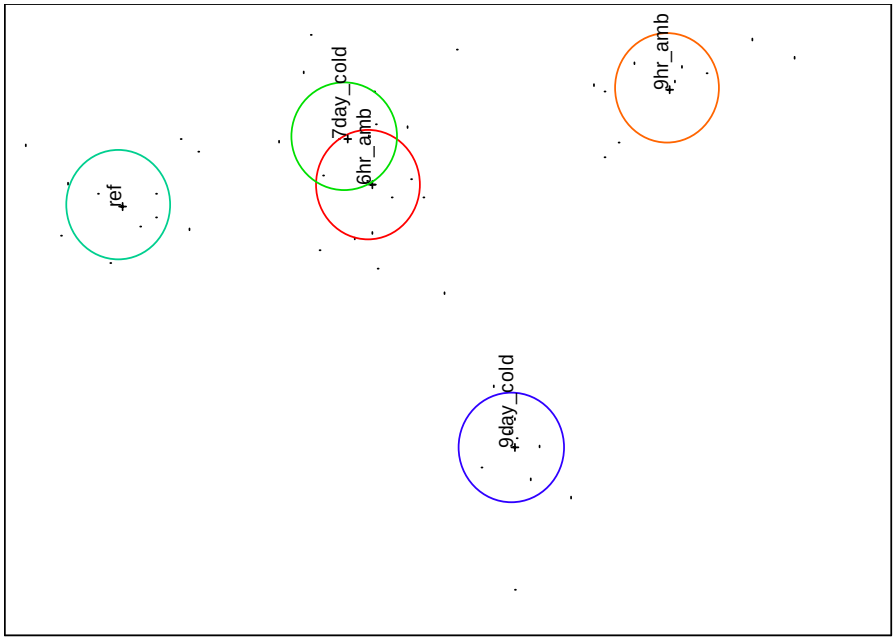


Figure 3. Canonical Discriminant Analysis Plot for Training Set Data for the Manufacturer's Recommended Method

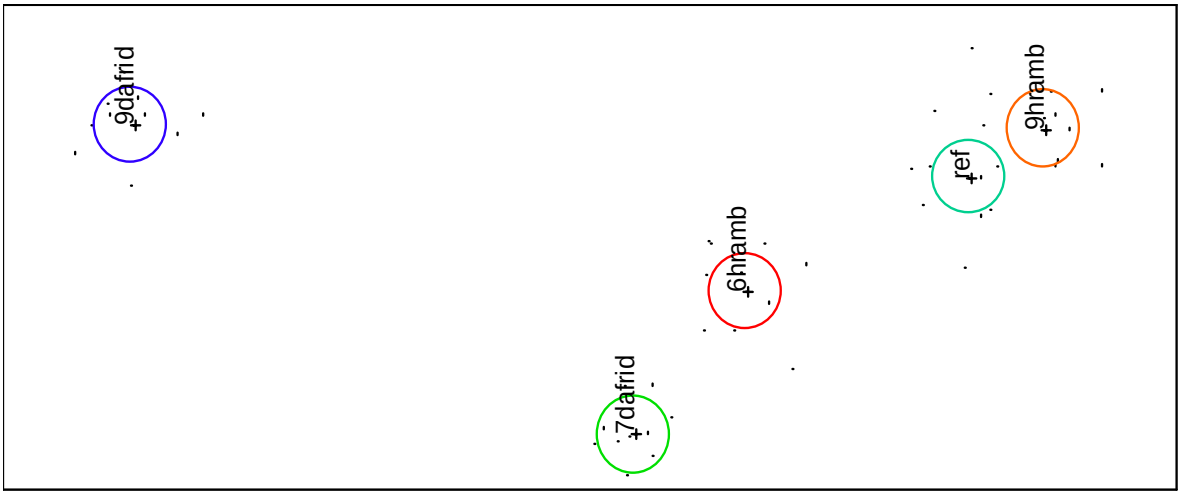


Figure 4. Canonical Discriminant Analysis Plot for Training Set Data for the Crab Reference Method

SPME-GC-MS Studies

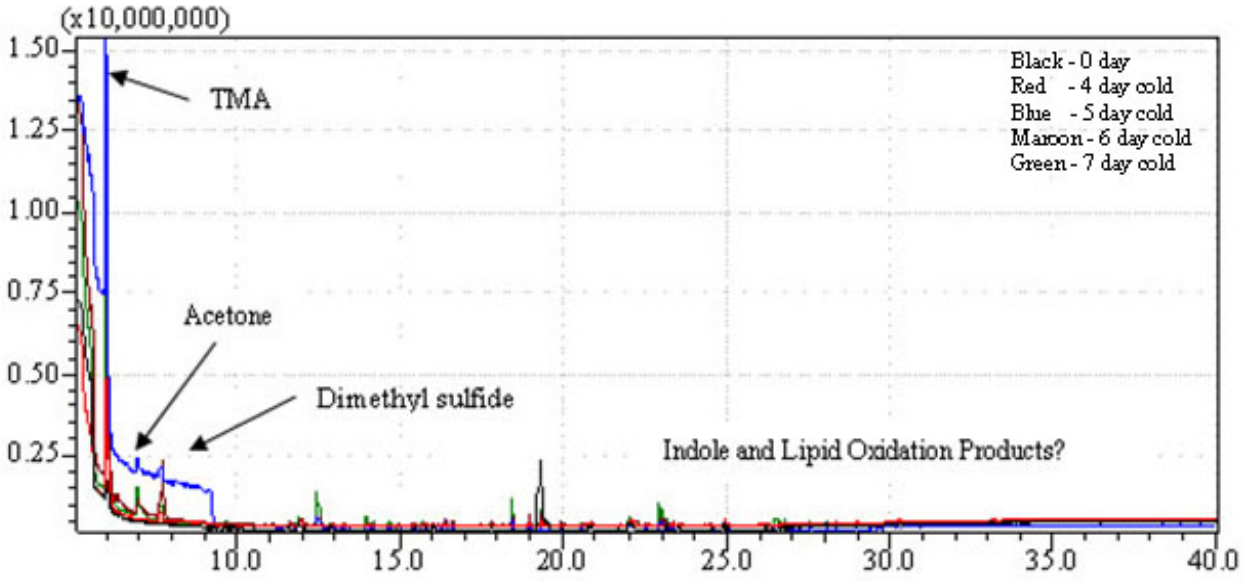


Figure 1 Scan mode results sampled at ambient (21 °C)

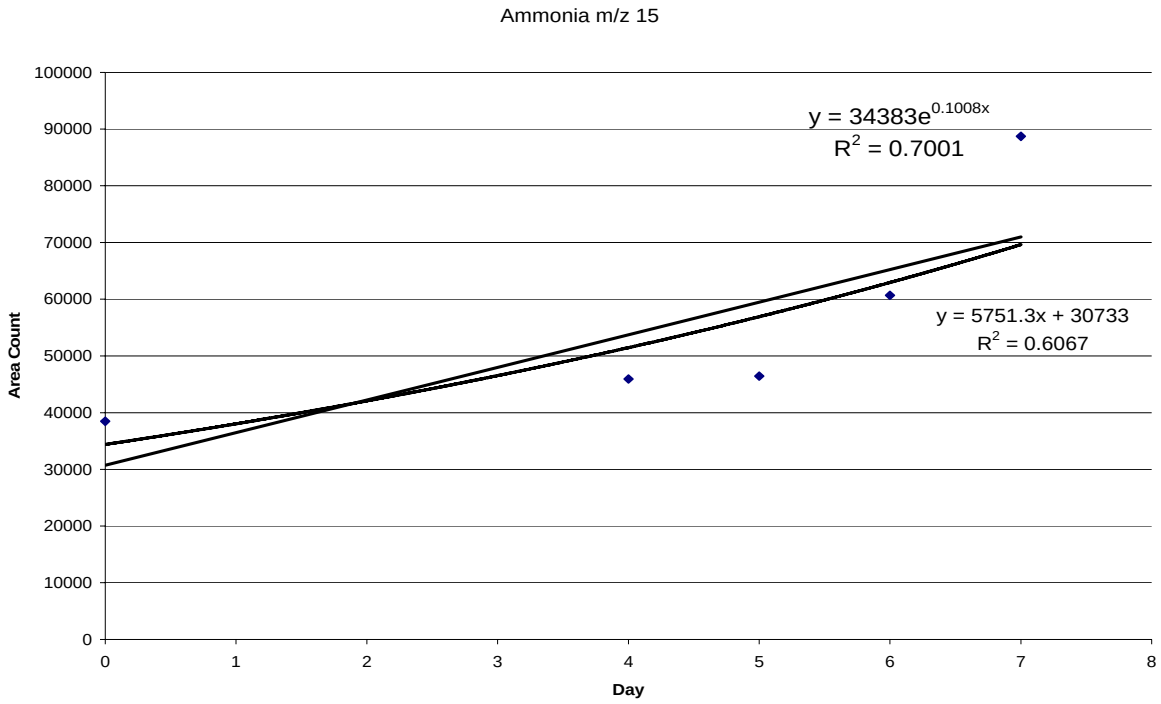


Figure 2 Ammonia amount as a function of Area Count. Each point is an average of three trials.

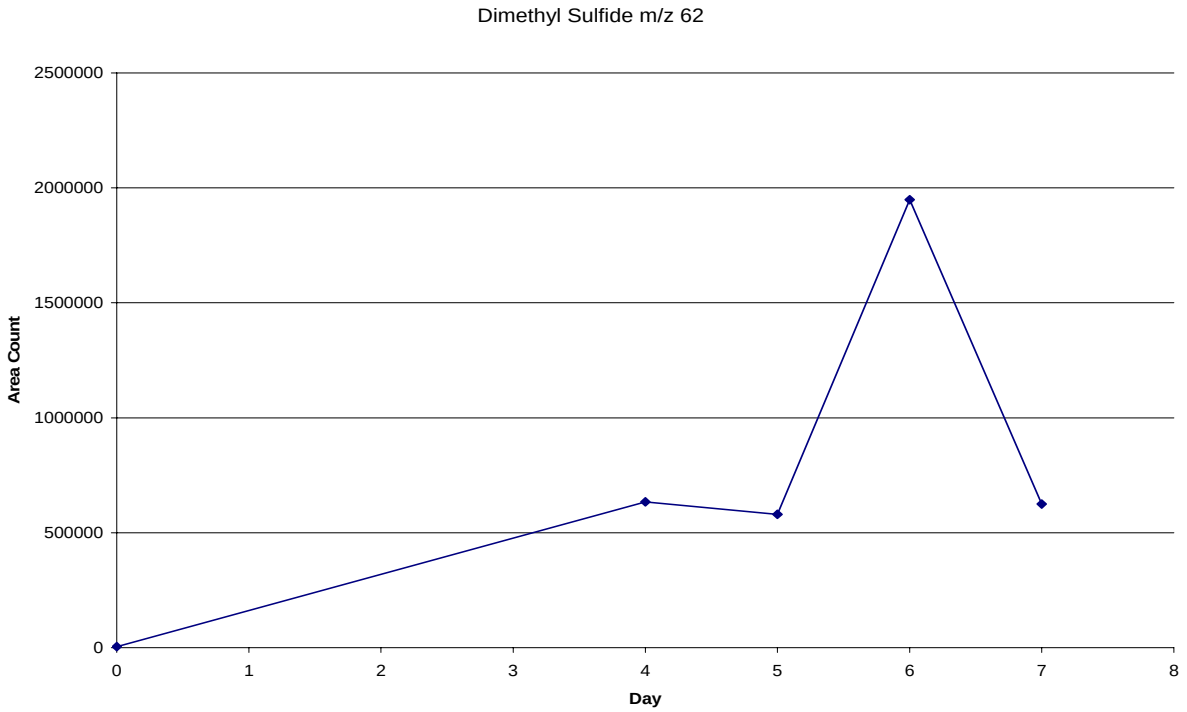
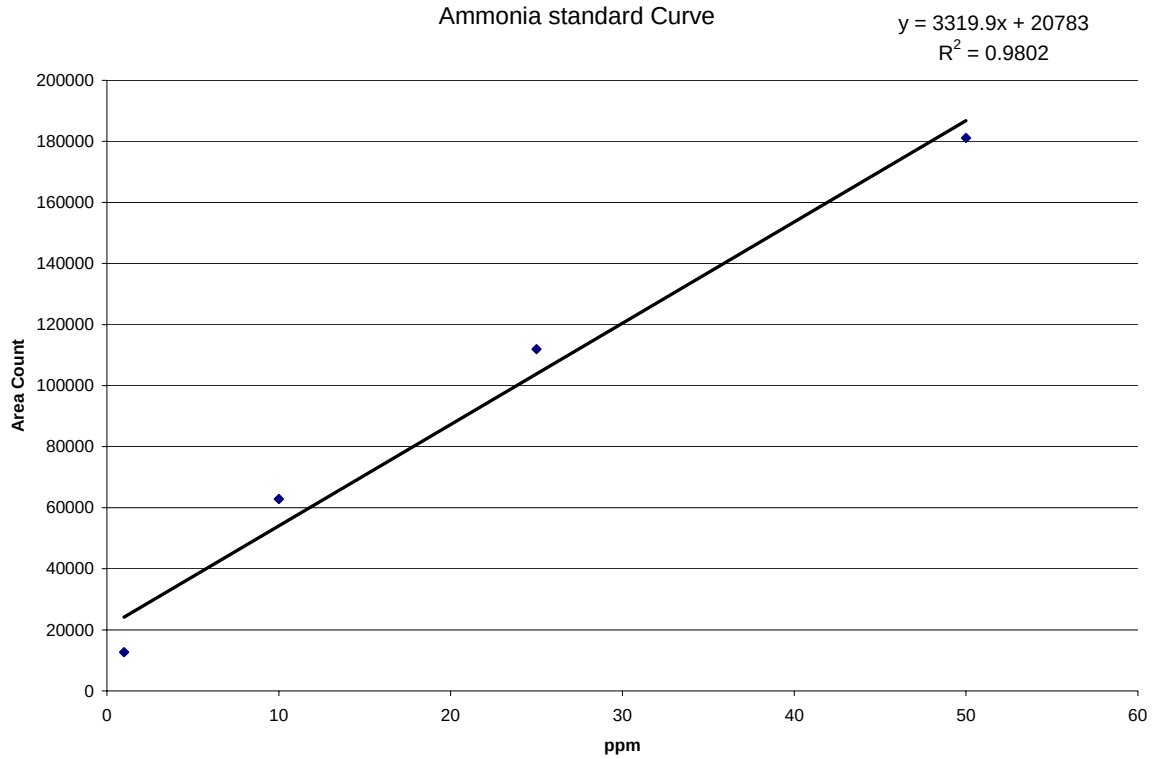
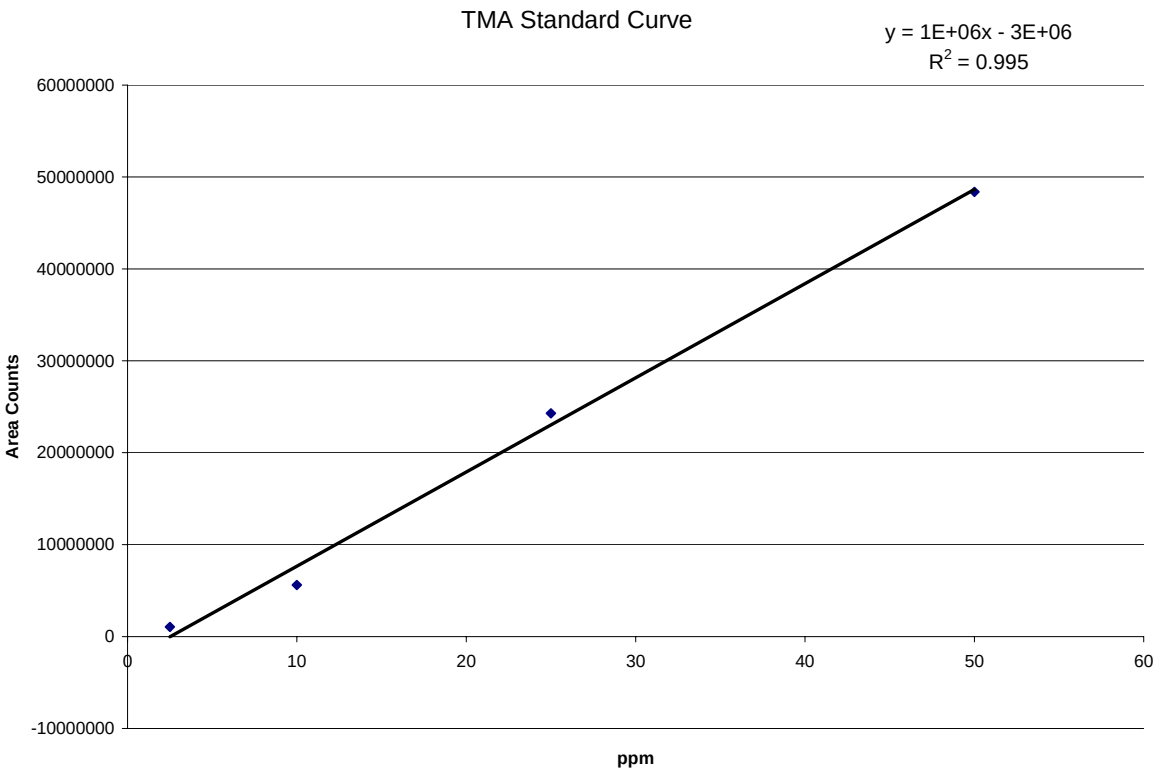
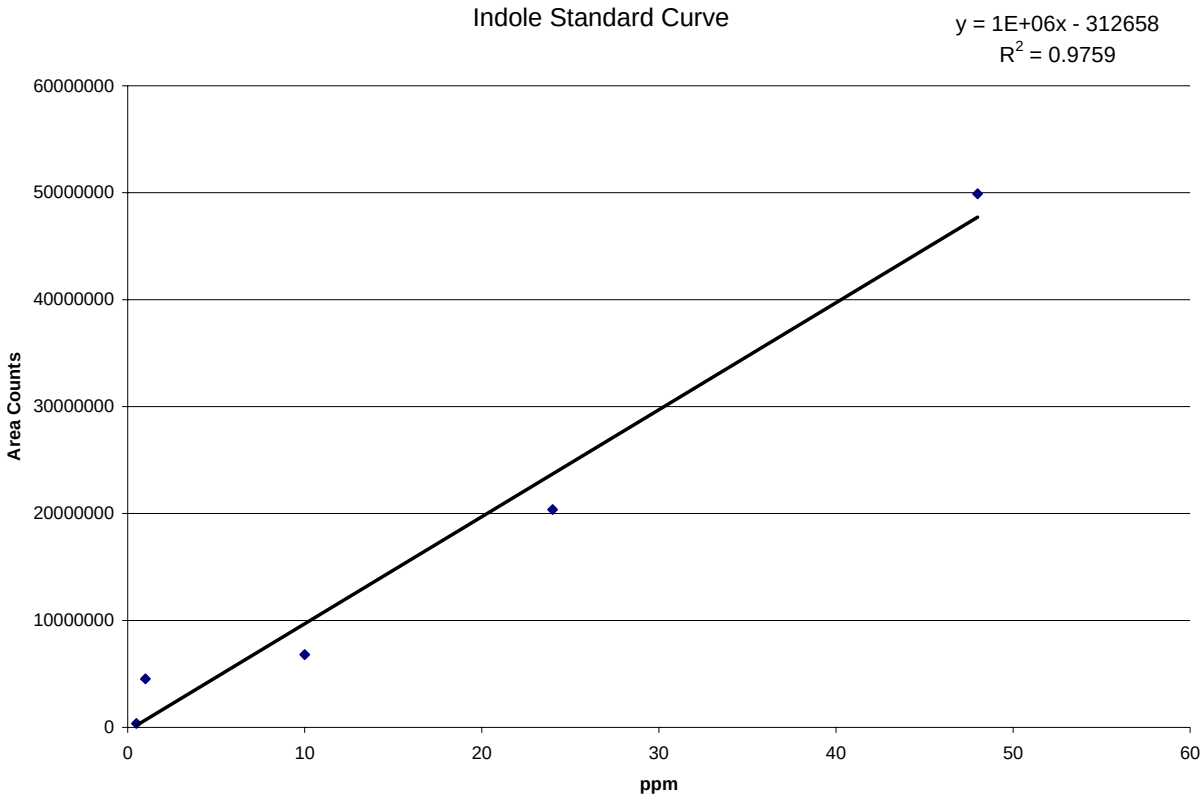
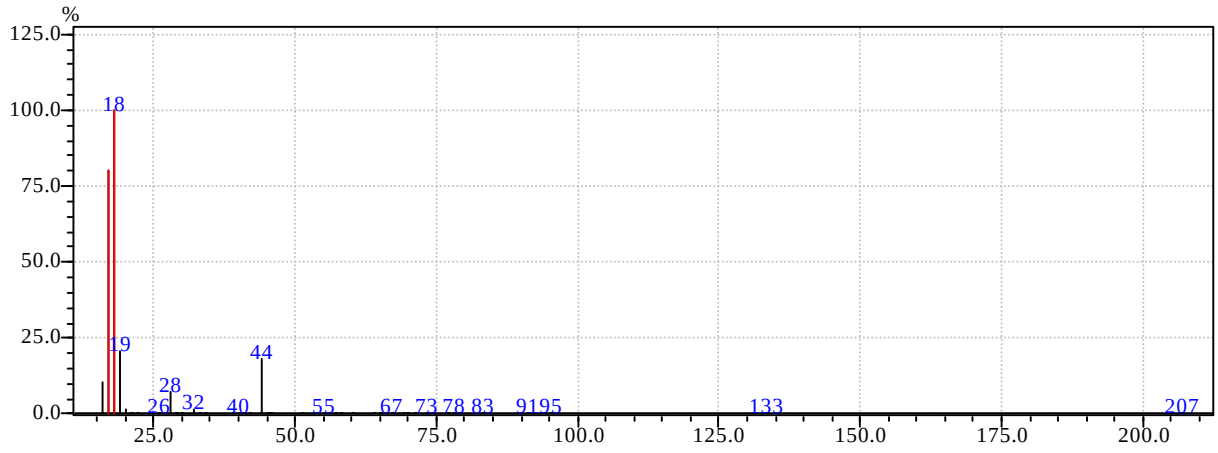


Figure 3 Dimethyl sulfide amount as a function of Area Count. Each point is an average of three trials.


Ammonia standard Curve
y = 3319.9x + 20783
R2 = 0.9802
Area Count
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
10
20
30
40
50
60
ppm


TMA Standard Curve
y = 1E+06x - 3E+06
R2 = 0.995
Area Counts
60000000
50000000
40000000
30000000
20000000
10000000
0
-10000000
0
10
20
30
40
50
60
ppm





Mass spectrum of the water peak

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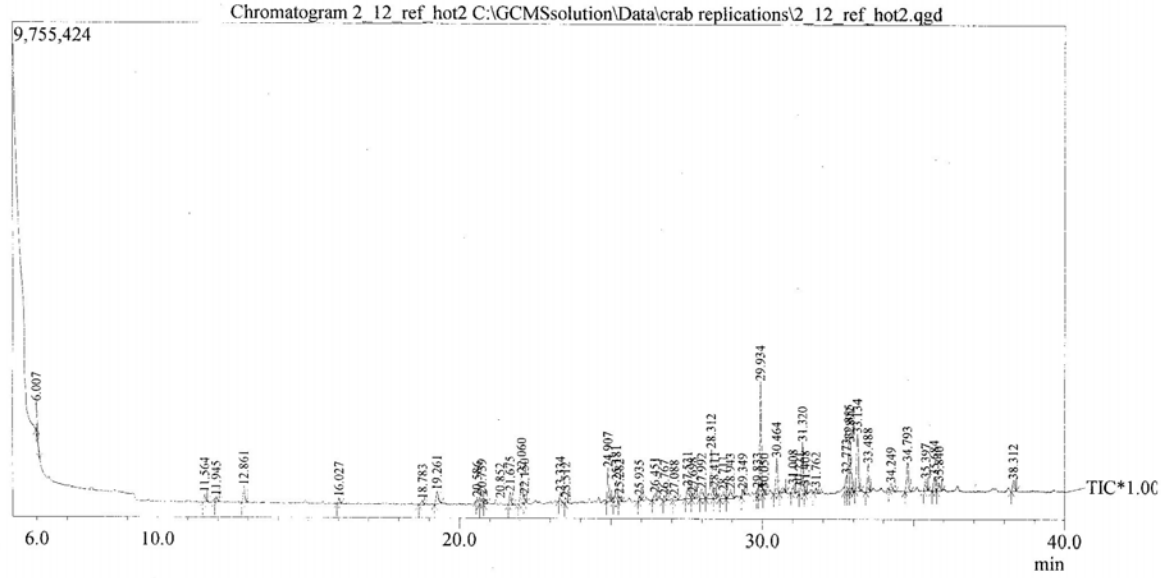

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	leight%	A/H	Mark	Name
1	6.007	5.992	6.100	1665232	2.36	640484	3.62	2.59		Methanamine, N,N-dimethyl- (CAS
2	11.564	11.500	11.650	621804	0.88	171672	0.97	3.62		Butanal, 3-methyl- (CAS) $$ 3-Metl
3	11.945	11.900	12.042	378197	0.54	100126	0.57	3.77	V	Silanediol, dimethyl- $$ Dihydroxy
4	12.861	12.792	12.983	1235252	1.75	314688	1.78	3.92		Butanal, 3-methyl- (CAS) $$ 3-Metl
5	16.027	15.950	16.092	465654	0.66	116984	0.66	3.98		Hexanal (CAS) $$ n-Hexanal $$ He
6	18.783	18.650	18.842	440014	0.62	92304	0.52	4.76		Heptanal (CAS) $$ n-Heptanal $$ 2
7	19.261	19.175	19.392	1418976	2.02	264555	1.50	5.36		2(3H)-Furanone, dihydro- (CAS) $$
8	20.586	20.550	20.667	329705	0.47	97666	0.55	3.37		1-Octen-3-ol
9	20.739	20.667	20.800	660139	0.94	180939	1.02	3.64		Benzaldehyde (CAS) $$ Phenylmetl
10	20.852	20.800	20.908	304124	0.43	100350	0.57	3.03		
11	21.675	21.608	21.767	918211	1.30	248781	1.41	3.69		1-Hexanol, 2-ethyl- (CAS) $$ 2-Eth
12	22.060	21.942	22.117	2647623	3.76	584790	3.31	4.52		Benzene, 1,3-dichloro-
13	22.150	22.117	22.217	566837	0.81	173963	0.98	3.25	V	D-Limonene
14	23.334	23.275	23.400	611870	0.87	187978	1.06	3.25		Nonanal
15	23.512	23.467	23.583	375421	0.53	100329	0.57	3.74		
16	24.907	24.850	25.008	2412872	3.43	672709	3.80	3.58	V	1-Dodecene $$ .alpha.-Dodecene $$
17	25.181	25.075	25.242	1854725	2.63	408526	2.31	4.54	V	
18	25.282	25.242	25.350	449737	0.64	134392	0.76	3.34	V	
19	25.935	25.892	26.000	331875	0.47	95745	0.54	3.46		
20	26.451	26.358	26.508	571776	0.81	134862	0.76	4.23		
21	26.767	26.717	26.833	448348	0.64	101283	0.57	4.42	V	
22	27.088	27.033	27.200	595018	0.85	91880	0.52	6.47	V	
23	27.531	27.450	27.650	1173504	1.67	229074	1.30	5.12		1H-Indole (CAS) $$ Indole $$ Ketol
24	27.698	27.650	27.758	397775	0.56	110468	0.62	3.60	V	
25	27.992	27.925	28.117	1194157	1.70	196577	1.11	6.07	V	5-Tetradecene, (E)- $$ (5E)-5-Tetra
26	28.312	28.117	28.375	4241940	6.02	982213	5.56	4.31	V	1-Hexadecene
27	28.411	28.375	28.483	976655	1.39	222276	1.26	4.39	V	Tetradecane
28	28.711	28.583	28.800	888298	1.26	141195	0.80	6.29	V	
29	28.943	28.800	29.058	1065289	1.51	190969	1.08	5.57	V	
30	29.349	29.300	29.408	408819	0.58	125265	0.71	3.26		
31	29.833	29.792	29.867	500858	0.71	139186	0.79	3.59	V	
32	29.934	29.867	30.008	8004299	11.37	2268877	12.83	3.52	V	Pentadecane
33	30.050	30.008	30.100	398562	0.57	114375	0.65	3.48	V	
34	30.464	30.367	30.550	2727961	3.87	707388	4.00	3.85		Butylated Hydroxytoluene $$ Pheno
35	30.721	30.550	30.767	404047	0.57	93826	0.53	4.30	V	
36	31.008	30.942	31.092	650195	0.92	152720	0.86	4.25		
37	31.320	31.200	31.383	4416436	6.27	1033142	5.84	4.27	V	
38	31.408	31.383	31.467	414667	0.59	155158	0.88	2.67	V	
39	31.762	31.667	31.858	466578	0.66	105213	0.60	4.43	V	Octadecanal $$ Stearaldehyde $$ O
40	32.773	32.708	32.792	1271519	1.81	339245	1.92	3.74	V	
41	32.855	32.792	32.875	3906874	5.55	1041988	5.89	3.74	V	
42	32.892	32.875	33.008	3234958	4.59	983758	5.56	3.28	V	
43	33.134	33.050	33.233	4920014	6.99	1128251	6.38	4.36		
44	33.488	33.408	33.567	2081448	2.96	566602	3.20	3.67		
45	34.249	34.175	34.383	670133	0.95	137598	0.78	4.87		
46	34.793	34.708	34.892	2631602	3.74	594152	3.36	4.42	V	Tetradecanal
47	35.397	35.325	35.475	903059	1.28	217778	1.23	4.14		
48	35.684	35.608	35.758	1169660	1.66	292624	1.66	3.99		
49	35.840	35.758	35.933	837664	1.19	173636	0.98	4.82	V	
50	38.312	38.233	38.400	1151744	1.64	221290	1.25	5.20		Tetradecanal
				70412125	100.00	17679850	100.00			

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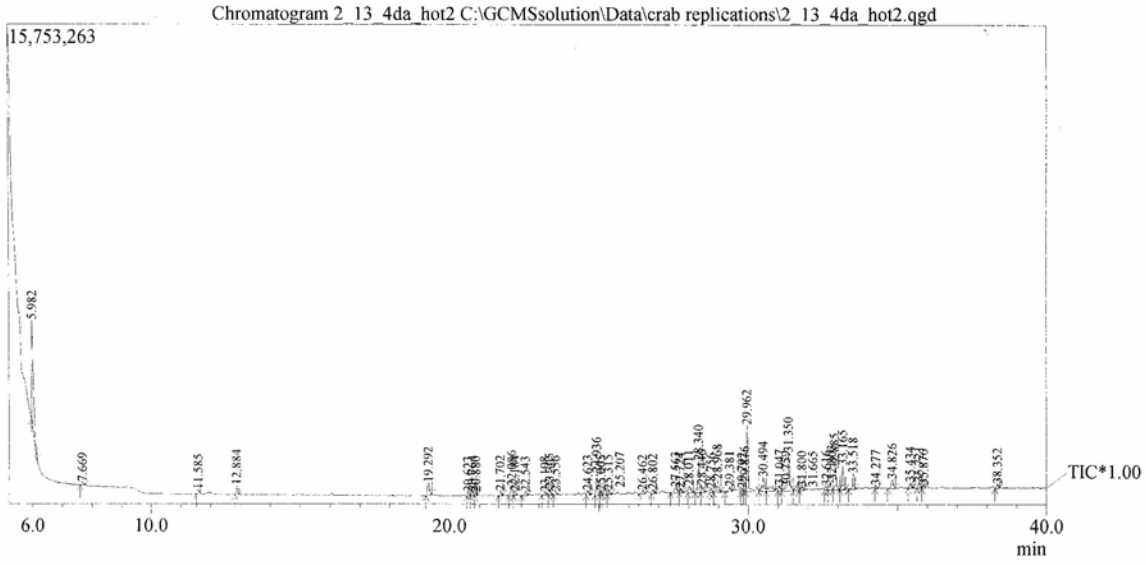

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.982	5.958	6.142	12834062	15.96	3424825	19.18	3.74		Methanamine, N,N-dimethyl- (CAS) $
2	7.669	7.600	7.758	412458	0.51	94773	0.53	4.35		Dimethyl sulfide $$ Methane, thiobis-
3	11.585	11.525	11.658	432592	0.54	123366	0.69	3.50		Hexanal (CAS) $$ n-Hexanal $$ Hexa
4	12.884	12.825	12.958	911009	1.13	275071	1.54	3.31		Pentanal (CAS) $$ n-Pentanal $$ n-Va
5	19.292	19.208	19.408	2002030	2.49	398834	2.23	5.01		2(3H)-Furanone, dihydro- (CAS) $$ B
6	20.623	20.575	20.692	441452	0.55	105490	0.59	4.18	V	Decane (CAS) $$ n-Decane $$ Isodeca
7	20.764	20.692	20.825	543321	0.68	128696	0.72	4.22	V	Benzaldehyde
8	20.880	20.825	20.925	380389	0.47	112733	0.63	3.37	V	1-Decene (CAS) $$ Dec-1-ene $$ n-1-
9	21.702	21.633	21.792	503472	0.63	134416	0.75	3.74		1-Hexanol, 2-ethyl- (CAS) $$ 2-Ethylh
10	22.086	21.975	22.142	1535305	1.91	295848	1.66	5.18	V	Benzene, 1,3-dichloro-
11	22.184	22.142	22.250	426697	0.53	122958	0.69	3.47	V	D-Limonene
12	22.543	22.400	22.617	664030	0.83	107597	0.60	6.17		
13	23.198	23.083	23.242	415218	0.52	90537	0.51	4.58	V	
14	23.365	23.308	23.442	660598	0.82	181667	1.02	3.63	V	Nonanal
15	23.556	23.475	23.608	378989	0.47	90547	0.51	4.18		
16	24.623	24.567	24.700	530982	0.66	120785	0.68	4.39	V	
17	24.936	24.867	25.025	2605149	3.24	702548	3.93	3.70	V	1-Dodecene $$ .alpha.-Dodecene $$ n-
18	25.065	25.025	25.108	372859	0.46	115682	0.65	3.22	V	
19	25.207	25.108	25.267	1113620	1.38	219974	1.23	5.06	V	
20	25.315	25.267	25.417	524316	0.65	126010	0.71	4.16	V	Decanal $$ n-Decaldehyde $$ n-Decar
21	26.462	26.333	26.542	596943	0.74	97166	0.54	6.14	V	
22	26.802	26.750	26.892	536853	0.67	119756	0.67	4.48	V	Tridecane (CAS) $$ n-Tridecane $$ Tr
23	27.563	27.392	27.683	1370917	1.70	176331	0.99	7.77	V	Indole
24	27.724	27.683	27.808	587944	0.73	143999	0.81	4.08	V	
25	28.011	27.958	28.150	1309995	1.63	221284	1.24	5.91	V	
26	28.340	28.208	28.408	4382312	5.45	1039187	5.82	4.21	V	1-Tridecene
27	28.446	28.408	28.558	1064131	1.32	230239	1.29	4.62	V	
28	28.756	28.708	28.825	505921	0.63	108574	0.61	4.65	V	
29	28.968	28.825	29.075	1569459	1.95	399932	2.24	3.92	V	
30	29.381	29.208	29.442	935530	1.16	166088	0.93	5.63	V	
31	29.792	29.733	29.825	458753	0.57	97119	0.54	4.72	V	
32	29.876	29.825	29.900	1052514	1.31	308380	1.73	3.41	V	
33	29.962	29.900	30.350	9124701	11.34	2187871	12.25	4.17	SV	Pentadecane $$ n-Pentadecane $$ CH
34	30.494	30.350	30.592	2534996	3.15	458623	2.57	5.52	V	Butylated Hydroxytoluene $$ Phenol,
35	30.759	30.592	30.892	1640527	2.04	135235	0.76	12.13	V	
36	31.047	30.983	31.117	963562	1.20	177310	0.99	5.43	V	
37	31.350	31.117	31.500	6041212	7.51	1245988	6.98	4.84	V	
38	31.665	31.500	31.700	834770	1.04	105831	0.59	7.88	V	
39	31.800	31.700	31.875	712672	0.89	104312	0.58	6.83	V	
40	32.616	32.550	32.658	594728	0.74	114233	0.64	5.20	V	
41	32.803	32.658	32.833	1780007	2.21	299197	1.68	5.94	V	
42	32.885	32.833	33.067	4016292	4.99	669660	3.75	5.99	V	Octadecane
43	33.165	33.067	33.258	3600589	4.48	783592	4.39	4.59	V	
44	33.518	33.350	33.592	2040045	2.54	514970	2.88	3.96	V	
45	34.277	34.242	34.325	328365	0.41	97239	0.54	3.37	V	
46	34.826	34.667	34.933	1397189	1.74	291154	1.63	4.79	V	Tetradecanal
47	35.434	35.358	35.517	728085	0.91	170478	0.95	4.27		
48	35.721	35.642	35.808	821737	1.02	179903	1.01	4.56	V	
49	35.876	35.808	35.933	486651	0.61	101533	0.57	4.79	V	
50	38.352	38.258	38.417	731843	0.91	142901	0.80	5.12	V	Octadecanal $$ Stearaldehyde $$ Octa
				80437791	100.00	17860442	100.00			

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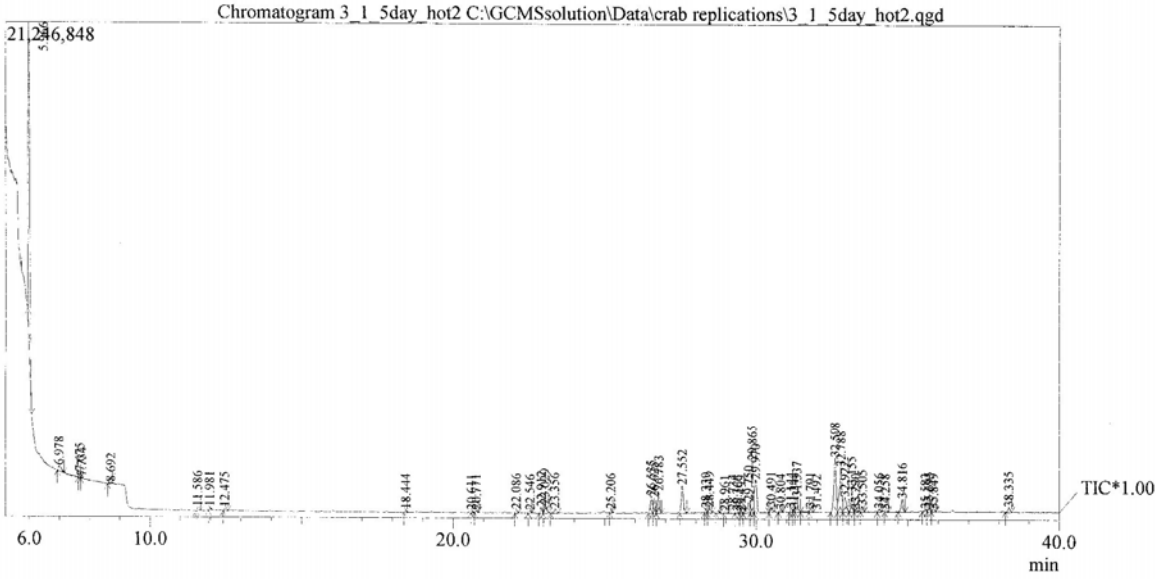
Chromatogram 3_1_5day_hot2 C:\GCMSsolution\Data\crab replications\3_1_5day_hot2.qgd



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.966	5.942	6.075	39392144	29.97	13091901	40.23	3.00		Methanamine, N,N-dimethyl- (CAS) $
2	6.978	6.925	7.142	1322676	1.01	217786	0.67	6.07		Acetone
3	7.675	7.617	7.692	747200	0.57	251305	0.77	2.97	V	1,2,3-Propanetriol (CAS) $$ Glycerol $
4	7.734	7.692	7.758	335622	0.26	64974	0.20	5.16	V	
5	8.692	8.592	8.742	405567	0.31	66685	0.20	6.08	V	Water $$ Water vapor $$ Distilled wat
6	11.586	11.533	11.675	481055	0.37	138488	0.43	3.47		Butanal, 3-methyl-
7	11.981	11.933	12.033	302706	0.23	106460	0.33	2.84		Silanediol, dimethyl- $$ Dihydroxydin
8	12.475	12.417	12.583	449429	0.34	115371	0.35	3.89		2-Butanone, 3-methyl- (CAS) $$ 3-Me
9	18.444	18.367	18.492	377652	0.29	107493	0.33	3.51		2-Heptanone $$ n-Amyl methyl ketone
10	20.611	20.575	20.675	312717	0.24	87321	0.27	3.58	V	1-Octen-3-ol
11	20.771	20.700	20.833	311865	0.24	83205	0.26	3.74		Benzaldehyde (CAS) $$ Phenylmethar
12	22.086	22.025	22.150	674171	0.51	149252	0.46	4.51	V	Benzene, 1,4-dichloro-
13	22.546	22.475	22.600	421377	0.32	89613	0.28	4.70	V	
14	22.912	22.833	22.975	915185	0.70	244955	0.75	3.73		4-Hexen-1-ol, acetate $$ (4E)-4-Hexer
15	23.029	22.975	23.150	1937181	1.47	339089	1.04	5.71	V	2-Nonanone $$ Heptyl methyl ketone $
16	23.356	23.208	23.458	826384	0.63	177776	0.55	4.64	V	Nonanal
17	25.206	25.150	25.267	388033	0.30	120780	0.37	3.21		
18	26.535	26.442	26.583	3213140	2.44	711513	2.19	4.51	V	tert-Butyl iodide
19	26.625	26.583	26.700	2515565	1.91	643050	1.98	3.91	V	
20	26.783	26.700	26.867	3409184	2.59	932939	2.87	3.65	V	2-Undecanone
21	27.552	27.467	27.708	5885655	4.48	1191015	3.66	4.94	V	Indole
22	28.339	28.292	28.383	594963	0.45	171945	0.53	3.46	V	1-Hexadecene
23	28.449	28.383	28.542	1319517	1.00	219141	0.67	6.02	V	6-Dodecanone
24	28.734	28.667	28.808	450425	0.34	100272	0.31	4.49		3-Tridecen-1-yne, (Z)- $$ (3Z)-3-Tride
25	28.961	28.917	29.075	359228	0.27	74548	0.23	4.81	V	
26	29.366	29.292	29.442	354676	0.27	81047	0.25	4.37	V	
27	29.499	29.442	29.558	326557	0.25	71421	0.22	4.57	V	
28	29.750	29.558	29.800	2836071	2.16	476503	1.46	5.95	V	3-Decen-1-ol, acetate, (Z)- $$ Z-3-Dec
29	29.865	29.800	29.917	8156801	6.21	2231687	6.86	3.65	V	1-Tridecene
30	29.970	29.917	30.417	7731042	5.88	1411560	4.34	5.47	SV	Hexadecane
31	30.491	30.417	30.583	868557	0.66	196872	0.61	4.41		Butylated Hydroxytoluene $$ Phenol,
32	30.804	30.717	30.867	422393	0.32	90645	0.28	4.65		1-Tetracosanol
33	31.144	31.083	31.200	646556	0.49	147539	0.45	4.38		
34	31.244	31.200	31.275	528156	0.40	147548	0.45	3.57	V	
35	31.337	31.275	31.458	2590020	1.97	628338	1.93	4.12	V	
36	31.492	31.458	31.733	661838	0.50	71118	0.22	9.30	V	2-Dodecanone (CAS) $$ Dodecan-2-o
37	31.791	31.733	31.875	640479	0.49	162221	0.50	3.94	V	Tetradecanal
38	32.598	32.450	32.675	12142426	9.24	2344667	7.21	5.17		1,13-Tetradecadiene
39	32.788	32.675	32.858	10864771	8.27	1937904	5.96	5.60	V	8-Heptadecene
40	32.922	32.858	33.058	4279873	3.26	624965	1.92	6.84	V	Pentadecane, 2,6,10,14-tetramethyl- (C
41	33.155	33.058	33.242	3714824	2.83	838258	2.58	4.43	V	
42	33.290	33.242	33.367	355904	0.27	92633	0.28	3.84	V	Octadecanal
43	33.505	33.433	33.567	845832	0.64	246058	0.76	3.43	V	
44	34.056	33.992	34.125	518099	0.39	112267	0.35	4.61	V	
45	34.258	34.225	34.358	311773	0.24	76557	0.24	4.07	V	
46	34.816	34.683	34.908	2544427	1.94	540470	1.66	4.70	V	Tetradecanal
47	35.583	35.475	35.625	456831	0.35	86099	0.26	5.30		
48	35.695	35.625	35.775	760362	0.58	146579	0.45	5.18	V	
49	35.849	35.775	35.925	471029	0.36	84863	0.26	5.55	V	
50	38.335	38.217	38.425	1069560	0.81	194773	0.60	5.49	V	Octadecanal $$ Stearaldehyde $$ Octa
				131447498	100.00	32539469	100.00			

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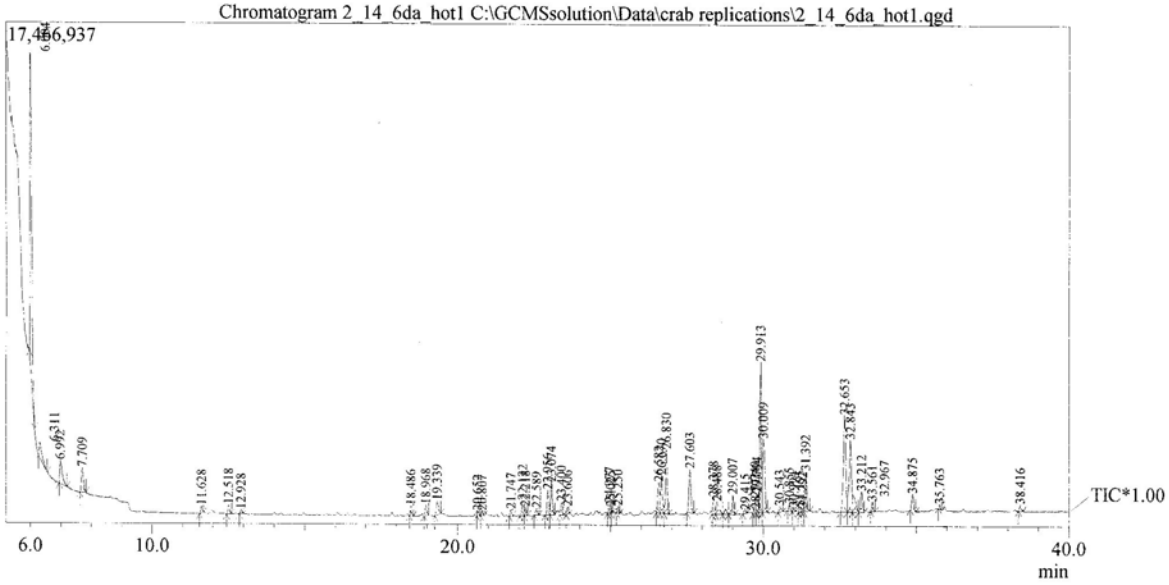
Chromatogram 2_14_6da_hot1 C:\GCMSsolution\Data\crab replications\2_14_6da_hot1.qgd



Peak Report TIC

Peak#	R.Time	l.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	6.004	5.975	6.150	40250278	19.47	10847674	22.07	3.71		Methanamine, N,N-dimethyl- (CAS) $
2	6.311	6.275	6.558	3277001	1.59	526889	1.07	6.21		Ethanol
3	6.992	6.950	7.208	4542494	2.20	817847	1.66	5.55		Acetone
4	7.709	7.625	7.825	4021402	1.95	871333	1.77	4.61		Dimethyl sulfide $$ Methane, thiobis-
5	11.628	11.567	11.692	904643	0.44	257243	0.52	3.51		Butanal, 3-methyl-
6	12.518	12.450	12.617	1283620	0.62	337760	0.69	3.80		2-Pentanone
7	12.928	12.867	13.008	547238	0.26	152537	0.31	3.58		Pentanal
8	18.486	18.425	18.558	1187947	0.57	358350	0.73	3.31		2-Heptanone $$ n-Amyl methyl ketone
9	18.968	18.900	19.050	1440227	0.70	400077	0.81	3.59		Styrene $$ Benzene, ethenyl- (CAS) $$
10	19.339	19.250	19.458	2739333	1.33	509777	1.04	5.37		2(3H)-Furanone, dihydro- (CAS) $$ B
11	20.652	20.625	20.725	575209	0.28	178379	0.36	3.22	V	1-Octen-3-ol
12	20.807	20.750	20.867	594712	0.29	172533	0.35	3.44		
13	21.747	21.683	21.808	839143	0.41	252591	0.51	3.32		1-Hexanol, 2-ethyl- $$ Ethylhexanol $$
14	22.132	22.033	22.183	2421854	1.17	546659	1.11	4.43		Benzene, 1,3-dichloro-
15	22.218	22.183	22.292	1063257	0.51	325060	0.66	3.27	V	D-Limonene
16	22.589	22.500	22.667	1095811	0.53	258058	0.53	4.24		
17	22.956	22.867	23.017	3538756	1.71	896549	1.82	3.94		
18	23.074	23.017	23.183	4971052	2.41	1159389	2.36	4.28	V	2-Nonanone
19	23.400	23.342	23.475	1843250	0.89	477066	0.97	3.86	V	9,12,15-Octadecatrienal $$ (9E,12E,15
20	23.606	23.550	23.667	914720	0.44	268656	0.55	3.40	V	
21	24.977	24.908	25.017	1396112	0.68	363709	0.74	3.83		1-Dodecene
22	25.025	25.017	25.075	506200	0.24	240132	0.49	2.10	V	
23	25.250	25.192	25.308	1032995	0.50	268496	0.55	3.84		
24	26.582	26.500	26.625	4769696	2.31	1090296	2.22	4.37	V	
25	26.670	26.625	26.758	5702808	2.76	1355794	2.76	4.20	V	
26	26.830	26.758	26.917	8186580	3.96	2267226	4.61	3.61	V	2-Undecanone (CAS) $$ 2-Hendecano
27	27.603	27.500	27.733	7299304	3.53	1568102	3.19	4.65		Indole
28	28.378	28.325	28.442	2398023	1.16	609281	1.24	3.93	V	1-Tridecene
29	28.488	28.442	28.675	2883022	1.39	460550	0.94	6.25	V	
30	28.771	28.675	28.867	1057049	0.51	214189	0.44	4.93	V	3-Tridecen-1-yne, (Z)- $$ (3Z)-3-Tride
31	29.007	28.867	29.083	2434071	1.18	719075	1.46	3.38	V	
32	29.415	29.333	29.483	595358	0.29	138592	0.28	4.29		
33	29.740	29.658	29.758	1927630	0.93	566737	1.15	3.40		1,10-Undecadiene
34	29.794	29.758	29.842	2784827	1.35	724078	1.47	3.84	V	3-Decen-1-ol, acetate, (Z)- $$ Z-3-Dec
35	29.913	29.842	29.967	19412363	9.39	5274185	10.73	3.68	V	1-Tridecene
36	30.009	29.967	30.133	12317507	5.96	2588483	5.27	4.75	V	Hexadecane
37	30.543	30.483	30.642	967090	0.47	226739	0.46	4.26		Butylated Hydroxytoluene $$ Phenol,
38	30.855	30.792	30.925	949584	0.46	272034	0.55	3.49		
39	30.994	30.942	31.050	490245	0.24	146769	0.30	3.34		
40	31.195	31.142	31.250	918813	0.44	219278	0.45	4.19		
41	31.292	31.250	31.325	572603	0.28	144811	0.29	3.95	V	
42	31.392	31.325	31.508	5286243	2.56	1425665	2.90	3.70	V	
43	32.653	32.508	32.742	18344529	8.88	3408082	6.93	5.38		1,13-Tetradecadiene
44	32.843	32.742	32.908	13155962	6.37	2551989	5.19	5.15	V	8-Heptadecene
45	32.967	32.908	33.108	4302699	2.08	551970	1.12	7.79	V	
46	33.212	33.108	33.292	3502104	1.69	758289	1.54	4.61	V	
47	33.561	33.500	33.650	1240124	0.60	359697	0.73	3.44		
48	34.875	34.800	34.983	2524063	1.22	600293	1.22	4.20		Tetradecanal
49	35.763	35.717	35.825	544331	0.26	168903	0.34	3.22		
50	38.416	38.342	38.500	1130363	0.55	255727	0.52	4.42		Octadecanal $$ Stearaldehyde $$ Octa
				206684245	100.00	49153598	100.00			

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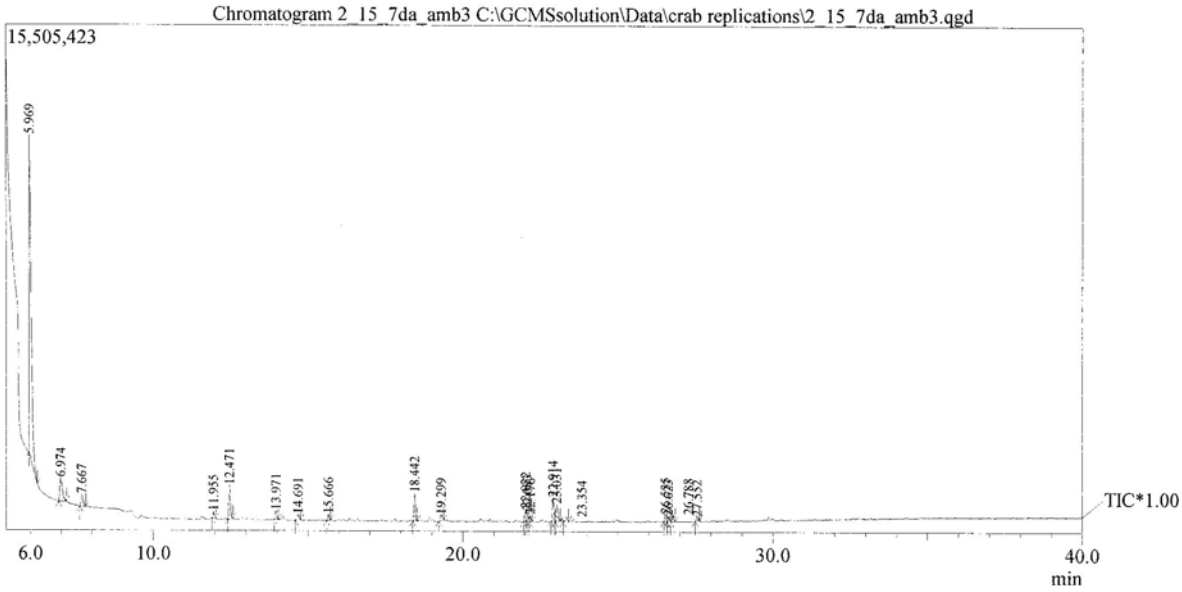

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	leight%	A/H	Mark	Name
1	5.969	5.942	6.217	42304645	60.57	9844388	59.12	4.29		Methanamine, N,N-dimethyl- (CAS) $
2	6.974	6.908	7.158	4258837	6.10	722704	4.34	5.89		2-Propanone (CAS) $$ Acetone $$ PR
3	7.667	7.600	7.800	1642903	2.35	356443	2.14	4.60		Dimethyl sulfide $$ Methane, thiobis-
4	11.955	11.908	12.033	600096	0.86	189994	1.14	3.15		Silanediol, dimethyl- $$ Dihydroxydin
5	12.471	12.408	12.583	3859632	5.53	1029240	6.18	3.74		2-Pentanone
6	13.971	13.908	14.050	947571	1.36	259768	1.56	3.64		1-Butanol, 3-methyl-
7	14.691	14.592	14.767	855838	1.23	165709	1.00	5.16		Disulfide, dimethyl
8	15.666	15.608	15.725	556345	0.80	177079	1.06	3.14		2-Hexanone
9	18.442	18.375	18.517	2769952	3.97	840938	5.05	3.29		2-Heptanone
10	19.299	19.217	19.375	820172	1.17	169699	1.02	4.83		Butanoic acid, 4-chloro- $$ Butyric ac
11	22.032	21.958	22.058	1423842	2.04	381561	2.29	3.73		
12	22.083	22.058	22.142	996544	1.43	296055	1.78	3.36	V	Benzene, 1,3-dichloro-
13	22.176	22.142	22.242	578416	0.83	183008	1.10	3.16	V	D-Limonene
14	22.914	22.842	22.975	2579634	3.69	722179	4.34	3.57		Butanoic acid,4-hexen-1-yl ester $$ (4
15	23.031	22.975	23.142	2513162	3.60	519071	3.12	4.84	V	2-Nonanone
16	23.354	23.233	23.408	482287	0.69	113439	0.68	4.25	V	6-Hexadecen-4-yne, (E)- $$ (6E)-6-He
17	26.535	26.475	26.583	814307	1.17	210354	1.26	3.87		
18	26.623	26.583	26.700	624226	0.89	160619	0.96	3.88	V	
19	26.788	26.700	26.842	711576	1.02	187151	1.12	3.80	V	2-Undecanone
20	27.552	27.492	27.625	500574	0.72	121669	0.73	4.11		Indole
				69840559	100.00	16651068	100.00			

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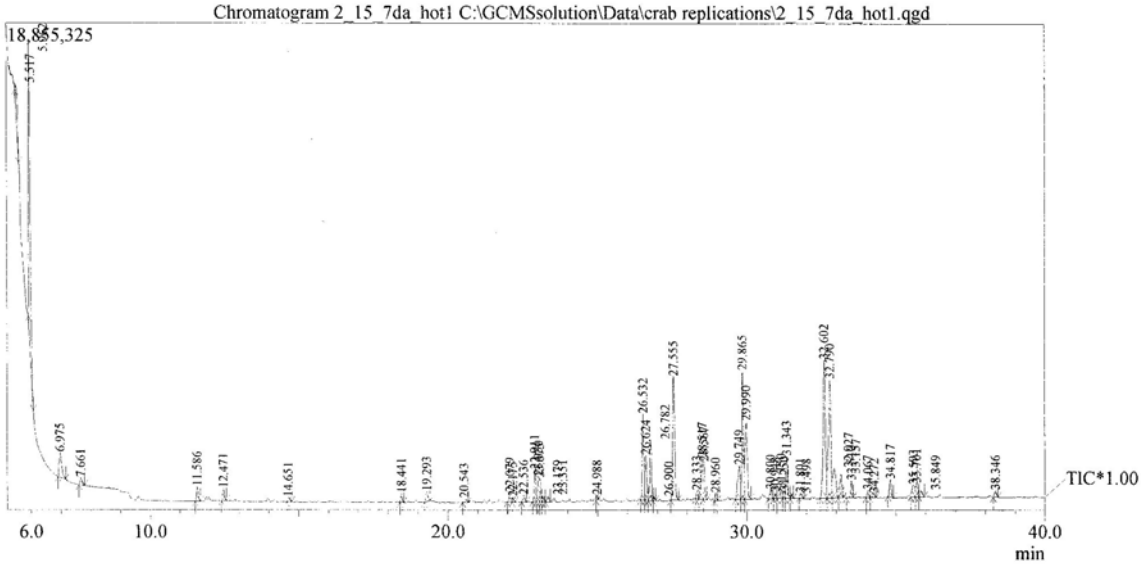

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.517	5.500	5.625	5133064	1.83	637887	0.99	8.04		2-Amino-3-quinolinecarbonitrile
2	5.962	5.933	6.092	38785248	13.81	11362213	17.70	3.41		Methanamine, N,N-dimethyl- (CAS) $
3	6.975	6.900	7.158	5756226	2.05	984115	1.53	5.84		Ethanol
4	7.661	7.600	7.792	1242530	0.44	281812	0.44	4.40		Dimethyl sulfide $$ Methane, thiobis-
5	11.586	11.517	11.700	2182152	0.78	555548	0.87	3.92		Butanal, 3-methyl-
6	12.471	12.400	12.575	1886528	0.67	483952	0.75	3.89		2-Pentanone
7	14.651	14.592	14.767	845649	0.30	160865	0.25	5.25		2-Pentanone, 3-methyl- (CAS) $$ 3-M
8	18.441	18.383	18.525	1111762	0.40	342634	0.53	3.24		2-Heptanone $$ n-Amyl methyl ketone
9	19.293	19.208	19.400	1966790	0.70	376329	0.59	5.22		2(3H)-Furanone, dihydro- (CAS) $$ B
10	20.543	20.483	20.675	830358	0.30	149160	0.23	5.56		Phenol (CAS) $$ Izal $$ ENT 1814 $$
11	22.079	21.983	22.142	1646454	0.59	341158	0.53	4.82		Benzene, 1,4-dichloro-
12	22.175	22.142	22.242	568576	0.20	184696	0.29	3.07	V	Limonene
13	22.536	22.458	22.625	1116917	0.40	259819	0.40	4.29		
14	22.911	22.833	22.967	4407959	1.57	1235927	1.93	3.56		4-Hexen-1-ol, acetate $$ (4E)-4-Hexer
15	23.030	22.967	23.058	3749212	1.33	1007595	1.57	3.72	V	2-Nonanone
16	23.075	23.058	23.142	2443326	0.87	904518	1.41	2.70	V	
17	23.179	23.142	23.258	968911	0.34	248325	0.39	3.90	V	
18	23.351	23.258	23.425	1149167	0.41	273361	0.43	4.20	V	3-Tetradecen-5-yne, (Z)- $$ (3Z)-3-Te
19	24.988	24.950	25.033	508728	0.18	181580	0.28	2.80	V	2-Decanone
20	26.532	26.442	26.583	12747273	4.54	3365941	5.24	3.78		
21	26.624	26.583	26.700	6781794	2.41	1746468	2.72	3.88	V	
22	26.782	26.700	26.858	9054708	3.22	2367633	3.69	3.82	V	2-Undecanone (CAS) $$ 2-Hendecano
23	26.900	26.858	26.950	509767	0.18	155752	0.24	3.27	V	2-Undecanol
24	27.555	27.450	27.708	22664114	8.07	4823526	7.51	4.69		Indole
25	28.333	28.275	28.383	960718	0.34	311289	0.48	3.08		1-Tridecene
26	28.517	28.383	28.542	7561096	2.69	1627289	2.53	4.64	V	
27	28.567	28.542	28.658	4703376	1.67	1471104	2.29	3.19	V	
28	28.960	28.908	29.025	935128	0.33	291736	0.45	3.20	V	
29	29.749	29.617	29.800	7794105	2.77	1335408	2.08	5.83		3-Decen-1-ol, acetate, (Z)- $$ Z-3-Dec
30	29.865	29.800	29.917	18530762	6.60	5023605	7.83	3.68	V	1-Tridecene
31	29.990	29.917	30.142	16082341	5.73	3063390	4.77	5.24	V	
32	30.800	30.725	30.875	1528917	0.54	340960	0.53	4.48	V	1-Tetradecene (CAS) $$ n-Tetradec-1-
33	30.948	30.892	31.008	827009	0.29	236802	0.37	3.49		1-Tetracosanol
34	31.150	31.008	31.200	1633087	0.58	372164	0.58	4.38	V	1,13-Tetradecadiene
35	31.250	31.200	31.275	1059666	0.38	266386	0.41	3.97	V	
36	31.343	31.275	31.450	6086822	2.17	1648958	2.57	3.69	V	
37	31.498	31.450	31.558	637681	0.23	169283	0.26	3.76	V	2-Tetradecanone
38	31.801	31.742	31.908	1014313	0.36	195345	0.30	5.19		Tetradecanal
39	32.602	32.458	32.683	29011105	10.33	5404614	8.42	5.36		1,13-Tetradecadiene
40	32.790	32.683	32.858	25847381	9.20	4631974	7.22	5.58	V	9-Nonadecene $$ (9E)-9-Nonadecene
41	32.927	32.858	33.067	8673387	3.09	1175130	1.83	7.38	V	Pentadecane, 2,6,10,14-tetramethyl- $$
42	33.157	33.067	33.242	4079701	1.45	920003	1.43	4.43	V	
43	33.510	33.333	33.575	2335482	0.83	667901	1.04	3.49	V	
44	34.067	34.000	34.133	1167945	0.42	274582	0.43	4.25		1,13-Tetradecadiene
45	34.272	34.133	34.333	716379	0.26	149948	0.23	4.77	V	
46	34.817	34.733	34.917	2907424	1.04	679771	1.06	4.27		Tetradecanal
47	35.593	35.492	35.650	3318126	1.18	470528	0.73	7.05	V	
48	35.701	35.650	35.775	2649256	0.94	523259	0.82	5.06	V	
49	35.849	35.775	35.967	1512137	0.54	253556	0.39	5.96	V	Hexadecanal (CAS) $$ PALMITIC AL
50	38.346	38.267	38.433	1248818	0.44	261822	0.41	4.76		Octadecanal $$ Stearaldehyde $$ Octa
				280879375	100.00	64197621	100.00			