**Abstract**

DODD, THOMAS HERMAN. Monitoring of Fishery Product Quality Using Electronic Nose and Visible/Near-Infrared Spectroscopy. (Under the direction of Dr. S. Andrew Hale).

In order to evaluate new technologies that could improve quality determination of fishery products, this research investigated the application of electronic noses (e-nose) and Visible/Near Infrared (VIS/NIR) spectroscopy as possible sensing technologies. The quality of fishery products has always been hard to define, and is typically based on the general perception of the consumer evaluating the product. Expiration dates serve as a guide, but the sensory appeal of a fishery product is generally the deciding factor as to whether a product is deemed acceptable or not by the end consumer.

Various chemical and sensory methods to determine fish freshness are available to the food industry, but most are expensive, time consuming or destructive. A rapid, non-destructive method to ascertain fish quality would be of great benefit to both the industry that is eager to provide its consumers with a fresh, safe product and the consumer who is increasingly looking for a better guarantee of food quality.

The multivariate analysis (MVA) techniques used by both e-nose and VIS/NIR technologies are similar, but widespread use of these techniques has only become possible with the increased computing power of the past few years.

E-nose technology is a slightly newer technology than VIS/NIR. Due to its more recent introduction, a rapid decay study was first performed to evaluate the feasibility of this method to sense decay time in a readily available fishery product of tilapia. Linear discriminate analysis (LDA) was used as a feature extraction method. This allows for the class of a given sample to be taken into account when features are extracted to yield a much

better model. Separating the samples into 6 hour classes, a classification rate of 97.8% was achieved.

Once the e-nose had shown promise in quantifying fish decay, a more continuous model was chosen to more accurately model the continuous decay of fish products. It was also decided to perform the testing at actual storage conditions and choose a product that has a higher commercial value than tilapia, so that results would be more useful to the market. As such, blue crab meat was chosen for the study.

In order to compare the two technologies to a standard quality measurement, blue crab claw meat was sampled over its commercial storage period of 14 days on ice. Total Volatile Base Nitrogen (TVB-N) was used a baseline for measured meat quality and models were generated using data collect from both e-nose and VIS/NIR technologies. E-nose was found to be able to predict the TVB-N level in the meat with an accuracy of less than 5.0 mg TVB-N / 100 g. Using visible spectroscopy TVB-N levels were predicted to an accuracy of 4.8 mg TVB-N / 100 g. These values were found to be in the same range as that of the ion-specific electrode TVB-N measurements suggesting that these two technologies have the potential to be more accurate with better measurement of the calibration variable.

Since most research has shown a steady decrease in product quality with time, storage time was used a calibration variable. While this does not tie to a specific chemical indicator, time has the advantage of taking into account many different changes that might be missed by any specific indicator. This study investigated both blue crab lump meat and claw meat. E-nose and VIS/NIR readings were taken throughout the storage time of 14 days. These measurements were then used to create a model that could predict total storage time of a meat sample under specific storage conditions. With e-nose technology a standard error of

prediction (SEP) was achieved of 2.48 days for claw meat and 2.77 days for lump meat. VIS/NIR spectroscopy yielded significantly better results with 1.31 and 1.11 days for claw and lump meat respectively.

This research shows that both e-nose and VIS/NIR spectroscopy can be used to generate an estimate of fish quality. By being able to model both time and specific indicators, these two technologies show the robust nature that is normally seen in sensory panels. While maintaining these advantages, both technologies yield a repeatable and nondestructive testing method that has not been available in the past.

**Monitoring of Fishery Product Quality Using an Electronic Nose and Visible/Near-Infrared Spectroscopy**

By

**Thomas H. Dodd**

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**Dedication**

This dissertation is dedicated to my wife, Shannon Nicole Reynolds Dodd, without whom I would not have made it as far as I have. I love you.

**Biography**

Tom Dodd was born in Dalton, GA in 1974. He was raised in a very technical family where learning was put at the forefront of importance and influenced him to pursue higher education. He graduated from Walhalla High School in 1992 and enjoyed the small town education that he received there. Tom then studied Agricultural Engineering at Clemson University, with a particular interest in Biotechnology, and received his Bachelor's degree in 1996. He then began a joint graduate program between the mechanical engineering and biological engineering department at Clemson where he studied blood flow through a stented carotid artery. Married in 1997, Shannon his wonderful wife agreed to the grueling life as an engineer's wife. After graduating from Clemson, Tom was offered a national needs fellowship at NCSU where he studied under Dr. Andrew Hale to produce the following research. During that time he and his wife adopted two wonderful little boys; Jakob in 2002 from Azerbaijan, and Tae in 2005 from Korea.

**Acknowledgements**

I would like to acknowledge the vast help that I received in preparation of this dissertation. First I would like to thank Dr. Andy Hale. His patience has been very much appreciated and his guidance has been invaluable. I would also like to thank the BAE staff of NCSU. To Dr. Susan Blanchard, while your signature is not on the cover please know that without your help this would not have been possible. To our church family at Christ our Comfort, I would like to thank you for all your prayers and support, especially Kevin and Andrea Thumpston (and Andrew, Ally and Emma); and Alpesh and Urvi Patel (and Asha). Also I would like to thank my in-laws, Robert and Gloria Reynolds, for their constant prayers on my behalf. I would also like to thank my father, Dr. Roy Dodd, for taking all the random phone calls with strange questions, thanks for listening; and my mom, Mildred Dodd, for her encouragement, unwavering support and mostly gentle prodding. And to Mark, Jen, Kate, Will and Sam; and Cheri and Kevin and baby-on-the-way; thanks for your love.

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## 1. INTRODUCTION

In our consumer-driven society, food products are continually being evaluated for both quality and safety. Consumers want the food products they buy to be fresh and safe. But as customers become farther removed from their food sources, they can no longer readily obtain information about food product handling. The responsibility for food quality then is placed primarily on the provider of that food. The food industry in the United States posts revenues exceeding $290 billion dollars (Industry Profiles, 2006), with fresh and frozen seafood processing accounting for over $7.5 billion of those dollars (US Census Bureau, 2004). Thus food providers are highly motivated to ensure that their products are of sufficient quality to meet consumer expectations.

Innovation and the development of new technologies can be applied to increase the quality of food products. However, quality in food products is very difficult to define. Customers are increasingly requiring higher quality substances and demanding that stores and restaurants provide these products on a consistent basis. If a store can guarantee that certain quality standards are consistently met, then their customers are more likely to continue to buy products from that store and recommend those products to others.

Consumer demand drives the need for better determination of food quality. A majority of consumers in virtually all surveys voice significant concerns over pesticides in food. In “The Packer’s” 2003 Fresh Trends survey, 63 percent of shoppers buying organic food stated a preference for “fewer chemicals in food” and 51 percent said organic food is “Better for me/my family” (Benbrook, 2003). Meanwhile, food safety and security issues appear to be growing in scale and scope. The arrival of mad cow disease to this country is

heightening concerns, coupled with growing consumer awareness of global climate changes and the onslaught of dubious fertilizers, pesticides, and genetic engineering into the food chain (McFadden, 2003).

This is especially true for fishery products where short shelf life and strong odors are common. A recent study performed by *Consumer Reports* found more than one quarter of the fish samples that were tested were on the brink of spoilage, if not yet spoiled (Anonymous, 2001).

Demand for quality is leading many consumers to organic food and grocery stores, where food is expected to be fresher and safer. The US organic and natural foods business is growing at more than 20% a year, up to $13.5 billion in 2004 (Murphy, 2004). Whole Foods, the largest organic and natural foods grocer in the world, has the fastest overall and comparable-store sales growth in the grocery market; growing at almost double the rate of Wal-Mart (Fishman, 2004). And even Wal-Mart, the biggest seller of fish in the US, announced in February 2006 that it would begin to purchase all of its "wild-caught seafood from fisheries that have been certified as sustainable by an independent nonprofit called the Marine Stewardship Council"(Gunther, 2006). Of course, consumers expect to pay more for "good" fish, and sales show that they are more than willing to do so. In Britain, organic fish farms have just been recognized for the first time by the Soil Association, which certifies organic food processors. Sales of organic food rose 30% in Britain last year, fueled primarily by consumer concern with food safety and health (foodqualitynews.com, 2006).

With Hazard Analysis and Critical Control Point (HACCP) based regulations that require companies to monitor their processing operations (both in the US and the EU), the likelihood of consumers purchasing seafood of questionable quality should, in theory,

decrease. But with imports increasing, economically viable methods for rapidly determining safety and quality need to be developed to protect consumers and providers, and ensure that proper processing standards are followed. For most fishery products, critical control points are hard to define and monitor. The different quality measurements are usually defined by examining microbial count, sensory panel scores, and chemical indicators. Although these methods all show some overlap, there are differences between the quality levels that each one indicates. The standard that has been most widely accepted has been sensory panel scores. The disadvantage of sensory panels is that they required a highly trained panel that can be expensive to train and maintain. As labor rates increase and instrumentation cost decrease other options could become a much better economic decision.

Each one of these types of quality measurements senses a different aspect of quality, and while all will eventually show that a fishery product is going bad, there may not reach that conclusion at the same time. Examining microbial quality can show contamination in meat; however it is possible for meat to be "spoiled," or unfit for consumption, with no sign of microbial activity. Sensory panel scores, while often repeatable when using a trained panel, are time-consuming and expensive, making them cost prohibitive for most food manufacturers. Sensory panels are also impractical to use on a large scale, such as at a processing plant where many lots of food need to be tested. Chemical analysis, which measures the chemical breakdowns in a food product, may not correlate with sensory scores. While chemical indicators provide a good overall measurement of food quality, the chemical makeup of each meat is different, so it is difficult to establish a standard chemical indicator.

An instrument that can rapidly and economically assess the quality of food products would provide store and restaurant owners and consumers alike a way to ensure that products

are of exceptional quality. Recent developments in multivariate analysis techniques along with advances in data acquisition and sensing capabilities have led to the creation of new technologies that can better monitor complex situations such as the freshness of fishery products. Research presented in this dissertation used Electronic Noses (e-noses), multivariate analysis (MVA), and Visible/Near-Infrared (VIS/NIR) spectroscopy to examine the freshness of fishery products. The goal of this research was to develop an instrument that would assess the quality of fishery products safely, quickly and economically, with the eventual goal of producing a safer and higher quality food supply for consumers.

E-noses use multiple gas sensors to create a fingerprint of a gas sample, while multivariate analysis can then be used to analyze and sort these fingerprints. The e-nose uses non-specific sensors with overlapping sensitivities. Looking at a combined output from all the sensors can show in much greater detail what one sensor is not able to detect. The sensors can be made from a range of different materials with varying sensitivities. They measure changes in a gas sample and relate those back to a signal response that can be either transient or steady state. This data is then analyzed using MVA to create a model that predicts the variables of interest.

NIR works similarly by looking at the spectra of light given off by a material in both the visible and NIR regions of the electromagnetic spectrum. The visible part of NIR mimics human senses. Since quality is defined so much by consumer opinion, using technologies that mimic human senses should provide an accurate assessment of product quality. NIR improves upon these senses by adding to them and increasing their accuracy. Thus adding an invisible region of the spectrum to the visible light spectrum that is perceived by the consumer should yield an even better instrument measurement. NIR works by viewing the

physical characteristic manifestations of changes in the meat that cause changes in the reflective spectrum. The spectrum is then treated by a similar MVA technique as used in e-nose sensors. Using these three types of analysis, it is possible to develop an instrument that can quickly assess food quality.

# 2. REVIEW OF LITERATURE

## 2.1 QUALITY MEASUREMENT

The safety and quality of fishery products has been of particular concern in recent years. With the increasing globalization of fishery product sales, processors, consumers, and regulatory officials have been seeking improved methods for determining freshness and quality (Dalgaard, 2000). A study performed by Consumers Union found that more than one quarter of the fish samples tested were on the brink of spoilage (Consumer Reports, 2001). Seafood quality has always been very hard to quantify. The two main parts of overall quality are safety and freshness. A food is considered unsafe when a person eats a product and has an unpleasant physical side effect. A safe food should cause no unwanted physical side effects. Freshness is an individual opinion; it is how the consumer feels about the product based upon their senses. While there are basic sensory guidelines to follow when choosing fishery products, it usually comes down to how the consumer feels about the product's general appearance and/or odor. Consumers normally examine color, flavor, odor and texture when evaluating fishery products (Alaskaseafood.org, 2006). This research will help fishery providers ensure their product will be both safe and fresh for the consumer.

### 2.1.1. Safety

Most safety concerns in food products are from microbial and chemical contamination. Both of these hazards have to be measured and controlled in order to increase the safety of the food supply. Hazard Analysis and Critical Control Point (HACCP) processing limits these concerns. Processors using HACCP must identify possible hazards and make detailed plans on how to detect and deal with these hazards. A primary goal of HAACP involves keeping a record of control points and making sure that these points are kept within the desired range. New monitoring techniques such as e-nose and VIS/NIR spectroscopy provide two way of contributing to a HACCP plan to further improve its effectiveness. First is the ability to monitor control variables, such as odor, that were unable to be monitored before. Second is the ability to validate that the provided records are correct. These two monitoring improvements make for a more powerful HACCP plan that can provide an overall increase in product quality and safety.

Since 1995, the EU had implemented the HACCP principles by stating that a hazard analysis must be performed, but there were no laws regarding writing down the steps used in each hazard analysis. The US, which had used HACCP-based guidelines since the 1970s to regulate canned foods, followed suit in 1995 by also establishing HACCP guidelines regarding the processing of fishery products. To continue doing business after December 1997, U.S. seafood processors and importers had to have a written HACCP plan on file and an employee certified through FDA approved HACCP training (www.fda.gov, 2006). As of January 2006, the EU issued a new directive stating that "Food safety is a result of several factors: legislation should lay down minimum hygiene requirements; official controls should be in place to check food business operators' compliance and food business operators should

establish and operate food safety programmes and procedures based on the HACCP principles" ((EC) No 852/2004). In addition, the new EU guidelines emphasize that it is the "primary responsibility of food business operators to produce food safely" (Food Standards Agency, www.food.gov.uk). From 1988 to the present day, HACCP principles have been promoted and incorporated into food safety legislation in many countries around the world. The purpose of these regulations is to ensure safe processing and importing of food products, including fish and fishery products. This program arose because of growing public concern about seafood-borne illnesses and seafood safety as well as from industry requests for a practical, cost-effective solution.

Microbial contamination is of major concern in almost all food products but is especially important in low shelf-life foods such as meat. About one-third of the world's food production is lost due to microbial spoilage annually (Lund *et.al.*, 2000). Measuring microbial contamination is both expensive and time consuming. However, measuring microbial contamination is still very widely used and is a parameter that is often used in HACCP plans.

### 2.1.2. Freshness

Freshness is more of a nebulous concept. Ultimately the quality of a product is going to be determined by the consumer buying it. Therefore any quality measurement should correlate to sensory changes in the product. Two of the main senses that customers use are sight and smell. Most quality measurements performed in industry use trained personnel to get a sensory score for a product. These personnel are trained as to what to look for and smell for as product quality deteriorates. The FDA uses this process in determining the

acceptability of crab meat. Inspectors use their senses and existing records to make decisions about the acceptability of products.

2.1.2.1. Chemical indicators

In the past, chemical indices have been used as freshness indicators in different products. Several have worked well for specific products. K-value is one indicator that has been studied in the past. In this measure the breakdown of adnosine triphosphate (ATP) is measured as a percentage. The specific pathway is that ATP breaks down into adnosine diphosphate (ADP), adonosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx). The K-value is calculated using the following equation:

**Equation 2-1**

$$K = \frac{\text{Inosine} + \text{hypoxanthine}}{\text{Inosine} + \text{hypoxanthine} + \text{IMP}} \text{ x } 100$$

In Japan a K-value of 20% is used as the maximum acceptable limit for consumer fish served raw. For most fish, the K-value increases linearly for the first few days of storage. Although this would seem to make it a good quality indicator, two problems are associated with this procedure. The first is that the maximum K-value for a certain fish can be reached well before sensory rejection. The second is that the procedure requires trained personnel and expensive equipment to perform.

Total volatile base nitrogen (TVB-N), which mainly consists of Trimethlyamine (TMA), ammonia, and dimethylamine (DMA), are used as chemical indices of quality. The

European Commission (Council Regulation No. 95/149/EEC of March 1995) specified if there is any doubt about a fishery product's freshness then TVB-N is to be measured. Critical limits of 25, 30 and 35 mg-TVB-N/100g were established for different groups of fish species.A major problem with this methodology is an existence of a large discrepancy between sensory scores and TVB-N. Ranges from 10-75 mg-TVB-N/100g have been found at sensory rejection of fishery products (Irish Agriculture and Food Development Authority, 2006). Therefore while this method is acceptable to determine a general trend in the freshness of fish, it can not be used as a good quality test. TMA is a compound that is formed by microbial growth and is therefore an indication of spoilage not freshness.

Biogenic amines have also been used as a freshness index (BAI). One is defined as:

**Equation 2-2**

$$BAI = \text{histamine} + \text{cadaverine} + \text{tryrosine} + \text{putrescine}$$

This was found to have promise in grading tuna, where a limit of 50 mg/kg was found to coincide with end of shelf life. New biosensors for these compounds are being developed (University of California at Davis, 2006).

2.1.2.2. Sensory evaluation

Consumers use their senses in order to determine the quality of food. While taste is the ultimate evaluation of a food product, sight and smell are often used to determine how a product will taste. These senses can also be important in determining raw meat quality. Sensory panels are usually used to take out some of the subjectivity of these measurements. These panels of trained individuals produce consistent results but can be time consuming and

very expensive compared to other testing methods. Fishery products produce a very pungent odor as they decay. Dalgaard (2000) shows that a linear relationship generally exists between storage time and sensory panel score for many different fish species. Chen *et.al.* (1996) found that sensory scores for crab meat followed close to a linear relationship with storage time. Several other indicators were shown to have a non-linear relationship. Since the overall consumer score is what the consumer is going to use to evaluate the quality, the linear model seems to be the proper model to use in quality measurements.

## 2.2 ELECTRONIC NOSE

### 2.2.1. Biology of Smell

The sense of smell is very important to the way consumers perceive and rate food products. The sense of smell is achieved when small molecules (20-300 Daltons) are pulled into tiny structures called turbinates (Farbman, 1992). Volatile organic compounds (VOCs) are then conveyed to the surrounding epithelium. VOCs are conveyed through these cells to sensory cells which convert this data into nerve impulses. There are roughly one thousand different types of receptor cells in the human olfactory system. It is the way that these sensors cells respond that makes up the sense of smell.

### 2.2.2. Mimicking of the human nose

Since the human nose is used as a quality indicator in so many areas (perfumes, wines, coffee, and just about every food product that is consumed by humans) there has been a large push to try to mimic it. The difficulty of this rises from the variability and lack of quantitativeness of the human sense of smell.

One way this variability has been handled is using statistics. As with most systems, the more data points that are available, the better the overall accuracy. Trained sensory panels are used to provide a more statistically valid measurement than one person trying to quantify an odor. Although this method has shown itself to be very robust at handling a variety of problems, such as predicting how a consumer will respond to products and testing threshold levels, it has a multitude of drawbacks. The first would be the cost involved performing a sensory panel. Panelists require training and payment for their services. This would especially be expensive for a company that wants to monitor aroma in a process line at a significant rate.

Many instruments have been used in an attempt to model the human sense of smell. Gas Chromatography (GC) and Mass Spectrometry (MS) are widely used in the scientific community. These instruments operate by measuring the presence and concentration of specific chemicals in a sample. Although the obvious detriment of these techniques is their cost (both in equipment and manpower) there is another reason that these technologies have proven inadequate to model the human sense of smell. They do not correlate well with sensory panel scores. A possible explanation is found by examining the chemical composition of meat (or almost any other food product). The meat by itself has one flavor, but if a small amount of table salt is added, the flavor is changed considerably. Although the

chemical composition has not been considerably changed, the flavor was radically altered. This makes is very hard to find specific chemicals to measure which are associated with aroma. Individuals tend to associate a specific combination of flavors with an aroma, so flavor combinations would be as varied as the individuals themselves, leaving no easy way to set a benchmark for sensory panels.

### 2.2.3. Electronic nose

Development of electronic nose (e-nose) technology has made it possible to measure food odors rapidly and automatically. E-nose technologies are currently being developed in an effort to provide a rapid, inexpensive, and more quantitative means of monitoring odor. They have allowed scientists to distinguish among coffees, wines, and many other products where aroma is a quality factor strongly associated with smell (Schaller et al., 1998).

The e-nose mimics the human nose in that the human nose does not use one odorant receptor for each chemical. Instead, responses from combinations of receptors make up what is perceived as odor. The cross sensitivity of receptors allows a greater range of compounds to be detected. If each sensor in the e-nose only reacted to one specific compound, then many sensors would be needed to detect the hundreds of individual compounds associated with odor. Because of its ability to detect a very large range of compounds, the e-nose can theoretically detect any odor for which it has been trained.

An overview of applications for e-nose technologies can be found in Deisingh, et. al. (2004). For fish products the standard measurement for quality is QIM measurements determined by sensory panels. Olafsdottir et al.(2004) describes a method to model attributes

such as appearance smell and texture in order to generate an artificial quality index (AQI). Olafsson et al. (1992) performed an accelerated decay study that evaluated fish freshness by allowing haddock to decay at room temperature. Headspace gases above the decaying fish were sampled with an e-nose containing tin metal oxide sensors calibrated to respond to compounds associated with fish spoilage. The method was found to have promise for future development by providing quick results based upon the level of fish decay. However, caution was urged in the interpretation of these results because of the susceptibility of their accelerated decay study to anaerobic conditions. No classification of the decay in terms of oxygen requirements was reported.

Roussel et al. (1998) used tin metal oxide sensors to examine different techniques for feature extraction. Wines with satisfactory and unsatisfactory vinegar content were sampled, and classification was attempted. Twenty-nine feature extraction methods were tested. Methods that showed promise for compound discrimination were the steady-state value, maximum absorption slope, and minimum de-sorption slope. These methods were post-processing techniques, and data reduction was not examined.

Delpha et al. (1999) and Sarry and Lumbreras (1999) used principal component analysis (PCA) as a first discrimination technique for separating multivariate data into classes. PCA uses multivariate statistics to reduce a large multidimensional array down to its most important principal components.

Llobet et al. (1999) used artificial neural networks (ANN), which do not have a set way of looking for possible links, as a classification method. ANNs are very efficient at finding linkages in data that might not be observed by other methods. A neural network examines the entered data and the desired output and attempts to get its actual output to

match the desired one. This makes ANNs very powerful to use but complex to develop, since the network must first be trained.

Although some work has been performed with fish decay (Krzymien and Elias, 1990; Ohashi et al., 1991) and particularly with metal oxide sensors for fish decay (Egashira, et al., 1990; Olafsson et al., 1992), these technologies have not been used to classify fish decay.

Natale et al. (1996) used four quartz microbalance sensors and PCA analysis to examine freshness loss in cod fillets. The sensors measured the mass change as the odorant in the air was absorbed onto the surface of the sensor. The resulting data were found to have some differences but could not be separated at all time points. Implementation of neural networks resulted in a continuous curve that corresponded to decay time. Schweizer-Berberich et al. (1994) also analyzed the freshness of fish using eight different amperometric three-electrode gas sensors. They found a general trend for decay time by using PCA on the output data.

## 2.3 VISIBLE/NEAR INFRARED SPECTROSCOPY

### 2.3.1. Electromagnetic radiation and spectroscopy

The electromagnetic spectrum consists of a large range of radiation that travels at the speed of light. The range of wavelengths is shown in Figure 2-1. While the entire spectrum travels at the speed of light, the energy possessed by each radiation region is substantial. In general, higher frequencies contain greater energy levels.

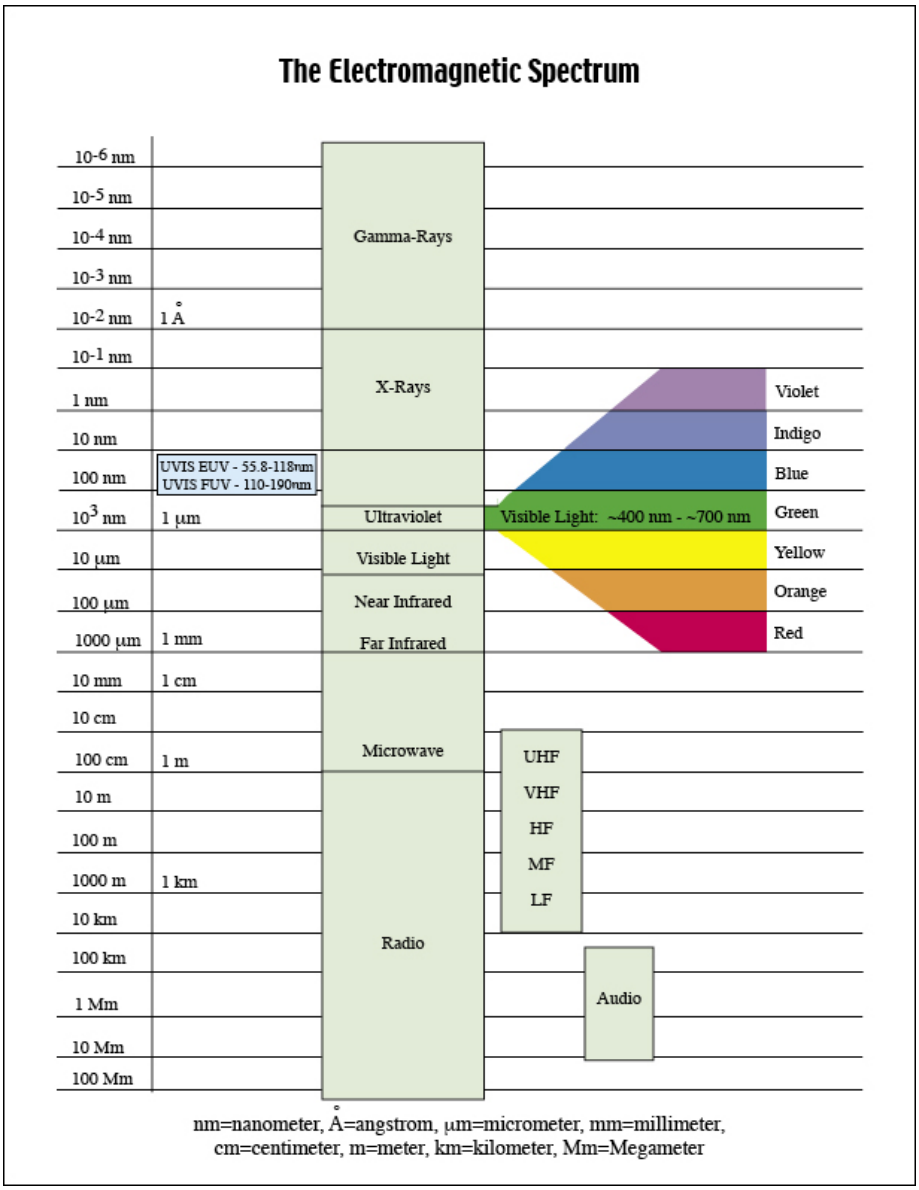



**Figure 2-1: Electromagnetic Spectrum**

When an electromagnetic wave comes in contact with matter it is either transmitted, absorbed, or reflected. Absorbed radiation can also be emitted by the material. The field of spectroscopy uses the information found in the spectrum that is emitted to predict certain qualities of the measured material.

The wavelength that is emitted or absorbed by the material is the result of changes in the energy state of electrons. The energy change can be described by the following equation:

**Equation 2-3**

$$\Delta E = E2 \ E1 = h\nu$$

Where h is Plank's Constant and ν is the frequency in Hz. So when a beam of radiation is focused on the material some of the energy is going to be absorbed by the material and some electrons will be excited into a different energy level. Spectroscopy is the study of how different compounds emit different spectra. This is possible because varied chemical combinations will absorb and reflect different wavelengths in numerous ways.

### 2.3.2. Visible Spectroscopy

The visible (VIS) spectra consist of wavelengths in the 380-700nm region. These wavelengths consist of what we are able to perceive visually. Chromophores are known to be distinctive groupings that absorb ultraviolet and visible radiation, usually conjugated double bonds, double bonds conjugated with carbonyl groups or aromatic rings. Quantitative spectroscopy is able to quantify or at least identify these chromophores.

The visible spectrum is a function of the entire structure of the compound rather than specific bonds. Other information should be used in conjunction with visible spectra in determining the specific properties of interest. Although little chemical data can be gathered from the visible spectra, it is widely used as a quality association with visual evaluation.

### 2.3.3. NIR Spectroscopy

The region of the electromagnetic spectrum from 700-2500 nm is known as the near-infrared (NIR) region. This radiation results from dipole vibration. This arises from covalent bonds stretching and vibrating when absorbing incoming electromagnetic radiation. Several excited states exist for each bond, and each one is caused by a specific frequency of radiation. The first excited state is known as the fundamental absorbance. It occurs in the mid-infrared region. An overtone occurs when a much smaller number of molecules are excited to the second energy level at a wavelength that is about half that of the fundamental absorbance wavelength. A second overtone occurs at one third the frequency and so on. Although in theory this leads to very specific bands for certain chemicals, in reality these bands end up being more spread out because real molecules do not adhere to the laws of simple harmonic motion. This is especially true for hydrogen bond vibrations. Hydrogen is a low molecular mass atom whose stretching fundamental absorbance occurs at 3600 – 2400 $cm^{-1}$. Hydrogen is a major component of most biological material, making its characteristics even more important. Covalent hydrogen bonds, C-H, O-H, and N-H, are likely responsible for the majority of the observed NIR spectra. Table 2-1 lists some important bonds that contain O-H, N-H and C-H bonds in foods and their respective wavelengths (Davies and Grant, 1987).

**Table 2-1: Important absorption bands and their tentative assignment for food components**

| Wavelength (nm) | Component | Bond |
|---|---|---|
| 1200 | Lipid | C-H |
| 1440 | Water and Carbohydrate | O-H |
| 1730 | Lipid | C-H |
| 1780 | Lipid | C-H |
| 1940 | Water | O-H |
| 1980 | Protein | N-H |
| 2080 | Carbohydrate | O-H |
| 2180 | Protein | N-H,C=O |
| 2320 | Lipid | C-H |
| 2350 | Lipid | C-H |

### 2.3.4. Applied VIS/NIR Spectroscopy

Much research has been done that has linked specific properties of meat products to VIS/NIR spectra. VIS/NIR data for cooked chicken patties were analyzed by Chen and Marks (1998). It was discovered that cooking loss and Kramer shear properties (yield force, yield deformation and yield energy) could be predicted using the VIS/NIR spectra of a sample. A standard normal variant (SNV) technique was used and then modified, and partial least squares regression was performed on the first derivative of the pretreated spectra. The data were then reduced into principal components (PCs) and calibration equations were established in terms of the PCs. Cooking loss and yield force were the most accurate predictions made with a standard error of prediction of 7.9% and 8.2%, respectively. The results showed that VIS/NIR spectroscopy could be used for rapid monitoring of physical characteristics in thermal processing of chicken patties.

Ding and Xu (1999) examined VIS/NIR spectroscopy to distinguish between beef and kangaroo meat. The study was conducted with meat samples that had been frozen and then thawed to room temperature. Both minced and cut meats were analyzed. For minced meat, scatter correction and derivative pre-treatment were used. For cut meat the original spectra were found to yield the best results. PCA was then performed on the data, and the first 13 principal components were used in Canonical Discriminant Analysis (CDA) for classification. Multiple Linear Regression (MLR) was also performed on the entire spectra, with beef samples assigned a target value of 1 and kangaroo samples assigned a value of 2. The worst classification rate obtained was 92.7% with CDA and 83.3% with MLR.

Ding and Xu (2000) detected adulteration of hamburger meat using NIR spectroscopy. Hamburger meat was artificially adulterated with 5-25% of mutton, pork, skim milk powder, and wheat flour. Adulteration level was also predicted by NIR spectroscopy once adulteration was detected. This was accomplished by first compressing the data with PCA. The first 20 principal components, which comprised 100% of the variation, were used for subsequent data analysis. CDA and K-nearest neighbor (KNN) were used to discriminate between samples that had not been adulterated and those that had been adulterated regardless of adulteration substance or level. The adulteration level was predicted by modified partial least squares (mPLS). All of these methods were performed on raw, cooked and minced hamburger. The classification accuracy for CDA was 76.6, 68.8, and 90.0. For KNN it was 83.5, 81.6, and 92.7. The classification accuracy increased with an increase in adulteration level. Prediction of adulteration level was then attempted. $R^2$ values of between 0.86 and 1.00 were obtained for minced sample regardless of the adulteration substances and levels.

NIR measurements have also been performed on fishery products. Downey (1996) used the NIR spectra (700-1100 nm range) obtained from farm raised salmon to determine the oil and moisture content of the fish. Using a fiber optic probe on the surface of the fish, a standard error of prediction (SEP) for oil and moisture was found to be 2.0 and 1.45% respectively for the dorsal side and 2.4 and 1.9% respectively for the ventral side. Zhang and Lee (1997) also used NIR spectroscopy to determine the free fatty acid level in fish oil. It was found that the NIR spectra could be analyzed using n-point smoothing, first derivative and second derivative treatments, and MLR and PLS regression could be used for calibration. It was also found that the NIR spectra could also be used to predict the freshness of mackerel in so far as FFA change and the hypoxanthine (Hx) trend in the fish meat spoilage.

The effect of freezing on meat was examined by Downey and Beauchêne (1997). Beef samples were scanned using VIS/NIR spectroscopy, frozen-then-thawed and scanned again. It was found that this methodology could distinguish, in combination with factorial discrimination, between fresh and frozen-then-thawed meat. Using four principal components a classification rate of 64% was achieved. It was also found that color information (650-748nm) helped in the model but was insufficient to create its own model. MSC was also used and found that with a three-component model a classification of 64.1% of validation samples was achieved.

## 2.4 MULTIVARIATE ANALYSIS

Modeling the behavior of variables is the goal of many sciences. Most of statistics is based on using a few variables to model a desired response. The more variables that are introduced, the more complex the problem becomes. Using a large number of variables to produce a model is known as multivariate analysis. Multivariate statistical analysis is at the heart of both the electronic nose and VIS/NIR technologies. Both of these technologies produce datasets with a large number of variables. In contrast to a classical sensor that simply measures one variable, these technologies monitor large numbers of variables and use multivariate analysis to produce a model that explains a response. In general, the data will have the following structure:

**Equation 2-4**

$$
\begin{aligned}
Y_1 &= X_{11} + X_{12} + X_{13} \ldots + X_{1p} \\
Y_2 &= X_{21} + X_{22} + X_{23} \ldots + X_{2p} \\
&\vdots \\
Y_i &= X_{i1} + X_{i2} + X_{i3} \ldots + X_{ip}
\end{aligned}
$$

Where Y is a response variable and $X_1$ through $X_p$ are the explanatory variables measured.

Using this general data form, several techniques can be used to get a model that better predicts the response variable. These include supervised and unsupervised methods. Unsupervised models do not use the response variable in the model causation. This leads to a model that is independent of the response variable. This can be very beneficial when looking at multiple response variables or comparing the ability of the model to predict different response variables. Supervised methods use the response variable of interest in the

model calculations. This leads to a model that usually fits the response variable of interest better, but the model is more limited to that particular application.

### 2.4.1. Multiple Linear Regression

Multiple linear regression (MLR) is a supervised method that uses a linear combination of explanatory variables to model the response of a response variable. Using the form given in Equation 2-4 the model becomes a linear combination of $X_p$ variables which describe the *Y* response variable. The result is a model that looks very similar to that of a standard regression model:

**Equation 2-5**

$$Y_1 = a_{11}X_1 + a_{12}X_2 + a_{13}X_3 \ldots + a_{1p}X_p + \varepsilon_1$$
$$Y_2 = a_{21}X_1 + a_{22}X_2 + a_{23}X_3 \ldots + a_{2p}X_p + \varepsilon_2$$
$$\vdots$$
$$Y_i = a_{i1}X_1 + a_{i2}X_2 + a_{i3}X_3 \ldots + a_{ip}X_p + \varepsilon_p$$

**a** represents model parameters that best model the response variable Y and ε is a error that is associated with each observation. Usually ε is considered to be a random error with a mean of 0 and a standard deviation of σ. This leads to a general form of DATA = FIT + RESIDUALS. Expected performance of the model can be determined by calculating the difference between the Y calculated by the model and the measured Y. These values are called residuals and these can be used to calculate the standard error of Calibration (SEC). This value is calculated from samples that have been used in the model creation. In order to evaluate how the model performs with unseen examples, a subset is usually withheld and then given to the model. The model's output is then compared with the known value of the response variable. This resulting difference is called the standard error of prediction (SEP).

### 2.4.2. Principal Component Analysis

Principal Component Analysis (PCA) is an unsupervised multivariate technique that is frequently used for complex problems. PCA reduces the way that data is presented by calculating principal components (PCs) for the data set. These PCs are linear combinations of each data point from a particular sampling event. The first component is the combination that represents the largest variation in the data, thereby giving the data the best class separation, whereas the second component gives the next best class separation, and so on. Typically, the first several components are graphed for a visual representation of the data. PCA is considered an unsupervised process because it uses no information about the sample to influence PC computations. This process determines the most variation regardless of its source. This can be both beneficial and detrimental to analysis with biological products. Biological products usually have a large variability. Limiting this can be problematic and limit the scope of research. Using an unsupervised method like this helps identify what variations are present in the dataset and how they can be accounted for in further analysis. By graphing the first few components these values can be assigned different values according to the desired response variable and the same graph shows the results of different variables. This can be useful to verify that the response variable of interest does account for the majority of the variation, or to discover what responses are contributing more to the PCA model.

Since PCA does not assume any knowledge of the response variable, the data keep the form of equation 2-4 as the data structure but without the Y variable. The principal components can be calculated using the following equation (Manly, 1986):

**Equation 2-6**

$$PC_i = \sum (a_{ij} x_j) \{ i = 1, \ldots, n; \; j = 1, \ldots, p \; \}$$

Where $PC_i$ = the $i^{th}$ principal component

x = input variables

a = values of eigenvector **a**

The principal components have the flowing constraints:

$$\sum a^2{}_{ij} = 1$$

All of the PCs are uncorrelated.

Therefore the PC will be a particular linear combination of the *p* variables in a matrix $x_{ip}$ where *i* and *p* represent the number of samples and variables respectively. Geometrically speaking these combinations are a new coordinate system obtained by rotating the original matrix. The resulting matrix axes provide a simpler description of the variability in the original matrix by sorting the linear combinations of variables according to how much variation is expressed in each combination. Mathematically this involves calculating the eigenvalues of the sample covariance matrix. The covariance matrix has the following form

**Equation 2-7**

$$Cov(A) = \begin{bmatrix} c_{11} & c_{12} & \cdots & c_{1p} \\ c_{21} & c_{22} & \cdots & c_{2p} \\ \vdots & \vdots & \ddots & \vdots \\ c_{p1} & c_{p2} & \cdots & c_{pp} \end{bmatrix}$$

Where the diagonal represents the variance of X and the off diagonal values are the covariance values between the variables. The eigenvalues of Cov(A), $\lambda_i$, represent the variances of the principal components ($var(PC_{i)=} \lambda_i$). The eigenvalues are then sorted to

correspond to the largest variances. This in turn leads to the first few PCs accounting for most of the variation found in the dataset.

### 2.4.3. Linear Discriminate Analysis

Linear Discriminate Analysis (LDA) is similar to PCA in that both take a multivariate set of data and reduce it to a set of components representing the variance that is found in the data. However LDA is different in that it is a supervised method where the vectors (or components) are chosen based upon a class assigned to each sample. LDA then groups the samples according to the assigning classes and returns linear combinations of the variables that shows the most special distance between the groups. In general if the samples can be assigned a group that is known, LDA gives a better model that separated the variables of interest than PCA. But if groups are unknown LDA can not be used.

Mathematically the data has the form of Equation 2-1 but the variable is replaced with a classification instead of a value. This would give the data the following form, where $X_{nmp}$ is the $p^{th}$ variable of the $n^{th}$ sample of a given class m:

**Equation 2-8**

$$
\begin{aligned}
&x_{111} + x_{112} + x_{113} \ldots + x_{11p} \\
&x_{211} + x_{212} + x_{213} \ldots + x_{21p} \\
&\vdots \\
&x_{n11} + x_{n12} + x_{n13} \ldots + x_{n1p} \\
&x_{121} + x_{122} + x_{123} \ldots + x_{12p} \\
&x_{221} + x_{222} + x_{223} \ldots + x_{22p} \\
&\vdots \\
&x_{n21} + x_{n22} + x_{n23} \ldots + x_{n2p} \\
&x_{1m1} + x_{1m2} + x_{1m3} \ldots + x_{1mp} \\
&\vdots \\
&x_{nm1} + x_{nm2} + x_{nm3} \ldots + x_{nmp}
\end{aligned}
$$

Three matrices are calculated from this data W, the within sample matrix, B the between sample matrix, and T the total sample matrix (Manly, 1986). The elements of the T and W matrix are calculated using the following equations:

**Equation 2-9**

$$t_{rc} = \sum_{j=1}^{m} \sum_{i=1}^{n_j} (x_{ijr} - \overline{x_r})(x_{ijc} - \overline{x_c})$$

**Equation 2-10**

$$w_{rc} = \sum_{j=1}^{m} \sum_{i=1}^{n_j} (x_{ijr} - \overline{x_{jr}})(x_{ijc} - \overline{x_{jc}})$$

Where $x_{ijk}$ represents the value of the $i^{th}$ row of the class j

$\overline{x_{jr}}$ represents the mean of the $j^{th}$ sample

$\overline{x_r}$ represents the mean of Xr

The between sample matrix B is found by subtracting matrix T from matrix W. The eigenvalues of the matrix $W^{-1}B$ generate the coefficients for the canonical discriminant

functions (DFs). As with PCA, the returned functions are sorted such that the most variation is in the first few DFs. The returned number of function is equal to the number of classes minus one. Sarry and Lumbreras (1999) found that this method provided excellent separation of carbon dioxide, freon refrigerant (R134a), and mixtures of the two.

### 2.4.4. Principal Component Regression

Principal component regression (PCR) builds upon PCA. The result is a supervised analysis that yields a multiple linear regression equation based on a specified number of principal components. This can be very useful because of the limitation of MLR that it can only be used where there are more samples than variables of interest. In spectroscopy where there are a large number of variables, PCA can be used to concentrate as much variation into the first few components and then PCR is performed on those first few components to give a prediction model that can be evaluated mathematically. This allows for a model to be built using these PCs as a continuous output signal. The output of PCA has the same form as equation 2-1 with the factors rearranged into those which explain the most data variation in the first few columns. The principal components are multiplied by the original data to generate scores for each of the samples. By plotting the first few of the principal components, a graph can be generated that shows some of the major variation that was found by using PCA analysis. Since PCA is an unsupervised method, the scores that are generated are independent of the response variable of interest. Therefore the same graph can be used and the points can be defined by different response variables.

### 2.4.5. Partial Least Squares

PLS is an extension of MLR, but the limitation of requiring more samples than variables is removed. Using the data in the form of equation 2-1 the first step in PLS is to center the data by subtracting the mean of each variable from all the observations. This is because with other regression tools when there are more variables than samples (n<p) the covariance matrix $\mathbf{X}^t\mathbf{X}$ is singular. PLS can be used based in the basic latent component decomposition:

**Equation 2-11**

$$Y = TQ^T + F$$

**Equation 2-12**

$$X = TP^T + E$$

Where T is a matrix giving the latent components for the n observations, P and Q are the matrices of coefficients and E and F are random error terms. The goal is again to generate a model of the form T=XW where W is weight matrix.

The latent components are then used in place of the original variables. $Q^T$ is the calculated as the least squares solution to Equation 2-13.

**Equation 2-13**

$$Q^T = (T^T T)^{-1} T^T Y$$

The regression coefficients B for the model Y = XB +F are given by:

**Equation 2-14**

$$B = WQ^T = W(T^T T)^{-1} T^T Y$$

and the model response matrix $\hat{\mathbf{Y}}$ may be may be written as

**Equation 2-15**

$$\overline{\mathrm{Y}} = \mathrm{T}(\mathrm{T}^{\mathrm{T}}T)^{-1}T^{T}Y$$

The coefficients of B can then be used to calculate the model performance for unseen examples.

### 2.4.6. Least Squares Classifier

Least squares classifier (LSC) uses data in the general form of equation 2-1, but instead of using a continuous response variable, a class is used to calculate the model. A subset of the data will be separated to designate a training set and a matrix is generated with classes being assigned to each observation and assigned a column in the Y response variable. The model that is generated using the training set takes the form

**Equation 2-16**

$$B = CA$$

where **B** is a matrix describing the class of the triaging dataset, **C** contains the observations of the training dataset, and **A** is the regression parameters calculated by the model.

Once **A** has been calculated the test dataset is used in Equation 2-16 in place of the training set and a new response matrix is calculated. By examining the columns of the new **B,** a class is assigned to each observation. This class corresponds to the row having the highest output.

### 2.4.7. K-Nearest Neighbor Classifier

The classification method known as K-Nearest Neighbor (KNN) has been used in many multivariate problems. KNN does not use knowledge of a sample in its calculation but uses the model that has been generated from MSA and gets a score value for the unknown sample. This value is then compared with samples of known classes. The distances between the unknown sample and the known samples are compared and the k nearest samples are evaluated. The value of k is variable. The class that is found most often in the k nearest samples is then assigned to the unknown variable.

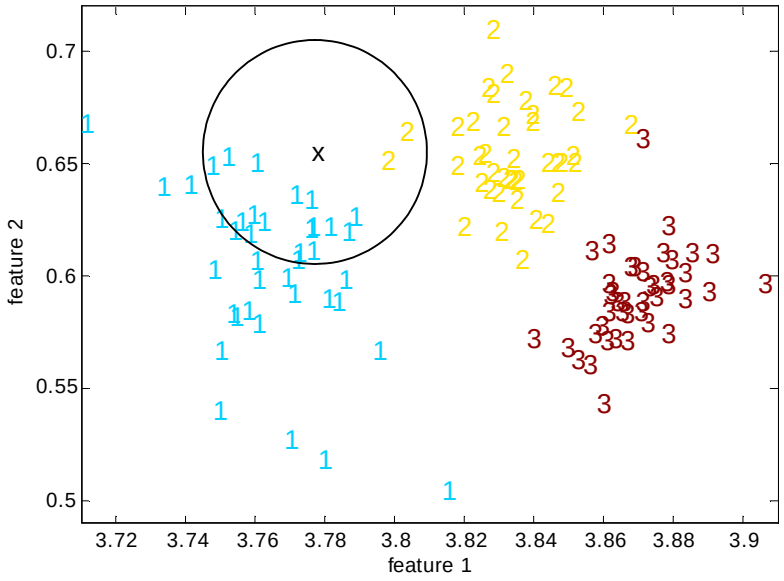



**Figure 2-2: Example of KNN classification**

An example is given in Figure 2-2. An unknown sample is designated by an 'x.' It can be seen that the majority of the points closest to the unknown sample are classified as a 1. Therefore KNN would classify the unknown sample as a 1.

### 2.4.8. Artificial Neural Network

The classification method known as Artificial Neural Networks (ANNs) is very efficient at finding linkages in data that might not be observed by other methods. A neural network examines the entered data and the desired output and attempts to get its actual output to match the desired one. This makes ANNs very powerful to use but complex to develop, since the network must first be trained. Neural networks work by attempting to mimic the way the human brain works. Simply put, this involves taking a group of functions that have the ability to change, and, knowing the desired response to a given input set, the group changes to adapt to this.

## 2.5 RESEARCH OBJECTIVE

The objective of this research was to investigate the applicability of e-nose and VIS/NIR technologies to provide a rapid and non-destructive measurement of fishery product quality.

## 2.6 REFERENCES

Bartlett PN, Elliott JM, Gardner JW. 1997. Electronic noses and their application in the food industry. *Food Technol* 51(12): 44-8.

Benbrook, Charles M. Why Food Safety Will Continue Driving Growth in Demand for Organic Food. 24 Jan. 2003. 25 Feb. 2005 <http://www.biotech-info.net/Ecofarm_Food_Safety.pdf>.

Bene A, Hayman A, Reynard E, Luisier JL, Villettaz JC. 2001. New method for the rapid determination of volatile substances: The SPME-direct method. Part II. Determination of the freshness of fish. *Sensors and Actuators, B: Chemical* 72(3): 204-7.

*Consumer Reports*. 2001. America's fish: Fair or foul? *Consumer Reports* (Feb.): 25-31.

Dalgaard, P. 2000. *Freshness, Quality, and Safety in Seafoods*. Flair-Flow Europe Technical Manual F-FE 380A/00. Dublin, Ireland: The National Food Centre.

(EC) No 852/2004 "REGULATION (EC) No 852/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on the hygiene of foodstuffs" November 28, 2006 <http://www.fsai.ie/legislation/food/eu_docs/Food_hygiene/Reg852_2004.pdf>

Deisingh A, Stone D, Thompson M. 2004. Application of electronic noses and tongues in food analysis. *International Journal of Food Science and Technology* 39(587-604.

Delpha C, Lumbreras M, Siadat M. 2001. Discrimination of Forane 134a and carbon dioxide concentrations in an air conditioned atmosphere with an electronic nose: Influence of the relative humidity. *Sensors and Actuators, B: Chemical* 80(1): 59-67.

Delpha C, Siadat M, Lumbreras M. 1999. Relative humidity: An interfering parameter for the characterization of a TGS sensor array. *Proc SPIE Int Soc Opt Eng* 3857(223-30.

Di Natale C, Brunink JAJ, Bungaro F, Davide F, d'Amico A, Paolesse R, Boschi T, Faccio M, Ferri G. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas Sci Technol* 7(8): 1103-14.

Di Natale C, Macagnano A, Mantini A, Davide F, D'Amico A, Paolesse R, Boschi T, Faccio M. Ferri G. 1997. Advances in food analysis by electronic nose. 1(122-7.

Dodd TH, Hale SA, Blanchard SM. 2004. Electronic nose analysis of tilapia storage. *Transactions of the American Society of Agricultural Engineers* 47(1): 135-40.

Downey G, 1996. Authentication of food and food ingredients by near infrared spectroscopy. *J. Near Infrared Spectrosc*. 4, p. 47–61.

Egashira M, Shimizu Y, Takao Y. 1990. Trimethylamine sensor based on semiconductive metal oxides for detection of fish freshness. *Sensors and Actuators, B: Chemical* B1(1-6): 108-12.

Ellis PC, Pivarmk LF, Thiam M, Berger L, Field S, Green D, Hewes D, Lemerise D, Lyttle C, Maciel J, Soper K. 2000. Determination of volatile bases in seafood using the ammonia ion selective electrode: Collaborative study. *J AOAC Int* 83(4): 933-43.

Fishman, Charles. "The Anarchist's Cookbook." Fast Company 84 (2004): 70

FoodQualityNews.com. Organic Fish Farms Get Green Light in UK. 26 Aug. 2006. 26 Nov. 2006 <http://www.foodqualitynews.com/news/ng.asp?id=69913>.

Gao D, Wu S. 1998. Development of artificial olfactory system and its prospects on evaluating flavor of food. *Nongye Jixie Xuebao/Transactions of the Chinese Society of Agricultural Machinery* 29(4): 167-72.

Gutierrez-Osuna R, Nagle HT. 1999. Method for evaluating data-preprocessing techniques for odor classification with an array of gas sensors. *IEEE Transactions on Systems, Man, and Cybernetics, Part B: Cybernetics* 29(5): 626-32.

Gunther, Marc. Saving Seafood. 31 July 2006. 25 Nov. 2006 <http://money.cnn.com/2006/07/25/news/companies/pluggedin_gunther_fish.fortune/index.htm>.

Gupta S, Misra TN. 1997. Manganese phthalocyanine for the detection of fish freshness by its trimethylamine emission. *Sensors and Actuators, B: Chemical* B41(1-3): 199-202.

Hammond J, Marquis B, Michaels R, Oickle B, Segee B, Vetelino J, Bushway A, Camire ME, Davis-Dentici K. 2002. A semi conducting metal-oxide array for monitoring fish freshness. *Sensors and Actuators, B: Chemical* 84(2-3): 113-22.

Hammond J, Mlsna T, Smith D, Fruhberger B. 1999. Fish freshness sensor. *Proc SPIE Int Soc Opt Eng* 3856(88-96.

Haugen JE, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Olafsdottir G. 2006. Rapid control of smoked Atlantic salmon (Salmo salar) quality by electronic nose: Correlation with classical evaluation methods. *Sensors and Actuators, B: Chemical* 116(1-2): 72-7.

Hofmann T, Schieberle P, Krummel C, Freiling A, Bock J, Heinert L, Kohl D. 1997. High resolution gas chromatography/selective odorant measurement by multisensor array (HRGC/SOMSA): A useful approach to standardize multisensor arrays for use in the detection of key food odorants. *Sensors and Actuators, B: Chemical* B41(1-3): 81-7.

Irish Agriculture and Food Development Authority. Freshness, Quality and Safety in Seafoods.. 25 Nov. 2006 <http://www.teagasc.ie/nfc/research/preparedfoods/quality_seafoods.pdf>.

Krzymien, M. E., and L. Elias. 1990. Feasibility study on the determination of fish freshness by trimethylamine headspace analysis. *J. Food Science* 55(5): 1228-1232.

Legin A, Rudnitskaya A, Seleznev B, Vlasov Y. Velikzhanin V. 2001. Electronic tongue for recognition of flesh food. 76-81.

Lindsay RC. 1988. Flavor chemistry and seafood quality factors. 1(61-5.

Llobet E, Hines EL, Gardner JW, Bartlett PN, Mottram TT. 1999. Fuzzy ARTMAP based electronic nose data analysis. *Sensors and Actuators, B: Chemical* B61(1-3): 183-90.

MacAgnano A, Careche M, Herrero A, Paolesse R, Martinelli E, Pennazza G, Carmona P, D'Amico A, Di Natale C. 2005. A model to predict fish quality from instrumental features. *Sensors and Actuators, B: Chemical* 111-112(SUPPL): 293-8.

McClure W, Crowell B. 1996. Quantitative information in near-infrared spectra: Part 1. Effects of smoothing and combing. *J Near-Infrared Spectroscopy* 4(129-137.

McFadden, Steven. The History of Community Supported Agriculture, Part II CSA's World of Possibilities. 2003. 26 Nov. 2006 <http://www.newfarm.org/features/0204/csa2/part2.shtml>.

Mitsubayashi K, Kubotera Y, Yano K, Hashimoto Y, Kon T, Nakakura S, Nishi Y, Endo H. 2004. Trimethylamine biosensor with flavin-containing monooxygenase type 3 (FMO3) for fish-freshness analysis. *Sensors and Actuators, B: Chemical* 103(1-2): 463-7.

Murphy, Richard. Food Safety--Why People Are Turning to Organics. 1 Mar. 2004. 25 Nov. 2006 <http://www.organicconsumers.org/organic/demographics031604.cfm>.

Nagle HT, Schiffman SS, Gutierrez-Osuna R. 1998. How and why of electronic noses. *IEEE Spectrum* 35(9): 22-34.

Natale CD, Olafsdottir G, Einarsson S, Martinelli E, Paolesse R, D'Amico A. 2001. Comparison and integration of different electronic noses for freshness evaluation of cod-fish fillets. *Sensors and Actuators, B: Chemical* 77(1-2): 572-8.

Natale C., J. A. J. Brunink, F. Bungaro, F. Davide, A. d'Amico, R. Paolesse, T. Boschi, M. Faccio, and G. Ferri. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensors array. *Measurement Science and Tech*. 7(8): 1103-1114.

Nilsen H, Esaiassen M. 2005. Predicting sensory score of cod (Gadus morhua) from visible spectroscopy. *Food Science and Technology* 38(1): 95-9.

Ohashi, E., Y. Takao, T. Fujita, Y. Shimizu, and M. Egashira. 1991. Semiconductive trimethylamine gas sensor for detecting fish freshness. *J. Food Science* 56(5): 1275-1278.

Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschlager J, Tryggvadottir SV, Schubring R, Kroeger M, Heia K, Esaiassen M, Macagnano A, Jorgensen BM. 2004. Multisensor for fish quality determination. *Trends in Food Science and Technology* 15(2): 86-93.

Olafsdottir G, Li X, Lauzon HL, Jonsdottir R. 2002. Precision and application of electronic nose for freshness monitoring of whole redfish (Sebastes marinus) stored in ice and modified atmosphere bulk storage. *J Aquat Food Prod Technol* 11(3-4): 229-49.

Olafsson, R., E. Martinsdottier, G. Olafsdottir, P. I. Sigfussonand, and J. W. Gardner. 1992. Monitoring of fish freshness using tin oxide sensors. In *Sensors and Sensory Systems for an Electronic Nose*, 257-272. J. W. Gardner and P. N. Bartlett, eds. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Pacquit A, Lau KT. Diamond D. 2004. Development of a colorimetric sensor for monitoring of fish spoilage amines in packaging headspace. 1(365-7.

Roussel S, Forsberg G, Grenier P, Bellon-Maurel V. 1999. Optimization of electronic nose measurements. Part II: Influence of experimental parameters. *J Food Eng* 39(1): 9-15.

Roussel S, Forsberg G, Steinmetz V, Grenier P, Bellon-Maurel V. 1998. Optimisation of electronic nose measurements. Part I: methodology of output feature selection. *J Food Eng* 37(2): 207-22.

Sarry F, Lumbreras M. 1999. Gas composition determination in an air conditioned system using a sensor array: Characterization of three different TGS sensors. *Sensors and Actuators, B: Chemical* 59(2-3): 94-9.

SearchEngines.com. Industry Profiles.. 26 Nov. 2006 <http://www.searchengines.com/industry_resources/industry_profiles2.html#food>.

Schaller, E., J. O. Bosset, and F. Escher. 1998. Electronic noses and their application to food. *Lebensm. Wiss. Technol*. 31(4): 305-316.

Schweier-Berberich PM, Vaihinger S, Gopel W. 1994. Characterization of food freshness with sensor arrays. *Sensors and Actuators, B: Chemical* B18(1-3 pt 1): 282-90.

Takao Y, Iwanaga Y, Shimizu Y, Egashira M. 1993. Trimethylamine-sensing mechanism of $TiO_2$-based sensors 1. Effects of metal additives on trimethylamine-sensing properties of $TiO_2$ sensors. *Sensors and Actuators, B: Chemical* B10(3): 229-34.

University of California Davis. Freshness, Quality and Safety in Seafoods.. 25 Nov. 2006 <http://seafood.ucdavis.edu/pubs/qualitysafety.doc>.

U.S. CENSUS BUREAU. Fresh and Frozen Seafood Processing: 2002. Dec. 2004. 26 Nov. 2006 <http://www.census.gov/prod/ec02/ec0231i311712.pdf>.

Watanabe KH, Desimone FW, Thiyagarajah A, Hartley WR, Hindrichs AE. 2003. Fish tissue quality in the lower Mississippi River and health risks from fish consumption. *Sci Total Environ* 302(1-3): 109-26.

Zhang Y, Zhang Z, Sugiura N, Maekawa T. 2002. Monitoring of methanogen density using near-infrared spectroscopy. *Biomass Bioenergy* 22(6): 489-95.

Zhao C, Pan Y, Ma L, Tang Z, Zhao G, Wang L. 2001. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal. *Sensors and Actuators, B: Chemical* 81(2-3): 218-22.

# 3. ELECTRONIC NOSE ANALYSIS OF TILAPIA STORAGE

**T. H. Dodd, S. A. Hale, S. M. Blanchard**

## 3.1 ABSTRACT.

An electronic nose (e-nose), containing 16 tin metal oxide sensors with various sensitivities, was used to classify decay times in an 18 h accelerated decay study of tilapia (Oreochromis niloticus). Data collected were split into three 6 h base classes for training. Principal component analysis was tested for feature extraction to be used in classification but was found to be inadequate. Linear discriminate analysis was also used and found adequate for feature extraction. Both least squares and K-nearest neighbor classifiers were explored. Least squares and K-nearest neighbor produced classification rates of 86.4% and 87.0%, respectively. Data combing techniques were used to increase classification rates from 87.0% to 97.8% for K-nearest neighbor. Optimum classification performance was achieved with classes corresponding to 0-1.9 h, 6-7.9 h, and 12-13.9 h. The dataset was also classified into six 3 h classes. Data classifications for the 3 h classes followed trends expected for decaying freshwater fish. Data combing was again employed to increase the classification that was possible. A final classification was achieved of 78.8% for least squares and 83.8% for K-nearest neighbor.

***Keywords.*** *Aroma, Electronic nose, Fish freshness, Tilapia.*

## 3.2 INTRODUCTION

The safety and quality of fishery products has been of particular concern in recent years. With the increasing globalization of fishery product sales, processors, consumers, and regulatory officials have been seeking improved methods for determining freshness and quality (Dalgaard, 2000). A recent study performed by Consumers Union found that more than one quarter of the fish samples tested were on the brink of spoilage (*Consumer Reports*, 2001). Odor is one of the most commonly used quality indicators. Products with unpleasant odors are likely to be rejected as unsafe. Development of electronic nose (e-nose) technology has made it possible to measure food odors rapidly and automatically. It has allowed scientists to distinguish among coffees, wines, and many other products where aroma is a factor strongly associated with quality (Schaller et al., 1998).

Measurement of odor has always presented researchers with a difficult problem because of its complexity and dependence on individual perceptions. Sensory panels, gas chromatography, and mass spectrometry have been widely used in odor classification (Nagle et al., 1998). While these techniques have been somewhat effective, they are time consuming, expensive, and can produce results with high degrees of variability. E-nose technologies are currently being developed in an effort to provide a rapid, inexpensive, and more quantitative means of monitoring odor.

The e-nose mimics the human nose in that the human nose does not use one odorant receptor for each chemical. Instead, responses from combinations of receptors make up what is perceived as odor. The cross sensitivity of receptors allows a greater range of compounds to be detected. If each sensor in the e-nose only reacted to one specific compound, then many sensors would be needed to detect the hundreds of individual compounds associated with

odor. Because of its ability to detect a very large range of compounds, the e-nose can theoretically detect any odor for which it has been trained.

Olafsson et al. (1992) performed an accelerated decay study that evaluated fish freshness by allowing haddock to decay at room temperature. Headspace gases above the decaying fish were sampled with an e-nose containing tin metal oxide sensors calibrated to respond to compounds associated with fish spoilage. The method was found to have promise for future development by providing quick results based upon the level of fish decay. However, caution was urged in the interpretation of these results because of the susceptibility of their accelerated decay study to anaerobic conditions. No classification of the decay in terms of oxygen requirements was reported.

Roussel et al. (1998) used tin metal oxide sensors to examine different techniques for feature extraction. Wines with satisfactory and unsatisfactory vinegar content were sampled, and classification was attempted. Twenty-nine feature extraction methods were tested. Methods that showed promise for compound discrimination were the steady-state value, maximum absorption slope, and minimum desorption slope. These methods were post-processing techniques, and data reduction was not examined.

Multivariate techniques are widely used for the analysis of e-nose data. With these methods, statistical features are extracted from the dataset as linear combinations of the data. The resulting statistical features are then used as inputs for analysis and classification algorithms. Delpha et al. (1999) and Sarry and Lumbreras (1999) used principal component analysis (PCA) as a first discrimination technique for separating multivariate data into classes. PCA uses multivariate statistics to reduce a large multidimensional array down to its most important principal components. The principal components consist of linear

combinations of each data point from a particular sampling event. The first component is the combination that represents the largest variation in the data, thereby giving the data the best class separation, whereas the second component gives the next best class separation, and so on. Typically, the first several components are graphed for a visual representation of the data.

Linear discriminant analysis (LDA), which is also referred to as discriminant factorial analysis (DFA), has also been used to distinguish between different classes. LDA works like PCA but adds knowledge of the classes that it is trying to separate. LDA then looks for the linear combination of data points that provide the best separation of those classes. It returns this combination as the first component providing the best separation between classes; the next component provides the second best class separation, and so on. Sarry and Lumbreras (1999) found that this method provided excellent separation of carbon dioxide, freon refrigerant (R134a), and mixtures of the two.

Llobet et al. (1999) used artificial neural networks (ANN), which do not have a set way of looking for possible links, as a classification method. ANNs are very efficient at finding linkages in data that might not be observed by other methods. A neural network examines the entered data and the desired output and attempts to get its actual output to match the desired one. This makes ANNs very powerful to use but complex to develop, since the network must first be trained. Although some work has been performed with fish decay (Krzymien and Elias, 1990; Ohashi et al., 1991) and particularly with metal oxide sensors for fish decay (Egashira, et al., 1990; Olafsson et al., 1992), these technologies have not been used to classify fish decay.

Natale et al. (1996) used four quartz microbalance sensors and PCA analysis to examine freshness loss in cod fillets. The sensors measured the mass change as the odorant in

the air was absorbed onto the surface of the sensor. The resulting data were found to have some differences but could not be separated at all time points. Implementation of neural networks resulted in a continuous curve that corresponded to decay time. Schweizer-Berberich et al. (1994) also analyzed the freshness of fish using eight different amperometric three-electrode gas sensors. They found a general trend for decay time by using PCA on the output data.

### 3.3 RESEARCH OBJECTIVE

The overall objective of this research was to adapt current e-nose classification technologies for use in determining the decay time of tilapia (*Oreochromis niloticus*), a freshwater fish raised in commercial aquaculture systems. Specifically, an e-nose with 16 commercially available tin metal oxide sensors was to be used to determine decay time in a rapid-decay study.

### 3.4 MATERIALS AND METHODS

The e-nose used in this research was designed and built at North Carolina State University. The NC State University e-nose, developed by the Department of Electrical and Computer Engineering, uses a Labview-based control and acquisition program (Gutierrez-Osuna, 1998). It has 16 commercially available tin metal oxide sensors with sensitivities and cross sensitivities to different gases (table 1). A general flow diagram for the e-nose is presented in Figure 3-1.

**Table 3-1: Sensors used in the NC State University e-nose**

| Sensor Type | Part (Manufacture) | Cross Sensitivities |
|---|---|---|
| Ethanol | AAS14 (Capteur) | Oxidizable solvents and vapors |
| Isopropyl alcohol | AAS20 (Capteur) | Oxidizable solvents and vapors |
| Hydrogen sulfide | GS05 (Capteur) | Ammonia, propane, and CO |
| Toluene | AAS25 (Capteur) | Oxidizable solvents and vapors |
| Ammonia | GS06 (Capteur) | Hydrogen sulfide, propane, CO |
| Carbon monoxide | GL07 (Capteur) | -- |
| Propane | CTS03 (Capteur) | Oxidizable solvents and vapors |
| Hydrogen | CTS23 (Capteur) | Oxidizable solvents and vapors |
| Chlorine | LGS09 (Capteur) | Ozone and $NO_x$ |
| Nitrogen dioxide | LGS10 (Capteur) | Hydrogen sulfide, ozone, chlorine |
| Butane | CTS04 (Capteur) | Oxidizable solvents and vapors |
| Sulfur dioxide | GS22 (Capteur) | CO, some solvents |
| Solvent vapors | TGS2620 (Figaro) | -- |
| Combustible gases | TGS2610 (Figaro) | -- |
| Methane | TGS2611 (Figaro) | -- |
| Air contaminants | TGS2600 (Figaro) | -- |

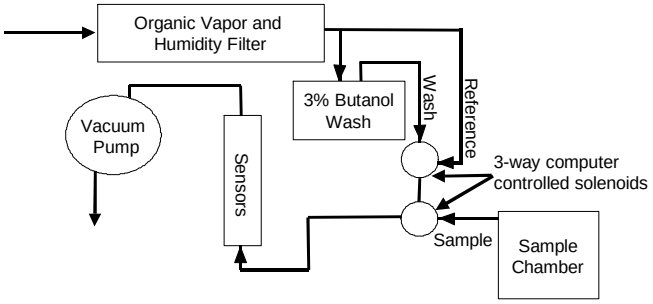



**Figure 3-1:Flow diagram for the NC State University e-nose.**

The e-nose sampling procedure included a wash cycle, a reference cycle, and a sampling cycle. The wash cycle consisted of a 30 s, 3% butanol wash to remove all of the odorants from the sensors before sampling. A 270 s reference cycle was then performed using air that had both humidity and organic vapors removed by a gas purification filter (model 8125, Alltech, Deerfield, Ill.). Thus, the sensors reached a reference value before sample testing. During the 60 s sampling cycle, voltage levels from each sensor were collected and stored at a 1 Hz sampling rate. Thus, each e-nose sample run contained 60 points per sensor, for a total of 960 points per run.

Fresh tilapia were obtained from the aquaculture research facility at North Carolina State University. After harvesting, fish were stored on ice, immediately filleted, and cut into $5 \times 5 \times 2$ cm rectangular parallelepiped samples within 5 h. Samples from a total of six different fish were subsequently stored at -18°C prior to analysis. When analyzed with the e-nose, individual samples were removed from the freezer and placed into the e-nose sampling chamber, a BioTransport carrier (Part No. 7135-0001, Nalgene, Rochester, N.Y.), and allowed to decay at room temperature (approximately 25°C) for 18 h. The BioTransport carrier was previously modified to allow the headspace gas to be collected by the e-nose and replaced with ambient air. Headspace gas samples were acquired by the e-nose at 7 min intervals for each sample over an 18 h period, for a total of 152 gas samples per fish sample. This resulted in a total of 912 gas samples taken from the six fish samples tested. The resulting data were separated into three distinct regions for classification training. The first region, or class, was comprised of data sampled in the 0 to 5.9 h time interval. The second class contained data taken between 6 and 11.9 h, and the third class contained data taken between 12 and 17.9 h.

Recorded data were analyzed using a customized MATLAB program. This began with a compression phase in which the number of measurements per sensor per sample was reduced from 60 to 4. Because both the transient and the steady-state portions of each sensor's response contained useful information, windowed time slicing was used (Gutierrez-Osuna and Nagel, 1999). This was done by multiplying the transient response by four smooth, bell-shaped window functions (Figure 3-2). By using this technique, information about the dynamic characteristics of the response was captured. The width, shape, and center of the windowing functions, $K_i(t_k)$, were defined by the parameters $a_i$, $b_i$, and $c_i$, respectively:

**Equation 3-1**

$$W^i = \sum_{k=1}^{N_T} R(t_k) K_i(t_k) \Delta t$$

**Equation 3-2**

$$K_i(t_k) = \frac{1}{1 + \left( \frac{t_k - c_i}{a_i} \right)^{2b_i}}$$

where

$R(t_k)$ = a sensor's response at time $t_k$

$\Delta t$ = time between samples (1 s)

$W^i$ = area under each curve (the four points to which the signal was reduced).

This reduced the number of data points for each sampling run from 960 to 64. The total number of points for all 912 gas samples then became 58,368. Feature extraction was accomplished with PCA and LDA (Gutierrez-Osuna, 1998). Data were then randomly grouped into two sets, with 60% (542 gas samples) assigned for training and 40% (365 gas samples) for testing. After training, testing data were classified using least squares (LS) and

K-nearest neighbor (KNN) classifiers. Following completion of the entire analysis, the entire 912 sample data set was randomly re-divided and re-analyzed. This procedure was repeated 20 times to statistically show how unseen data would be classified. The rates of correct test sample classifications were recorded and compared using Wilcoxon's signed-rank test (Ott, 1977). In this alternative to the paired t-test, the difference between pairs of measurements is calculated and ranked according to the absolute value of the difference. The appropriate sign is then assigned to the ranks, and they are added according to their sign. The smaller of these is used to compare with critical values to test for a statistical difference.

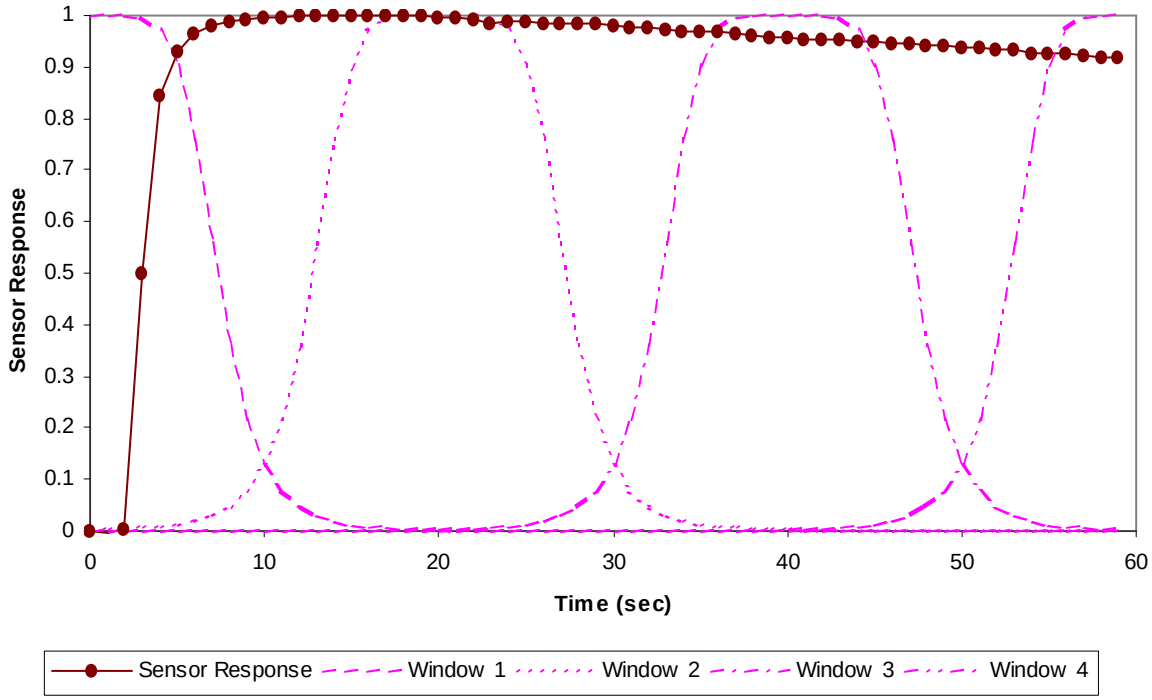



**Figure 3-2: Bell-shaped sections used for windowed time slicing**

A data combing technique, which has been used in chemometric analysis of spectroscopic data (McClure and Crowell, 1996), was also tested to determine its effect on sample classification. As implemented here, each 6 h region of data was separated into six 1

h regions (each containing 50 to 51 samples) that could be individually selected and grouped for analysis. The data were re-analyzed using the 6 h classifications for only the first 5 h of data from each region. By combing the data in this way, the transitioning effects induced by data bordering each region were minimized. This process was repeated using 4, 3, and 2 h data increments. This analysis procedure was repeated with the data divided into 3 h classes (0 to 2.9 h, 3 to 5.9 h, 6 to 8.9 h, 9 to 11.9 h, 12 to 14.9 h, and 15 to 17.9 h), for a total of six individual classes.

### 3.5 RESULTS AND DISCUSSION

PCA analysis of the 6 h classifications initially proved to be unsuccessful because there was no real statistical separation of the data when compared against decay time. When PCA results were compared with the individual fish samples, it was found that PCA was separating the data more by individual fish than by decay time. Some decay trends could be seen within certain individual fish sample groups. However, these were inadequate for classification into decay time groups. Therefore, PCA was determined to be an inadequate method for feature extraction from this data.

LDA feature extraction was also performed on the training set, with KNN and LS being used to classify the testing set. The rates of correct test sample classifications for KNN and LS in the 6 and 3 h groups with and without data combing are listed in table 2. Both classification routines were able to classify at a reasonable level with the given data. The results from the Wilcoxon signed-rank test (Ott, 1977) are also given. The critical value for this test with $\alpha = 0.05$ is 60. Thus, the means were statistically different in tests having a value lower than 60. Classification rates between KNN and LS were not found to be statistically different for the 6 h groups when data combing was not used. However, they

were significantly different in all other cases. In addition, KNN consistently yielded higher classification rates. Because of this, it was concluded that KNN classifications were best suited for this application.

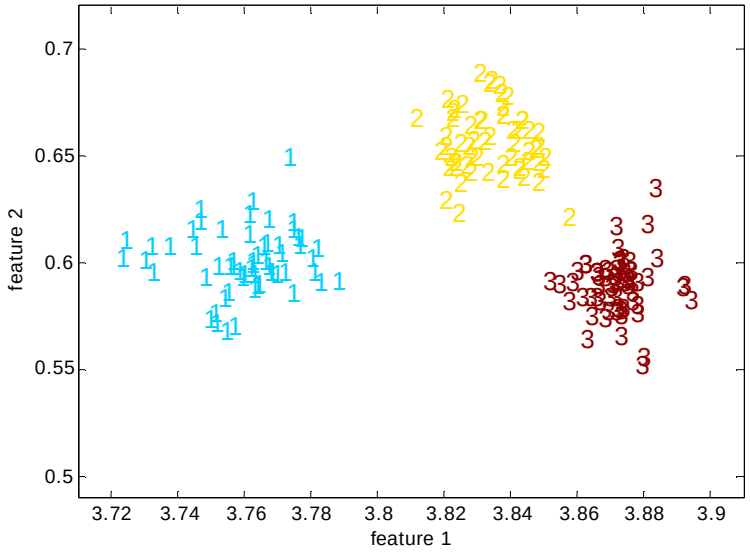
**Table 3-2: Classification rates for headspace gas samples with various amounts of data combing (mean ±standard deviation).**

| Time per Group (h) | No. of Data Samples Combed | KNN (%) | LS (%) | Wilcoxon Signed-Rank Test Value |
|---|---|---|---|---|
| 6 | 0 | 87.0 ±2.1 | 86.4 ±2.3 | 68[a] |
| | 51 | 90.0 ±1.7 | 88.7 ±1.7 | 34 |
| | 102 | 93.7 ±2.0 | 92.2 ±2.6 | 40 |
| | 153 | 95.9 ±1.3 | 93.1 ±1.6 | 0 |
| | 204 | 97.8 ±1.3 | 95.9 ±2.1 | 0 |
| 3 | 0 | 77.1 ±1.8 | 71.7 ±2.1 | 0 |
| | 51 | 83.3 ±2.6 | 76.3 ±3.0 | 0 |
| | 102 | 83.8 ±2.9 | 78.8 ±2.2 | 0 |

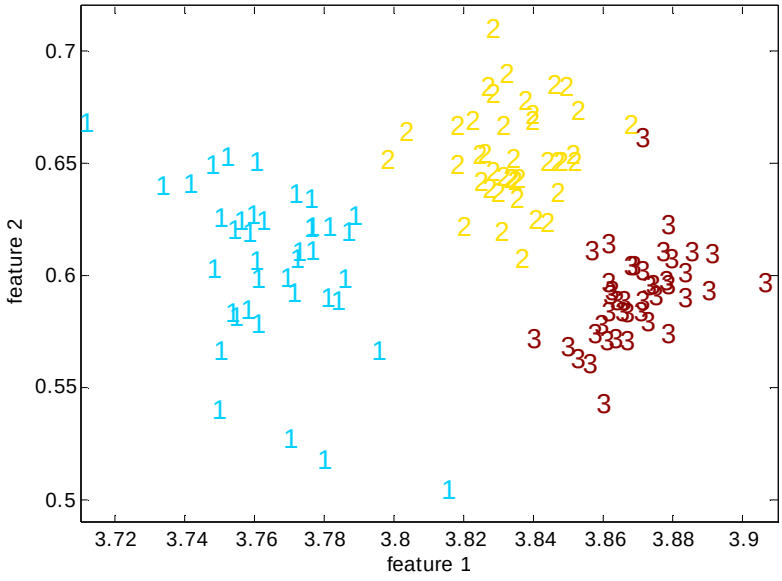
[a] Indicates that KNN and LS percentages of correctly classified samples were not significantly different ($\alpha = 0.05$).

Figure 3-3a shows the LDA features that were identified from the training set data after combing 204 samples from each region's data for the 6 h classes (group 1: 0 to 1.9 h, group 2: 6 to 7.9 h, and group 3: 12 to 13.9 h). Figure 3-3b shows the findings when these features were projected onto the testing set for the same groups. In both cases, the regions separated well when classifying by feature 1 and feature 2. These features corresponded to the scores generated using the first two components found in LDA. From these graphs, it can be seen that the testing data could be separated into appropriate groups, with a slight overlap between groups 2 and 3. Figure 3-4 shows the same analysis but with 3 h classes with 102 samples

combed from each region (group 1: 0 to 0.9 h, group 2: 3 to 3.9 h, group 3: 6 to 6.9 h, group 4: 9 to 9.9 h, group 5: 12 to 12.9 h, and group 6: 15 to 15.9 h). Groups 1 and 6 were almost completely separated by the analyses, while groups 2 through 5 overlap somewhat. However, there is a general trend of separation into three distinct regions. These regions correspond to what is known about aromatic profiles in decaying fishery products. Lindsay (1988) indicated that three distinct types of chemical aromas occur in freshwater fish that are associated with decay. Fresh fish have a very delicate marine-green aroma that is lost during the first stage of holding as a result of microbial decay. Further aerobic storage results in continued microbial production of esters that provide a sweet aroma. Eventually, a more putrid aroma will result from the microbial production of sulfur compounds.

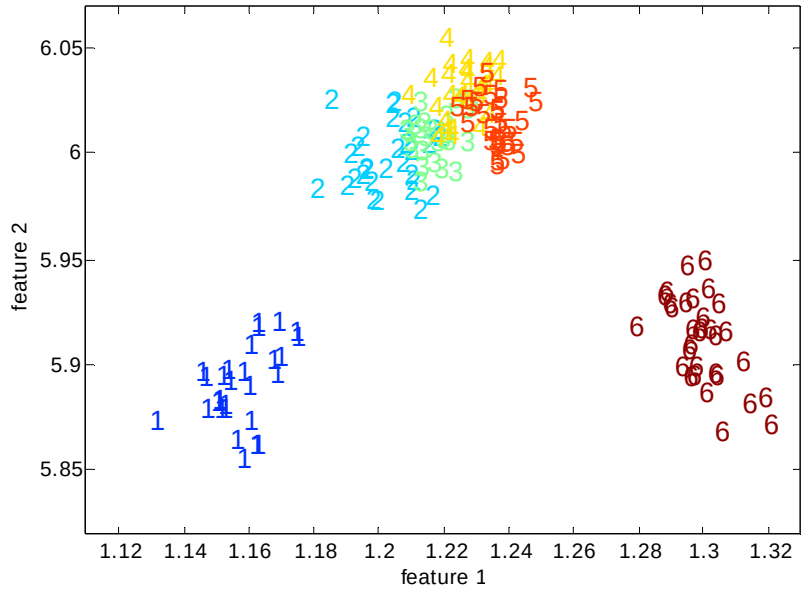



**(a)**

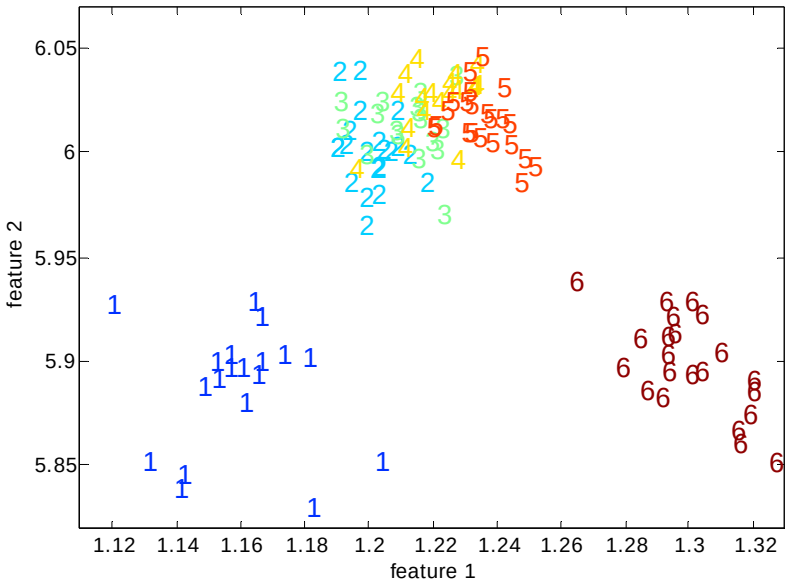



**(b)**

**Figure 3-3:LDA features for the (a) training set and (b) testing set showing discrimination among decay time points for tilapia with 6 h base classes and 204 data sample combed between classes (group 1: 0 to 1.9 h, group 2: 6 to 7.9 h, and group 3: 12 to 13.9 h).**




**(a)**




**(b)**

**Figure 3-4:LDA features for the (a) training set and (b) testing set showing discrimination among decay time points for tilapia with 3 h base classes and 102 data samples combed between classes (group 1: 0 to 0.9 h, group 2: 3 to 3.9 h, group 3: 6 to 6.9 h, group**

The classification rate dropped from 97.8% to 87.0% (with the KNN classifier) when the number of data points combed from the regions was reduced. This indicates that although the classification system used here was effective in identifying a sample's phase of spoilage (based on aroma), a parametric model could also be developed to indicate the total holding time. The classification rate for the 3 h classes was lower than that of the 6 h classes for KNN and LS classifiers (table 2). While this was expected, the fact that this method achieved this resolution indicates promise.

### 3.6 CONCLUSIONS

An e-nose consisting of 16 tin metal oxide sensors was successful in separating headspace gas samples taken from tilapia tissue into individual time-based classifications. By using LDA data analysis with KNN and LS classifiers, data were successfully classified into three 6 h regions. Through the use of data combing, classification rates were increased from 87.0% to 97.8%. When this analysis procedure was used to classify the samples into six 3 h regions, the data formed three distinct zones similar to what might be expected for decaying freshwater fish.

The development of this tool will be beneficial to both fishery product buyers and regulatory officials because of its ability to rapidly determine the stage of decay. This would be particularly useful when the use of appropriate prior storage conditions needs to be verified. Future work will be targeted at the development of parametric analysis procedures capable of both this type of classification and determining product quality as it is related to storage times and conditions.

### 3.7 REFERENCES


*Consumer Reports*. 2001. America's fish: Fair or foul? *Consumer Reports* (Feb.): 25-31.

Dalgaard, P. 2000. *Freshness, Quality, and Safety in Seafoods*. Flair-Flow Europe Technical Manual F-FE 380A/00. Dublin, Ireland: The National Food Centre.

Delpha, C., M. Siadat, and M. Lumbreras. 1999. Relative humidity: An interfering parameter for the characterization of a TGS sensor array. In *SPIE Conference on Chemical Microsensors and Applications II* 3857: 223-230. Bellingham, Wash.: SPIE.

Egashira, E., Y. Shimizu, and Y. Takao. 1990. Trimethylamine sensor based on semiconductive metal oxides for detection of fish freshness. *Sensors and Actuators B* 1(1-6): 108-112.

Gutierrez-Osuna, R. 1998. Signal processing and pattern recognition for an electronic nose. PhD diss. Raleigh, N.C.: North Carolina State University.

Gutierrez-Osuna, R., and H. T. Nagel. 1999. A method for evaluating data-preprocessing techniques for odor classification with an array of gas sensors. *IEEE Trans. Systems, Man, and Cybernetics - Part B* 29: 626-632.

Krzymien, M. E., and L. Elias. 1990. Feasibility study on the determination of fish freshness by trimethylamine headspace analysis. *J. Food Science* 55(5): 1228-1232.

Lindsay, R. C. 1988. Flavor chemistry and seafood quality factors. In *Oceans '88: Proc. MTS/IEEE Conference*, 61-65. Columbia, Md.: Marine Technology Society.

Llobet, E., E. L. Hines, J. W. Gardner, P. N Bartlett,. and T. T. Mottram. 1999. Fuzzy ARTMAP-based electronic nose data analysis. *Sensors and Actuators B* 61(1-3): 183-190.

McClure, W. F., and B. Crowell. 1996. Quantitative information in near-infrared spectra: Part 1. Effects of smoothing and combing. *J. Near-Infrared Spectroscopy* 4: 129-137.

Nagle, H. T., R. Gutierrez-Osuna, and S. S. Schiffman. 1998. The how and why of electronic noses. *IEEE Spectrum* 35(9): 22-31.

Natale C., J. A. J. Brunink, F. Bungaro, F. Davide, A. d'Amico, R. Paolesse, T. Boschi, M. Faccio, and G. Ferri. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensors array. *Measurement Science and Tech*. 7(8): 1103-1114.

Ohashi, E., Y. Takao, T. Fujita, Y. Shimizu, and M. Egashira. 1991. Semiconductive trimethylamine gas sensor for detecting fish freshness. *J. Food Science* 56(5): 1275-1278.

Olafsson, R., E. Martinsdottier, G. Olafsdottir, P. I. Sigfussonand, and J. W. Gardner. 1992. Monitoring of fish freshness using tin oxide sensors. In *Sensors and Sensory Systems for an Electronic Nose*, 257-272. J. W. Gardner and P. N. Bartlett, eds. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Ott, L. 1977. *An Introduction to Statistical Methods and Data Analysis*. Pacific Grove, Cal., Duxbury Press.

Roussel, S., G. Forsberg, V. Steinmetz, P, Grenier, and V. Bellon-Maurel. 1998. Optimization of electronic nose measurements: Part I. Methodology of output feature selection. *J. Food Eng.* 37(2): 207-222.

Sarry, F., and M. Lumbreras. 1999. Detection of two gases in an air-conditioned system with an array of TGS sensors. In *SPIE Conference on Chemical Microsensors and Applications II* 3857: 215-222. Bellingham, Wash.: SPIE.

Schaller, E., J. O. Bosset, and F. Escher. 1998. Electronic noses and their application to food. *Lebensm. Wiss. Technol*. 31(4): 305-316.

Schweizer-Berberich, P. M., S. Vaihinger, and W. Gopel. 1994. Characterization of food freshness with sensor arrays. *Sensors and Actuators B* 18(1-3): 282-290.

# 4. VOLATILE BASES LEVEL PREDICTION OF BLUE CRAB CLAW MEAT USING ELECTRONIC NOSE AND VISIBLE/NEAR-INFRARED SPECTROSCOPY

**T. H. Dodd and S. A. Hale**

## 4.1 ABSTRACT

. Electronic Nose (e-nose) and Visible/Near-Infrared (VIS/NIR) instruments were used to measure volatile bases in blue crab meat over a storage time of 14 days on ice. Total volatile bases – nitrogen (TVB-N) levels were also recorded and used as an overall quality measurement. Using an e-nose to estimate the concentration of volatile bases, a standard error of prediction of 4.9 mg/100 g apparent TVB-N was achieved. VIS/NIR spectroscopy produced a standard error of prediction of 4.8 mg/100 g apparent TVB-N. These numbers were in the range of error commonly associated with TVB-N measurements. Both technologies provided a quick, simple, non-destructive test method.

## 4.2 INTRODUCTION

Quality in fishery products has always been hard to define and even harder to measure. The US, through the FDA, has placed a particular emphasis on traceability of food products. The FDA has implemented hazard analysis and critical control point (HACCP) regulations in an effort to try to bring these concerns under control. According to HACCP regulations “All raw materials and products should be lot-coded and a recall system in place so that rapid and complete traces and recalls can be done when a product retrieval is

necessary." (FDA 2005) This traceability becomes even more important as consumers are further removed from their food supply and more food crosses international boundaries.

While traceability is important, it provides only an assumed product quality measurement. It can only provide an estimate of storage time and conditions. Overall product quality is much more difficult to define. The standard for monitoring quality is using a sensory panel. While using human senses has the advantage of being able to consider a large variation of factors, it has limitations due to the scale of effort that is required to perform a sensory panel analysis on a large number of samples. The two senses are used most to determine product quality are sight and smell. Many studies have linked sensory panel scores with various chemical indicators. Odor evaluation has been correlated to Total volatile bases – nitrogen (TVB-N) in mahi-mahi (Antoine, et. al. 2002), and used as a quality indicator. While odor is not the only sense that the consumer uses to evaluate products, it is one that is commonly used to indicate spoilage. FDA uses odor to evaluate products. It is listed in their "The Safe Food Chart" for meat, poultry, and seafood as one of the main ways to detect the freshness of seafood. As such, odor is also used by FDA inspectors as a quality indicator. If an inspector smells something out of the ordinary, the lot of meat can be condemned. Sight is used with color being a major component of quality in crab meat.

While chemical tests provide quantifiable results, they are normally destructive, time consuming, and expensive. They are also different for various fishery products. All this leads to confusion as to what to use as a standard indicator for 'quality.' One indicator is the volatile base concentration. Several studies have tied TVB-N values to storage time in various fishery products. These values have also been tied to sensory scores in mahi-mahi (Antoine *et. al.*, 2002). Studies have also shown that tests that look at a group of chemicals,

such as TVB-N, tend to correlate better with freshness than specific chemical indicators (Sikorski et. al. 1990). One method to estimate TVB-N is to use ammonia ion-specific electrodes (ISE). The AOAC has developed a method for determining the concentration of volatile bases using these probes (AOAC 2000). The ISE measurements show apparent g-$NH^3$/100ml. This value is really a sum of ammonia and Trimethylamine (TMA).

In order to better estimate these properties in an objective manner, alternative sensing technologies are being explored: e-noses and VIS/NIR spectroscopy. These use the same data sources as the human senses of smell and sight respectively, but with additional information and repeatability not found in individuals or sensory panels. For example, the NIR region of the electrometric spectrum is not seen by the human eye.

E-noses have shown promise in being able to analyze a wide variety of products. Research has been conducted on products ranging from coffee to perfumes, to wines and other products that have changes in their off gases. Evaluation of fishery products has also been explored. E-noses have been used to examine fish decay (Krzymien and Elias, 1990; Ohashi et al., 1991, Egashira, et al., 1990; Olafsson et al., 1992). These studies have shown that e-noses have the potential to sense changes in fish decay. Natale et al. (1996) used four quartz microbalance sensors and Principal Component Analysis (PCA) to examine freshness loss in cod fillets. The sensors measured the mass change as the odorants in the air were absorbed onto their surfaces. The resulting data were found to have some differences. PCA was not able to provide separation at all time points. Implementation of neural networks resulted in a continuous curve that corresponded to decay time. Schweizer-Berberich et al. (1994) also analyzed the freshness of fish using eight different amperometric, three-electrode, gas sensors. They found a general trend for decay time by using PCA on the output

data. An e-nose was used to predict the Total volatile bases (TVB) in fishmeal products (Olafsdottir, et. al., 2000). TVB was measured using a gas chromatograph (GC). It was found that gas sensors could predict TVB in the headspace above the product, with data combing yielding better results. The lowest Root-Mean-Square Error of Prediction (RMSEP) calculated was 7.6 mgN/100g of fish. Dodd *et. al.* (2004) showed that e-noses have the ability to classify tilapia by spoilage time. In this study tilapia was allowed to decay at room temperature and e-nose measurements were able to classify the response time of 3-hour time segments with an accuracy of 83%.

VIS/NIR technologies have been used to examine a wide variety of products. It has been successfully used to analyze everything from pharmaceuticals to food products to agricultural measurements. A review of applications and techniques can be found in Blancho and Villarraya (2002) and Pasquiuni (2003). It has also been used in a variety of fishery product quality evaluations. Downey (1996) used the NIR spectra (700-1100 nm range) to determine the oil and moisture content of salmon. Corresponding Standard Errors of Prediction (SEP)s calculated were all under 2.5%. Zhang and Lee (1997) also used NIR spectroscopy to determine the free fatty acid level in fish oil. First derivative NIR spectra were used to predict FFA and hypoxanthine (Hx) changes in the fish meat. A relative error of less than 10% was seen in all cases. Nilsen and Esaiassen (2005) examined the correlation between spectra in cod meat sensory scores. It was shown that the visible region provided the greatest correlation with Quality Index Method (QIM) scores. Adding the NIR region decreased the performance with the NIR region alone yielding the worst correlation.

## 4.3 RESEARCH OBJECTIVE

The objectives of this paper were as follows:

1. Measure TVB-N in claw crabmeat over a storage time of 14 days on ice.
2. Generate multivariate model to predict TVB-N values using e-nose and VIS/NIR spectroscopy as inputs.

## 4.4 MATERIALS AND METHODS

The experimental design used in this study was devised to model the storage conditions used in the seafood industry for Atlantic blue crab claw meat. The typical industry standard is to process crabs and store the meat on ice for a maximum of 14 days.

### 4.4.1. Sample Preparation

Atlantic blue crab meat was obtained from a North Carolina crab processor. Three, one-pound containers of claw meat were obtained. The meat was originally packaged under commercial conditions, then repackaged, in vacuum seal bags and stored at -80°C. Prior to analysis, one pound of meat was allowed to thaw in a 4°C refrigerator overnight. Once thawed, it was separated into samples for e-nose/TVB–N analysis and VIS/NIR analysis. This procedure was repeated for the three different pounds of crab meat. Each pound of meat was separated into 3 VIS/NIR samples and 7 /TVB-N samples. This yields a total of 9 VIS/NIR samples and 21 e-nose/TVB-N samples.

### 4.4.2. TVB-N level analysis

To measure TVB-N levels five (5) grams of meat were sealed in polyethylene bags and then stored on ice until sampling was to be performed. One sample was removed from ice every other day for measurement. E-nose sampling was first performed, followed by TVB analysis. The TVB measurement method requires the destruction of the samples to be performed. TVB levels were determined by the AOAC method for determining TVB levels in fishery products (AOAC 2000). First, the crab meat was mixed with 95 ml distilled water. This mixture was then ground by a food grinder for 2 minutes. In order to cause ammonia release, 2.0 ml ionic strength adjuster (ISA, 5M NaOH, 0.05M disodium EDTA and 10% (v/v) methanol) was added to the test solution and the amount of apparent TVB-N was recorded by a calibrated ammonia ion specific electrode.

### 4.4.3. Electronic nose data collection

Samples were prepared as previously discussed. On the day of analysis samples were removed from storage and placed in the e-nose sampling chamber where headspace gasses were pulled through a sensing chamber. The sampling sensing chamber consisted of a Nalgene box that had been modified to allow for headspace gas sampling at its top. E-nose sampling was performed as reported in Dodd et al. (2004) on a custom e-nose that uses 15 commercially available tin metal oxide sensors. The sensors were mounted in a small sensing chamber, and headspace and reference gases were pulled through the chamber with a vacuum pump. The sampling procedure involved washing the sensors with a 3% butanol solution for 30 seconds prior to exposure to a baseline gas of dehumidified and deodorized

air for 120 seconds. Headspace gasses above the e-nose sample were then pulled through the sample chamber for 60 seconds with sensor voltage measurements being recorded every second. Multiple headspace samples were taken in succession on a single meat sample. This gave more data on a single sample, countering sensor inaccuracies and meat variability. Each meat sample was measured five times every other day for a total of 140 odor fingerprints being used in the following analysis obtained.

Recorded data were pre-processed using a customized MATLAB program. This began with a compression phase in which the number of measurements per sensor per sample was reduced from 60 to 4. Because both the transient and the steady-state portions of each sensor's response contained useful information, windowed time slicing was used (Gutierrez-Osuna and Nagel, 1999). This was done by multiplying the transient response by four smooth, bell-shaped window functions (Figure 4-1). By using this technique, information about the dynamic characteristics of the response was captured. The width, shape, and center of the windowing functions, $K_i(t_k)$, were defined by the parameters $a_i$, $b_i$, and $c_i$, respectively:

**Equation 4-1**

$$W^i = \sum_{k=1}^{N_T} R(t_k) K_i(t_k) \Delta t$$

**Equation 4-2**

$$K_i(t_k) = \frac{1}{1 + \left( \frac{t_k - c_i}{a_i} \right)^{2b_i}}$$

where

$R(t_k)$ = a sensor's response at time $t_k$

$\Delta t$ = time between samples (1 s)

$W^i$ = area under each curve (the four points to which the signal was reduced).

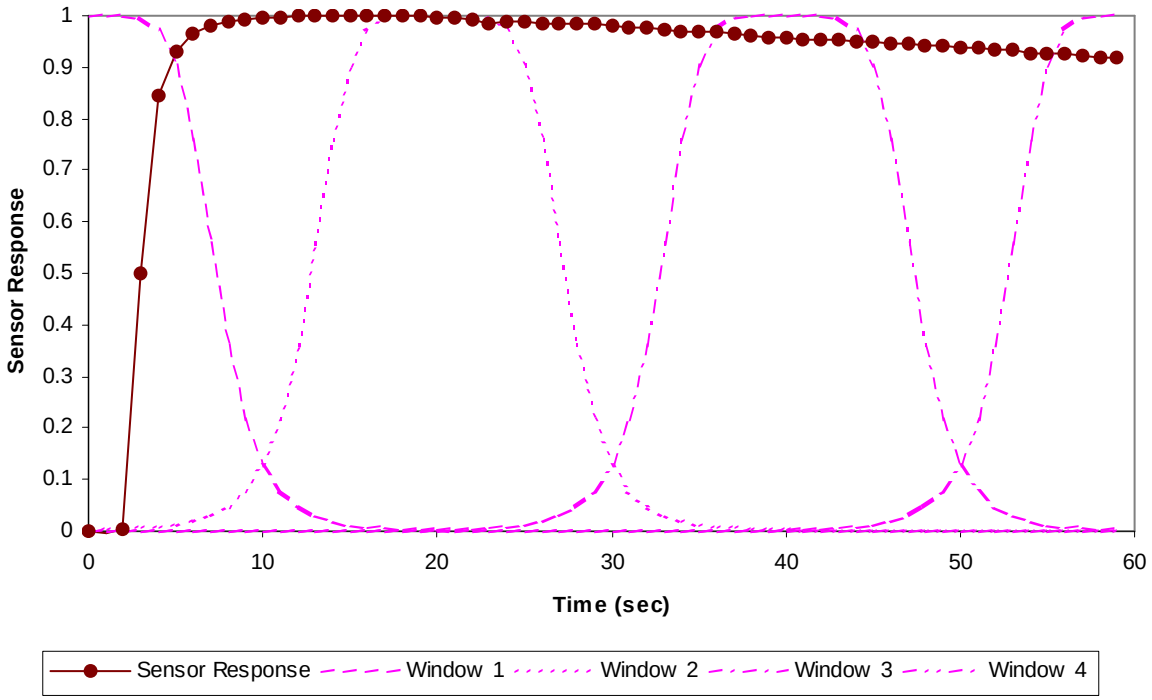



**Figure 4-1:Bell-shaped sections used for windowed time slicing**

4.4.4. VIS/NIR Data collection

For the VIS/NIR samples, 150 grams of meat was sealed in polyethylene bags and stored on ice. Once a day the samples were removed, scanned and returned to ice storage. They were scanned using a monochromator spectrometer (NIRSystems 6500, Perstorp Analytical, Silver springs, MD). Three spectra from 400 to 2498 nm in 2 nm increments were recorded each day. Each recorded spectra consisted of the average of 32 scans. The length of time the samples was minimized to less than one minute per scan before the meat was returned to storage on ice.

### 4.4.5. Data Analysis

Similar data analysis techniques were used on both e-nose and VIS/NIR datasets. Data were imported into the Unscrambler version 9.6 (CAMO software inc, OR) for analysis. Principal Component Regression (PCA) was first used to determine any underlying trends. PC scores were plotted to examine what factors were the major contributors to the first PCs.

Continuous models were generated using Partial Least Squares (PLS). In order to limit over fitting the maximum number of components used in the model set at 8. Full cross validation was used in order to generate a more robust model while still examining its ability to determine TVB-N concentrations in unseen examples. Model performance was evaluated using $r^2C$, SEC, $r^2P$ and SEP, where C stands for calibration and P stands with prediction.

For pretreatments on VIS/NIR spectra, samples were averaged to better control random noise effects. The effect of pretreatments (n-point smoothing, first and second derivatives) on the model were then explored. PLS models were generated for visible only, NIR only, and total VIS/NIR spectra.

## 4.5 RESULTS AND DISCUSSION

### 4.5.1. TVB-N

Measured TVB-N values for each meat sample are shown in Figure 4-2. It can be seen that while there is increase of TVB-N for each meat sample there is a different level found in each meat sample. It can be seen that there was a decrease in the starting level of TVB-N as frozen storage time increased.

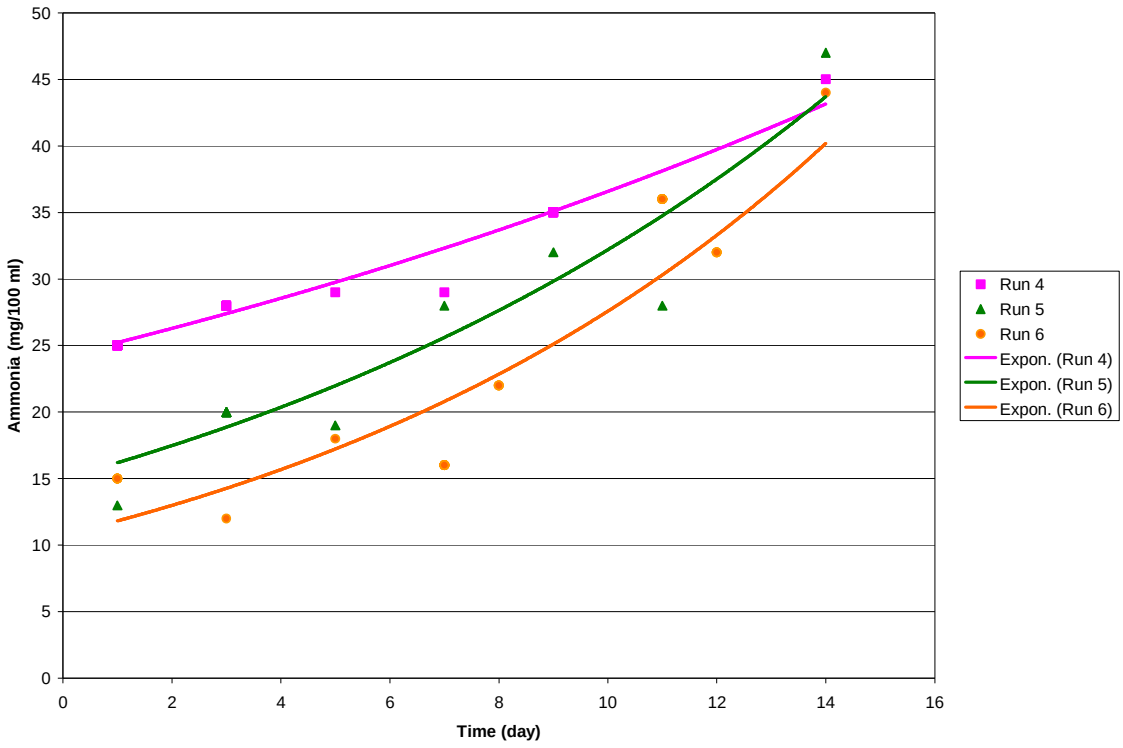



**Figure 4-2: TVB-N values vs. time**

Crab meat has been shown to remain commercially acceptable under frozen storage at -18 ºC for 10 months (Yerlikaya and Gokoglu, 2004). During this storage, TVB-N levels were seen to increase while sensory scores were seen to decrease. This tends to suggest that there are still changes occurring at -18 ºC.

Storage at -80 ºC still also showed changes in TVB-N over time with levels dropping over the storage time. Since higher levels of TVB-N are associated with lower quality the storage at -80ºC for 8 months would not effect on the commercial acceptability of the meat.

4.5.2. E-nose

One of the sensors in the e-nose array was designed for ammonia determination. Since ammonia is a component of TVB-N the sensor should have been able to make an estimation of TVB-N. The sensor response was compared with the TVB values obtained. There was no statistical correlation between this sensor and TVB ($r^2$ <0.6). This sensor was sold as an industrial sensor detector set up to measure much higher concentrations of ammonia and was calibrated for ammonia values from 10-100 ppm. The headspace above the fish sample had a much lower ammonia concentration that was undetectable with the ammonia sensor alone. Since this sensor was not able to determine changes in TVB, multivariate analysis on the e-nose data as a whole was examined.

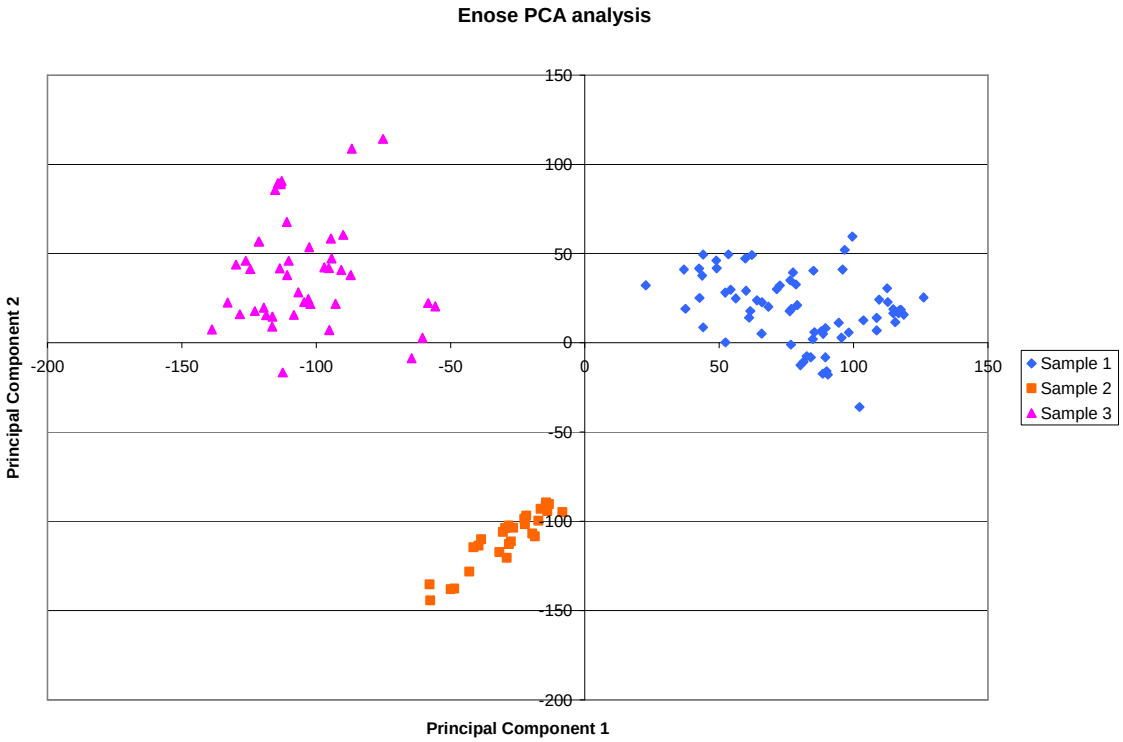



**Figure 4-3: PCA scores from e-nose data**

In an effort to examine the information contained in the e-nose data, PCA was employed. This is an unsupervised method involving overall variation which picks the linear combination of the variables that explains the most variation. Loading scores for the first two PCs in the PCA model are shown in Figure 4-3. The data are grouped according to meat sample. This suggests that the majority of the variability detected by the e-nose results from differences between the three meat samples. This also agrees with the fact that TVB-N levels changed with frozen storage time.

Since TVB-N values should change continuously over time a continuous model was produced using PLS. PLS is a modeling technique that uses predictive data to select components that have the variability that is of interest. In this case TVB-N measured values were used to generate the model. For calibration values, an $r^2$ value of 0.91 was obtained with a corresponding SEC of 4.0 mg/ 100 g TVB-N. Predication values were only slightly lower with an $r^2$ P value of 0.86 and a corresponding SEP of 4.9 mg / 100 g TVB-N. The low SEP shows that a model was generated that detected changes in volatile bases in crab meat to an accuracy of less than 5 mg/100 g TVB-N. Figure 4-4 shows measured values vs. predicted values of TVB-N.

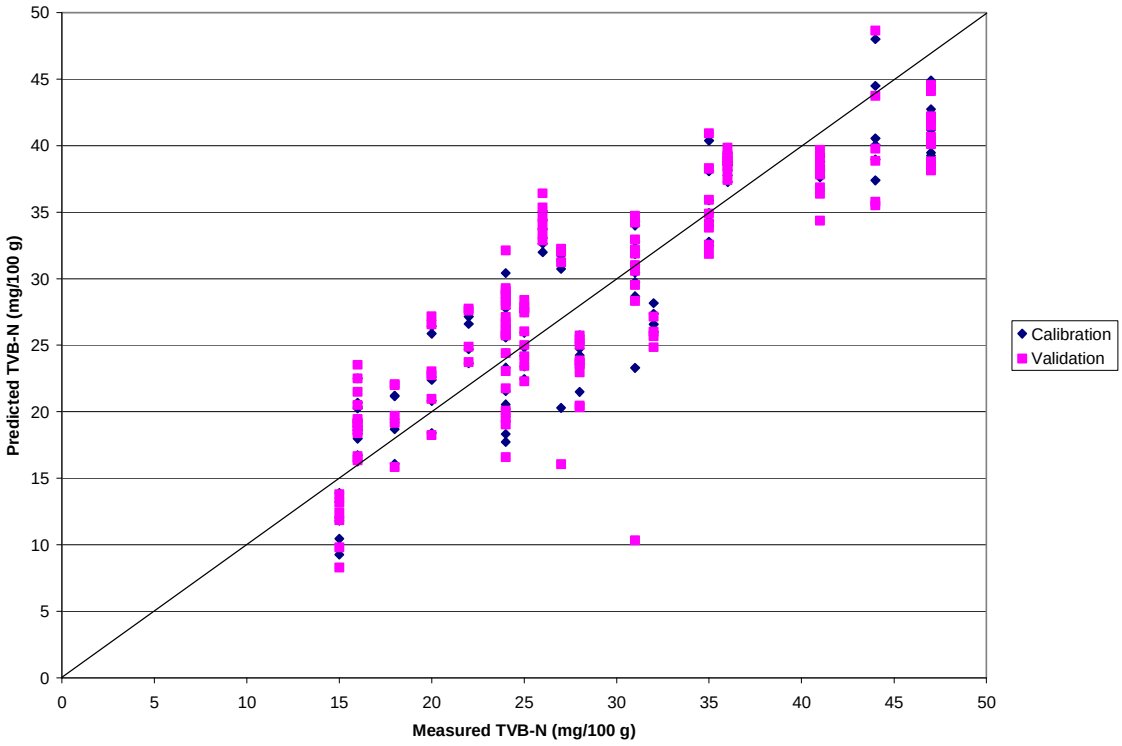



**Figure 4-4:Predicted vs. Measured values for e-nose**

The strong correlation between TVB-N and e-nose measurements shows that volatile bases were being liberated into the headspace gas of the sample and detected by the e-nose sensors. While this is expected in that one of the tin metal oxide sensors is made to determine ammonia concentrations, this sensor by itself was not able to measure the changes while the e-nose as a whole could. The cross sensitivities found in the sensors combined with the multivariate statistical data analysis permitted much smaller changes in TVB-N to be detected.

### 4.5.3. VIS/NIR

In spectral analysis pretreatments, choosing the smoothing and derivative gaps is critical. Wide gaps lead to peak shifting while narrow gaps allow noise to remain and decrease the effectiveness of the analysis (McClure, 1993). For the current experiment a smoothing average of 15 wavelengths was found to be optimum.

According to NIR theory, the primary absorption bands for aromatic amines are 1970 and 1490 nm. These were therefore used in preliminary analysis. However, these values showed no correlation to TVB ($r^2$ <0.1). While these wavelengths should in theory show some correlation to the changes in TVB, there were also many other chemicals in meat that would also have absorption in these wavelengths. Overtones from other chemicals also can interfere with the spectrum in different ranges. This demonstrated that more powerful multivariate methods were needed.

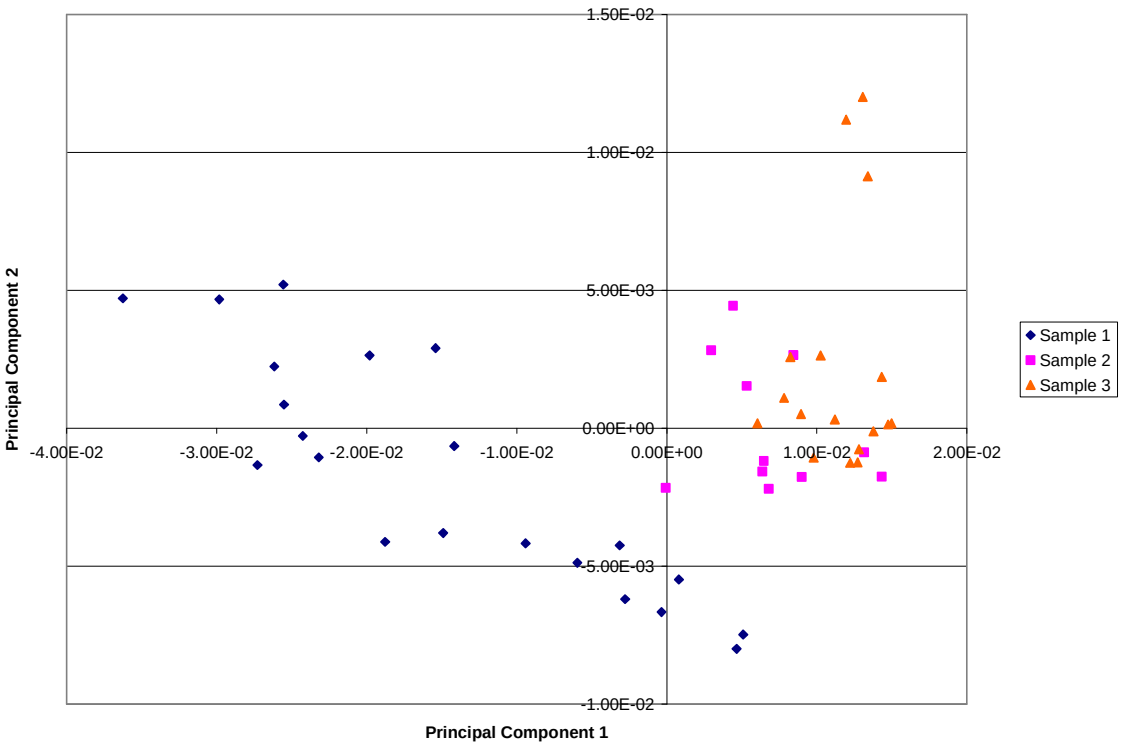



**Figure 4-5: PCA scores for VIS/NIR data**

PCA was initially used to evaluate sample variation. Figure 4-5 shows the first two PC loadings for the PCA analysis of the meat samples. While some separation between samples can be seen, the difference is not as profound as in the e-nose data. The e-nose relies solely on volatile compounds for its measurements. VIS/NIR spectroscopy differs in that a surface measurement is made of all compounds found in the material. VIS/NIR data have more information adding complexity to the multivariate analysis, thus making it more difficult to extract the influences on any particular variable. Therefore PLS was employed to obtain the best model performance in regard to TVB-N.

**Table 4-1:Summary PLS statistics using VIS/NIR spectroscopy**

| | $r^2$ C | SEC | $r^2$ P | SEP |
|---|---|---|---|---|
| VIS | 0.95 | 3.1 | 0.86 | 4.8 |
| NIR | 0.88 | 4.4 | 0.79 | 5.8 |
| Combined | 0.91 | 3.9 | 0.79 | 5.9 |

Table 4-1 shows summary PLS statistics for the spectroscopic analysis. The PLS model generated on only the VIS spectra generated the lowest SEP. This suggests that the NIR spectrum was being influenced by other chemical changes. The addition of this additional information further complicated the model and led to a lower SEP. While TVB-N changes should be able to be detected in the NIR spectrum because it is a chemical change, there are also a lot number of other chemical changes occurring at the same time. This can make it much more difficult for the desired response variable to be modeled.

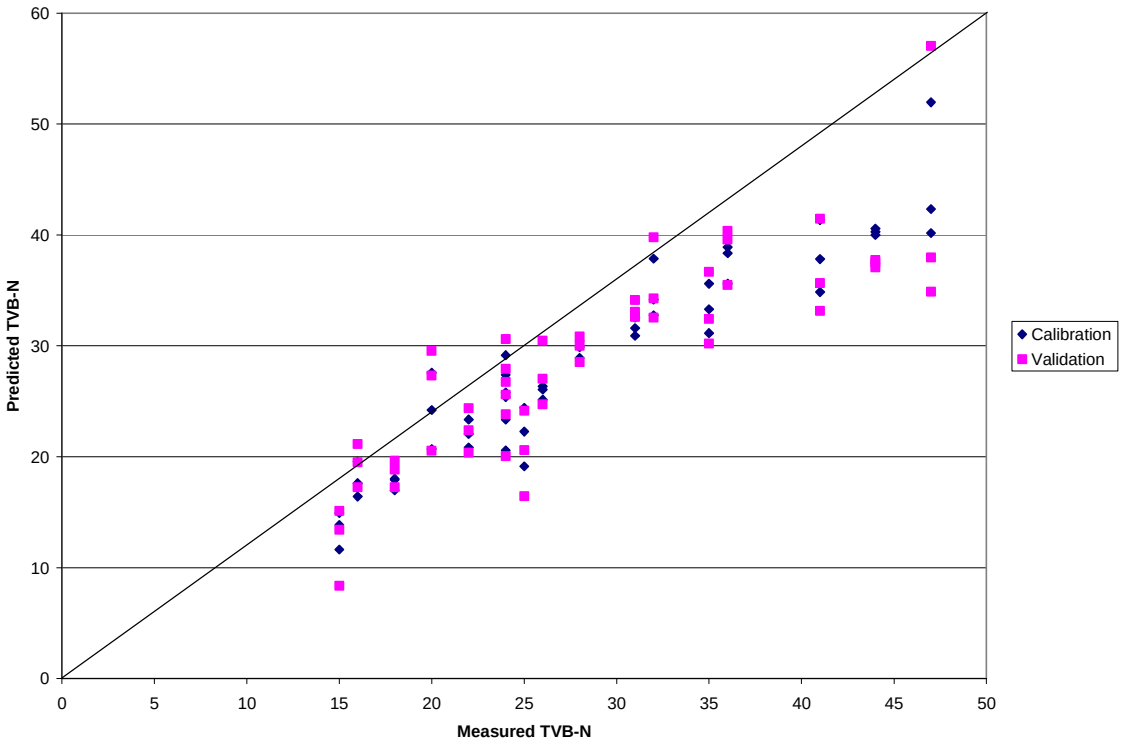



**Figure 4-6:Predicted vs. measured for VIS spectra**

Figure 4-6 shows the predicted vs. measured values for TVB-N using the VIS spectral data. An excellent correlation can be seen in the lower TVB-N values. But it falls off as the measured TVB-N values increase.

Nilsen and Esaiassen (2005) examined the correlation between spectra and sensory scores in cod meat. It was found that only using the VIS region yielded better results than using either the NIR or total VIS/NIR spectrum. With both volatile bases and sensory scores being used as quality indicators, similar patterns in model behavior would be expected.

According to the AOAC procedure for measuring volatile bases with an ammonia specific electrode, there were some cross sensitivities. This leads to decreased accuracy for this testing method. The AOAC reported reproducibility standard deviations (RSDR) ranged from 8.8 to 21%. These values were higher than the range of the SEPs presented. This leads to the conclusion that both technologies can predict TVB-N levels in crab meat with at least the same level of accuracy as that of TVB-N measurements recorded by an ion specific electrode.

These two technologies examine meat samples in very different ways. While the e-nose measures headspace gases, VIS/NIR measurements are surface measurements. Both have advantages and disadvantages. Utilizing an e-nose to measure headspace gases is a benefit in that a large sample can be analyzed at once. If you have a sample in a cup, NIR can only measure the surface of the cup sample. However, the e-nose can detect a volatile in a sample no matter where the volatile is being created. If a volatile is released into the atmosphere, whether on the surface or internal in the sample, the e-nose will detect it. This could allow for monitoring of large quantities of meat without requiring sampling of each

container. The disadvantage is that off gases from the meat need to be collected. Measurements in a sealed container are not possible.

VIS/NIR measurements have the advantage that they can be obtained through a transparent surface. With a properly designed container, measurements could be taken throughout the life of the product without unsealing it. The disadvantage of this method is that only the surface of the sample is examined. If the meat sample quality is not uniform then the measurements will not give a proper quality indication.

## 4.6 CONCLUSION

These experiments show that both e-nose and VIS/NIR spectroscopy can predict volatile bases in blue crab claw meat. Both technologies were adequate for the rapid estimation of TVB-N. The SEPs that were generated were along in the same order of magnitude as that of the calibration measurements. This suggests that both the e-nose and VIS/NIR data can be used to detect more than just volatile bases. Both of these sensing technologies can detect many different chemicals. This would be useful in modeling more complex problems, such as that of storage time which have many overlapping mechanisms.

## 4.7 REFERENCES

Bartlett PN, Elliott JM, Gardner JW. 1997. Electronic noses and their application in the food industry. *Food Technol* 51(12): 44-8.

Bene A, Hayman A, Reynard E, Luisier JL, Villettaz JC. 2001. New method for the rapid determination of volatile substances: The SPME-direct method. Part II. Determination of the freshness of fish. *Sensors and Actuators, B: Chemical* 72(3): 204-7.

Deisingh A, Stone D, Thompson M. 2004. Application of electronic noses and tongues in food analysis. *International Journal of Food Science and Technology* 39(587-604.

Delpha C, Lumbreras M, Siadat M. 2001. Discrimination of Forane 134a and carbon dioxide concentrations in an air conditioned atmosphere with an electronic nose: Influence of the relative humidity. *Sensors and Actuators, B: Chemical* 80(1): 59-67.

Delpha C, Siadat M, Lumbreras M. 1999. Relative humidity: An interfering parameter for the characterization of a TGS sensor array. *Proc SPIE Int Soc Opt Eng* 3857(223-30.

Di Natale C, Brunink JAJ, Bungaro F, Davide F, d'Amico A, Paolesse R, Boschi T, Faccio M, Ferri G. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas Sci Technol* 7(8): 1103-14.

Di Natale C, Macagnano A, Mantini A, Davide F, D'Amico A, Paolesse R, Boschi T, Faccio M. Ferri G. 1997. Advances in food analysis by electronic nose. 1(122-7.

Dodd TH, Hale SA, Blanchard SM. 2004. Electronic nose analysis of tilapia storage. *Transactions of the American Society of Agricultural Engineers* 47(1): 135-40.

Downey G, 1996. Authentication of food and food ingredients by near infrared spectroscopy. *J. Near Infrared Spectrosc.* 4, p. 47–61.

Egashira M, Shimizu Y, Takao Y. 1990. Trimethylamine sensor based on semiconductive metal oxides for detection of fish freshness. *Sensors and Actuators, B: Chemical* B1(1-6): 108-12.

Ellis PC, Pivarmk LF, Thiam M, Berger L, Field S, Green D, Hewes D, Lemerise D, Lyttle C, Maciel J, Soper K. 2000. Determination of volatile bases in seafood using the ammonia ion selective electrode: Collaborative study. *J AOAC Int* 83(4): 933-43.

Gao D, Wu S. 1998. Development of artificial olfactory system and its prospects on evaluating flavor of food. *Nongye Jixie Xuebao/Transactions of the Chinese Society of Agricultural Machinery* 29(4): 167-72.

Gupta S, Misra TN. 1997. Manganese phthalocyanine for the detection of fish freshness by its trimethylamine emission. *Sensors and Actuators, B: Chemical* B41(1-3): 199-202.

Gutierrez-Osuna R, Nagle HT. 1999. Method for evaluating data-preprocessing techniques for odor classification with an array of gas sensors. *IEEE Transactions on Systems, Man, and Cybernetics, Part B: Cybernetics* 29(5): 626-32.

Hammond J, Marquis B, Michaels R, Oickle B, Segee B, Vetelino J, Bushway A, Camire ME, Davis-Dentici K. 2002. A semi conducting metal-oxide array for monitoring fish freshness. *Sensors and Actuators, B: Chemical* 84(2-3): 113-22.

Hammond J, Mlsna T, Smith D, Fruhberger B. 1999. Fish freshness sensor. *Proc SPIE Int Soc Opt Eng* 3856(88-96.

Haugen JE, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Olafsdottir G. 2006. Rapid control of smoked Atlantic salmon (Salmo salar) quality by electronic nose: Correlation with classical evaluation methods. *Sensors and Actuators, B: Chemical* 116(1-2): 72-7.

Hofmann T, Schieberle P, Krummel C, Freiling A, Bock J, Heinert L, Kohl D. 1997. High resolution gas chromatography/selective odorant measurement by multisensor array (HRGC/SOMSA): A useful approach to standardize multisensor arrays for use in the detection of key food odorants. *Sensors and Actuators, B: Chemical* B41(1-3): 81-7.

Legin A, Rudnitskaya A, Seleznev B, Vlasov Y. Velikzhanin V. 2001. Electronic tongue for recognition of flesh food. 76-81.

Lindsay RC. 1988. Flavor chemistry and seafood quality factors. 1(61-5.

Llobet E, Hines EL, Gardner JW, Bartlett PN, Mottram TT. 1999. Fuzzy ARTMAP based electronic nose data analysis. *Sensors and Actuators, B: Chemical* B61(1-3): 183-90.

MacAgnano A, Careche M, Herrero A, Paolesse R, Martinelli E, Pennazza G, Carmona P, D'Amico A, Di Natale C. 2005. A model to predict fish quality from instrumental features. *Sensors and Actuators, B: Chemical* 111-112(SUPPL): 293-8.

McClure W, Crowell B. 1996. Quantitative information in near-infrared spectra: Part 1. Effects of smoothing and combing. *J Near-Infrared Spectroscopy* 4(129-137.

Mitsubayashi K, Kubotera Y, Yano K, Hashimoto Y, Kon T, Nakakura S, Nishi Y, Endo H. 2004. Trimethylamine biosensor with flavin-containing monooxygenase type 3 (FMO3) for fish-freshness analysis. *Sensors and Actuators, B: Chemical* 103(1-2): 463-7.

Nagle HT, Schiffman SS, Gutierrez-Osuna R. 1998. How and why of electronic noses. *IEEE Spectrum* 35(9): 22-34.

Natale CD, Olafsdottir G, Einarsson S, Martinelli E, Paolesse R, D'Amico A. 2001. Comparison and integration of different electronic noses for freshness evaluation of cod-fish fillets. *Sensors and Actuators, B: Chemical* 77(1-2): 572-8.

Nilsen H, Esaiassen M. 2005. Predicting sensory score of cod (Gadus morhua) from visible spectroscopy. *Food Science and Technology* 38(1): 95-9.

Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschlager J, Tryggvadottir SV, Schubring R, Kroeger M, Heia K, Esaiassen M, Macagnano A, Jorgensen BM. 2004. Multisensor for fish quality determination. *Trends in Food Science and Technology* 15(2): 86-93.

Olafsdottir G, Li X, Lauzon HL, Jonsdottir R. 2002. Precision and application of electronic nose for freshness monitoring of whole redfish (Sebastes marinus) stored in ice and modified atmosphere bulk storage. *J Aquat Food Prod Technol* 11(3-4): 229-49.

Pacquit A, Lau KT. Diamond D. 2004. Development of a colorimetric sensor for monitoring of fish spoilage amines in packaging headspace. 1(365-7.

Roussel S, Forsberg G, Grenier P, Bellon-Maurel V. 1999. Optimization of electronic nose measurements. Part II: Influence of experimental parameters. *J Food Eng* 39(1): 9-15.

Roussel S, Forsberg G, Steinmetz V, Grenier P, Bellon-Maurel V. 1998. Optimisation of electronic nose measurements. Part I: methodology of output feature selection. *J Food Eng* 37(2): 207-22.

Sarry F, Lumbreras M. 1999. Gas composition determination in an air conditioned system using a sensor array: Characterization of three different TGS sensors. *Sensors and Actuators, B: Chemical* 59(2-3): 94-9.

Schweier-Berberich PM, Vaihinger S, Gopel W. 1994. Characterization of food freshness with sensor arrays. *Sensors and Actuators, B: Chemical* B18(1-3 pt 1): 282-90.

Takao Y, Iwanaga Y, Shimizu Y, Egashira M. 1993. Trimethylamine-sensing mechanism of $TiO_2$-based sensors 1. Effects of metal additives on trimethylamine-sensing properties of $TiO_2$ sensors. *Sensors and Actuators, B: Chemical* B10(3): 229-34.

Watanabe KH, Desimone FW, Thiyagarajah A, Hartley WR, Hindrichs AE. 2003. Fish tissue quality in the lower Mississippi River and health risks from fish consumption. *Sci Total Environ* 302(1-3): 109-26.

Zhang Y, Zhang Z, Sugiura N, Maekawa T. 2002. Monitoring of methanogen density using near-infrared spectroscopy. *Biomass Bioenergy* 22(6): 489-95.

Zhao C, Pan Y, Ma L, Tang Z, Zhao G, Wang L. 2001. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal. *Sensors and Actuators, B: Chemical* 81(2-3): 218-22.

# 5. STORAGE TIME PREDICTION OF BLUE CRAB LUMP AND CLAW MEAT USING ELECTRONIC NOSE AND VISIBLE/NEAR-INFRARED SPECTROSCOPY

**T. H. Dodd and S. A. Hale**

## 5.1 ABSTRACT

This study examined the ability of an electronic nose (e-nose) and visible/near-infrared spectroscopy (VIS/NIR) to predict storage time in Atlantic blue crab meat. Both claw and lump meat were examined. The meat was stored on ice and sampled throughout the spoilage period of 14 days. Using an e-nose to estimate storage time, a standard error of prediction of 2.48 and 2.77 days were obtained for claw and lump meat respectively. VIS/NIR spectroscopy was then used to produce a continuous model for predicting the storage time of the crab meat. Overall standard errors of prediction of 1.31 days and 1.11 days were obtained for claw and lump meat respectively.

## 5.2 INTRODUCTION

Food safety has always been at the forefront of public concern. People in general are becoming more interested in the quality of the food supply and have begun placing more importance on food safety. Safety and quality have always been linked. An unsafe product would be considered of low quality, although a low quality product could be absolutely safe. One constant in most food products is that quality deteriorates over time.

Food quality measurements have often been likened to sensory scores and chemical analysis. In most cases, storage time is used as a quality indicator. This is seen in the use of expiration dates found on most packaged foods sold in the US. Expiration dates give the consumer a clear indicator by which to tell that a food may no longer be safe for consumption. The US, through the FDA, has placed a particular emphasis on traceability of food products, so that a food can be traced from the time it is produced to when it enters the market and is finally sold.

The FDA has implemented hazard analysis and critical control point (HACCP) regulations in an effort to address consumer concerns and ensure that food sold to consumers is safe. According to HACCP regulations "All raw materials and products should be lot-coded and a recall system in place so that rapid and complete traces and recalls can be done when a product retrieval is necessary." (FDA 2005) HACCP tries to establish guidelines by which records can be independently verified. Inspectors now currently use sensory perception or destructive chemical analysis to try to verify the accuracy of these records. Both of these techniques have major drawbacks. Sensory perception has the disadvantage of being subjective if not done by a trained individual or panel and chemical testing requires that some of the product be destroyed. A quick objective measurement could be of major benefit in ensuring a better quality product.

Because quality can be subjective, sensory panels are used to try to remove this variation. In this process, a panel of consumers, usually trained in how to score specific traits of the product, rates a product using a standard scale. The most common attributes used are appearance, aroma, taste and texture. Sensory scores have been measured over time in a variety of fishery products. The general trend is a decrease in quality over time.

Crab meat has been shown to have an almost linear decrease in sensory score over time (Chen 1996). While using human senses has the advantage of being able to consider a large variation of factors, it necessarily has limitations due to the scale of effort that is required to perform a sensory panel analysis on a large number of samples. The two senses most frequently used to determine product quality, both by sensory panels and the consumer, are sight and smell.

Many studies have linked sensory panel scores with various chemical indicators. Odor evaluation has been correlated to TVB-N in Mahi-Mahi (Antoine, et. al. 2002), and used as a quality indicator. While odor is not the only sense that the consumer uses to evaluate products, it is one that is commonly used to indicate spoilage. FDA uses odor to evaluate products. It is listed in "The Safe Food Chart" for meat, poultry, and seafood as one of the main ways to detect the freshness of seafood. As such, odor is also used by FDA inspectors as a quality indicator.

The sense of sight is also used to determine freshness, with color being a major component of quality in crab meat. One common measure is the lightness (L), red-green (a), and yellow-blue (b) values. In general, the lighter the color of the lump meat, the greater quality the consumer attributes to the meat. Other studies have shown that spectroscopy can be used to estimate various chemical parameters in crab meat (Requena, 1998).

While chemical indicators provide very repeatable and quantifiable results, this type of testing is normally destructive, time consuming, and expensive. The process of testing for chemical indicators also varies widely from product to product. This limits the practical use of chemical indicators as a quality indicator.

One thing that remains almost certain for any food product is that quality decreases over time. In order to better estimate food quality in an objective manner, alternative sensing technologies, such as e-noses and VIS/NIR spectroscopy, are being explored. These use the same data sources as the human senses of smell and sight respectively, but with additional information and repeatability not found in individuals or sensory panels. For example, the NIR region of the electrometric spectrum is not able to be detected by the human eye.

E-noses have been used to study a variety of food products. One of these has been the examination of fish spoilage (Krzymien and Elias, 1990; Ohashi et al., 1991,Egashira, et al., 1990; Olafsson et al., 1992). Natale et al. (1996) used four quartz microbalance sensors and PCA analysis to examine freshness loss in cod fillets. The sensors measured the mass change as the odorants in the air were absorbed onto their surfaces. While the resulting data were found to have some differences, PCA was not able to provide separation at all time points. Implementation of neural networks resulted in a continuous curve that corresponded to decay time. Schweizer-Berberich et al. (1994) also analyzed the freshness of fish using eight different amperometric, three-electrode, gas sensors. By using PCA on the data, general trends were observed but were not quantified.

Focusing more on specific chemicals indicators has also been attempted using e-noses. Total volatile bases (TVB) in fishmeal products were examined using an e-nose and comparing these values to gas chromatograph (GC) (Olafsdottir, et. al., 2000). It was found that gas sensors could predict TVB in the headspace above the product, with data combing yielding better results. The lowest RMSEP calculated was 7.6 mgN/100g of fish. Dodd *et. al.* (2004) showed that e-noses have the ability to classify tilapia by spoilage time. In this

study tilapia was allowed to decay at room temperature and e-nose measurements were able to classify the response time of 3-hour time segments with an accuracy of 83%.

VIS/NIR spectral analysis has also been used to examine a wide variety of products. It has been successfully used to analyze everything from pharmaceuticals to food products to agricultural measurements. A review of applications and techniques can be found in Blancho and Villarraya (2002) and Pasquiuni (2003). It has also been used in a variety of fishery product quality evaluations. Downey (1996) used the NIR spectra (700-1100 nm range) to determine the oil and moisture content of salmon. Corresponding SEPs calculated were all under 2.5%. Zhang and Lee (1997) also used NIR spectroscopy to determine the free fatty acid level in fish oil. First derivative NIR spectra were used to predict FFA and hypoxanthine (Hx) changes in the fish meat. A relative error of less than 10% was seen in all cases. Nilsen and Esaiassen (2005) examined the correlation between spectra in cod meat sensory scores. It was shown that the visible region provided the greatest correlation with Quality Index Method (QIM) scores. Adding the NIR region decreased the performance with the NIR region alone yielding the worst correlation.

### 5.3 RESEARCH OBJECTIVE

The objective of this research is to predict the storage time of Blue Crab lump and claw meat using e-nose and VIS/NIR measurements.

## 5.4 MATERIALS AND METHODS

The experimental design used in this study was devised to model the storage conditions used in the seafood industry for Atlantic blue crab lump and claw meat. The typical industry standard is to process crabs and store the meat on ice for a maximum of 14 days.

### 5.4.1. Sample Preparation

Atlantic blue crab meat was obtained from a North Carolina crab processor. Three one-pound containers of claw meat and three one-pound containers of lump meat were obtained. The meat was originally packaged under commercial conditions, then repackaged, in vacuum sealed bags and stored at -80°C. Prior to analysis, one pound of meat was allowed to thaw in a 4°C refrigerator overnight. Once thawed, it was separated into samples for e-nose analysis and VIS/NIR analysis. This procedure was repeated for the three different pounds of crab meat. Each pound of meat was separated into 3 VIS/NIR samples and 7 e-nose samples. This yields a total of 9 VIS/NIR samples and 21 e-nose samples for each pound of claw and lump meat.

### 5.4.2. E-nose sampling

Five (5) grams of meat were sealed in polyethylene bags and then stored on ice until sampling was to be performed. One sample was removed from ice every other day for measurement. On the day of analysis, samples were removed from storage and placed in the

e-nose sampling chamber where headspace gasses were pulled through a sensing chamber. The sampling chamber consisted of a Nalgene box that had been modified to allow for headspace sampling at its top. E-nose sampling was performed as reported in Dodd *et. al.* (2004) on a custom e-nose with 15 commercially available tin metal oxide sensors. The sensors were mounted in a small sensing chamber, and headspace and reference gases were pulled through the chamber with a vacuum pump. The sampling procedure involved washing the sensors with a 3% butanol solution for 30 seconds prior to exposure to a baseline gas of dehumidified and deodorized air for 120 seconds. Headspace gasses above the e-nose sample were then pulled through the sample chamber for 60 seconds with sensor voltage measurements being recorded every second. Multiple headspace samples were taken in succession on a single meat sample. This gave more data on a single sample, countering sensor inaccuracies and meat variability.

Recorded data were pre-processed using a customized MATLAB program. This began with a compression phase in which the number of measurements per sensor per sample was reduced from 60 to 4. Because both the transient and the steady-state portions of each sensor's response contained useful information, windowed time slicing was used (Gutierrez-Osuna and Nagel, 1999). This was done by multiplying the transient response by four smooth, bell-shaped window functions (Figure 5-1). By using this technique, information about the dynamic characteristics of the response was captured. The width, shape, and center of the windowing functions, $K_i(t_k)$, were defined by the parameters $a_i$, $b_i$, and $c_i$, respectively:

**Equation 5-1**

$$W^i = \sum_{k=1}^{N_T} R(t_k) K_i(t_k) \Delta t$$

**Equation 5-2**

$$K_i(t_k) = \frac{1}{1 + \left( \frac{t_k - c_i}{a_i} \right)^{2b_i}}$$

where

$R(t_k)$ = a sensor's response at time $t_k$

$\Delta t$ = time between samples (1 s)

$W^i$ = area under each curve (the four points to which the signal was reduced).

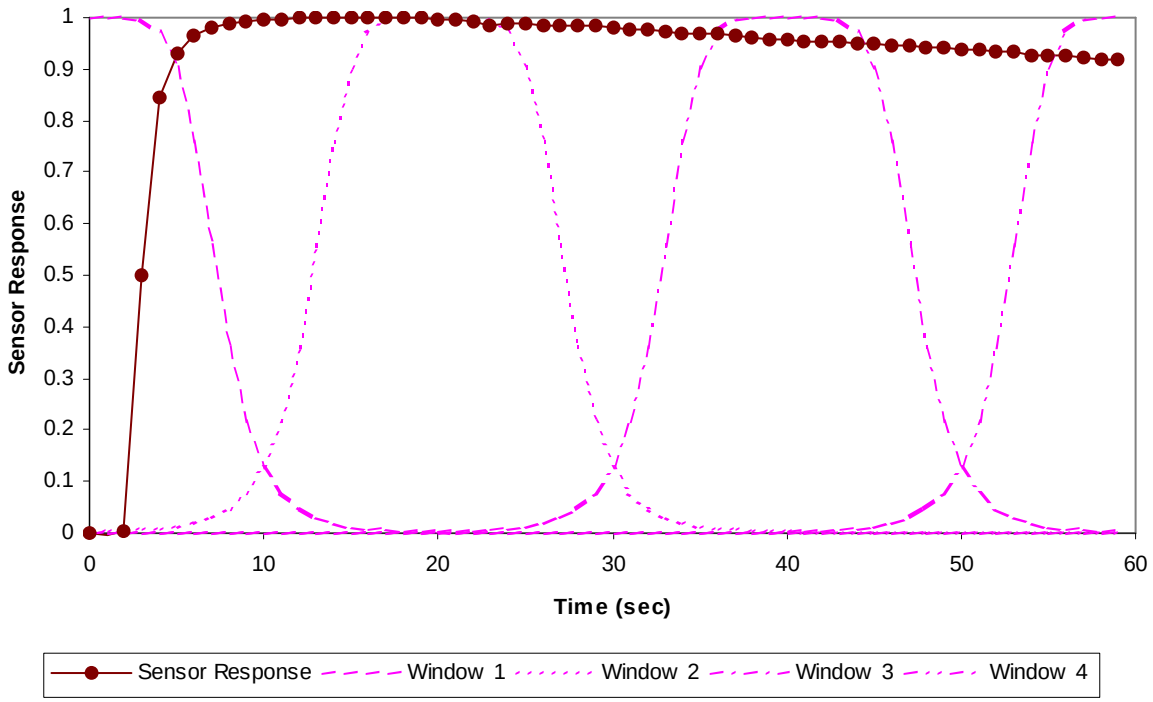



**Figure 5-1:Bell-shaped sections used for windowed time slicing**

### 5.4.3. VIS/NIR Data collection

For the VIS/NIR samples, 150 grams of meat was sealed in polyethylene bags and stored on ice. Once a day the samples were removed, scanned and returned to ice storage.

They were scanned using a monochromator spectrometer (NIRSystems 6500, Perstorp Analytical, Silver springs, MD). Three spectra from 400 to 2498 nm in 2 nm increments were recorded each day. Each recorded spectra consisted of an average of 32 scans.

### 5.4.4. Data Analysis

Similar data analysis techniques were used on both e-nose and VIS/NIR datasets. Data were imported into the Unscrambler version 9.6 (CAMO software inc, OR) for analysis. Principal Component Regression (PCA) was first used to determine any underlying trends. PC scores were plotted to examine what factors were the major contributors to the first PCs.

Continuous models were generated using Partial Least Squares (PLS). In order to limit over fitting, the maximum number of components used in the model set at 8. Full cross validation was used in order to generate a more robust model while still examining its ability to determine storage time in unseen examples. Model performance was evaluated using $r^2C$, SEC, $r^2P$ and SEP, where C stands for calibration and P stands for prediction.

For pretreatments on VIS/NIR spectra, samples were averaged to better control random noise effects. The effect of pretreatments (n-point smoothing, first and second derivatives) on the model were then explored. PLS models were generated for visible only, NIR only, and total VIS/NIR spectra.

## 5.5 RESULTS AND DISCUSSION

### 5.5.1. E-nose

In order to first examine the largest factors in the collected e-nose data, PCA was first performed. Since PCA is an unsupervised technique, the graph of the samples should be the same for all variables. Figure 5-2 shows the first two principal components labeled by meat sample.

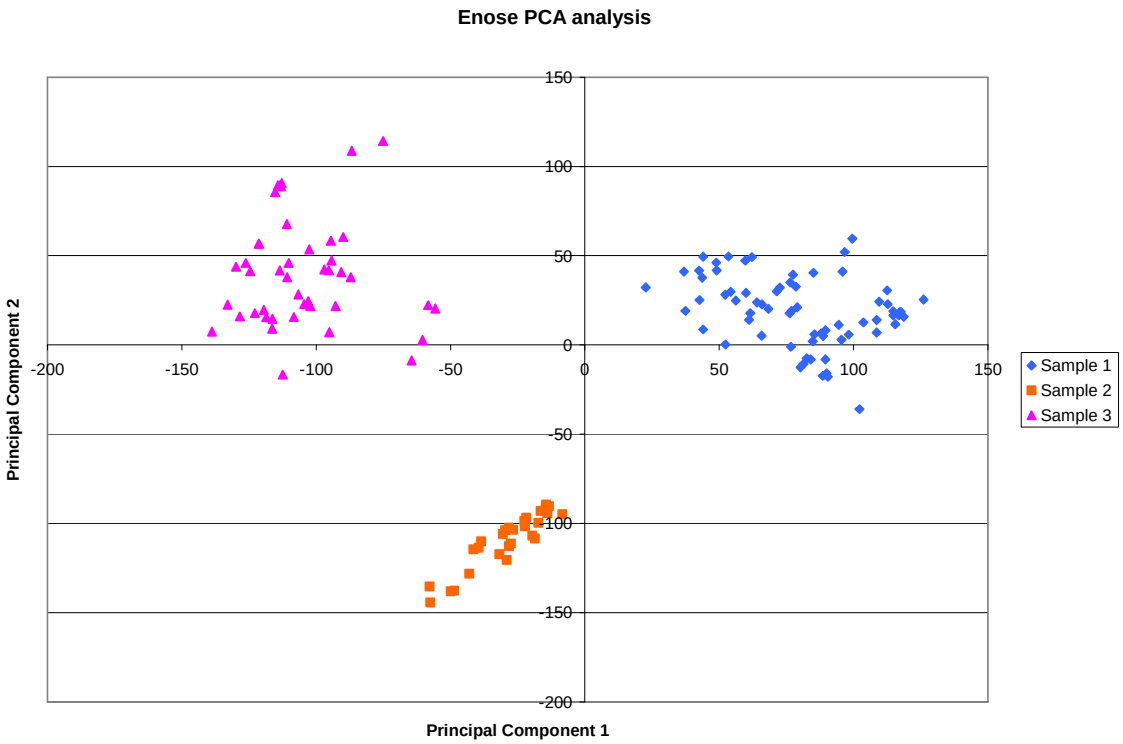



**Figure 5-2: PCA scores for claw meat from e-nose data**

The data are grouped according to meat sample. This suggests that the majority of the variability detected by the e-nose results from differences between the 3 meat samples.

Since an unsupervised method did not include the variable of interest, a supervised method should generate a more precise model. This would reduce the effect of differences seen between the meat samples.

Multivariate analysis was performed and a model was generated to predict the storage time of the meat samples from the e-nose data. Results are shown in table 5-1, which summarizes the model performance.

**Table 5-1: Summary E-nose Statistics for Storage Time**

| Sample | $r^2$ C | SEC | $r^2$ P | SEP |
|---|---|---|---|---|
| Claw | 0.841 | 2.233 | 0.801 | 2.484 |
| lump | 0.767 | 2.488 | 0.709 | 2.744 |

It can be seen from Table 5-1 that the electronic nose was able to determine aging storage time trends in both claw meat and lump meat. Both were able to be modeled to an SEP of under 3 days, indicating that an unknown sample may be classified to within 3 days. While a more accurate method would be preferred, the performance of the analysis shows that variations in the storage time could be monitored using the e-nose technology. This correlation should not be surprising since storage time is linked to sensory scores and aroma is one of the criteria used in calculating these scores.

It can also be noted that the claw meat was able to be modeled slightly better than the lump meat could be modeled. During the experimentation, it was observed that claw meat had a much stronger odor than lump meat. Since the e-nose is monitoring odor, differences in strong odors should be more distinguishable than those of less dilute odors.

5.5.2. VIS/NIR

Representative spectra of both claw and lump Atlantic blue crab meat are shown in Figure 5-3. Each spectrum is comprised of absorbance or Log (1/Reflectance) vs. Wavelength in the visible (400-720 nm) and NIR (720-2500 nm). Claw meat is seen as having a higher absorbance value in the visible region; this is expected because of its darker coloration.

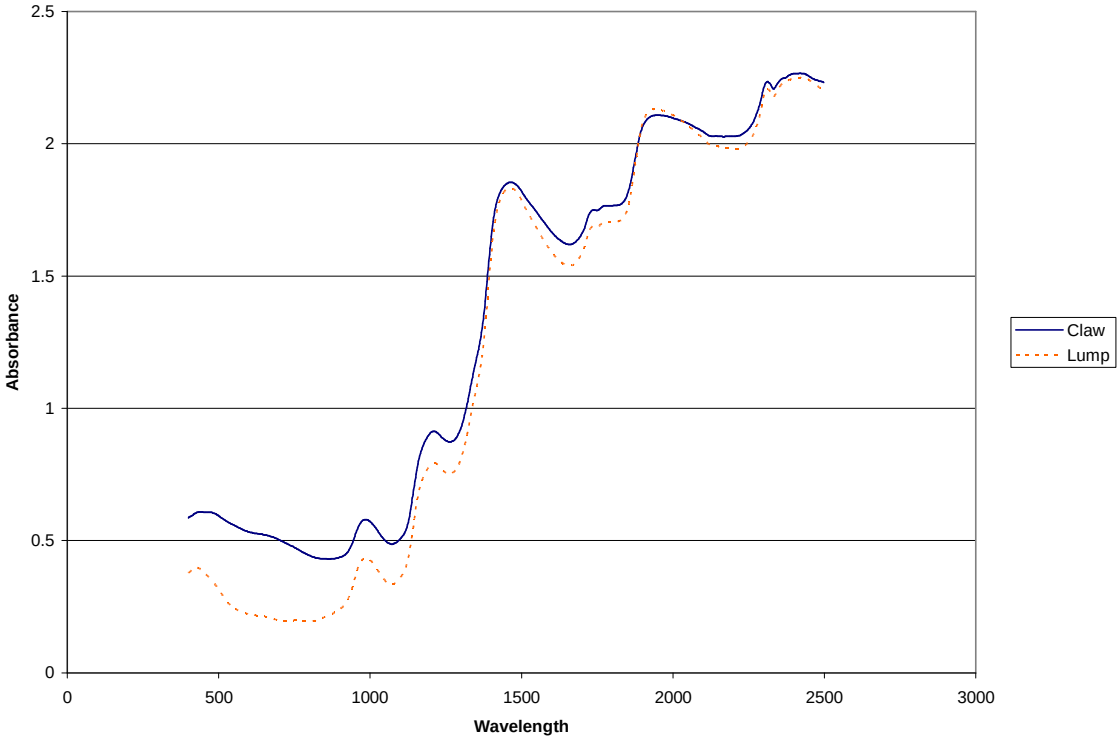



**Figure 5-3:Representative Initial Spectra of Claw and Lump Meat**

For data pretreatments, choosing the smoothing and derivatives gaps is critical for the analysis to be successful. If too wide of a gap is chosen then the peaks can be shifted, too small and the noise takes over and no real data can be gleaned from the data (McClure, 1993). For the current experiment a smoothing average of 15 wavelengths was used.

Pretreatments performed on the data are shown in Figure 5-4. This Figure shows a representative sample of lump meat in smoothed absorbance, first and second derivatives.

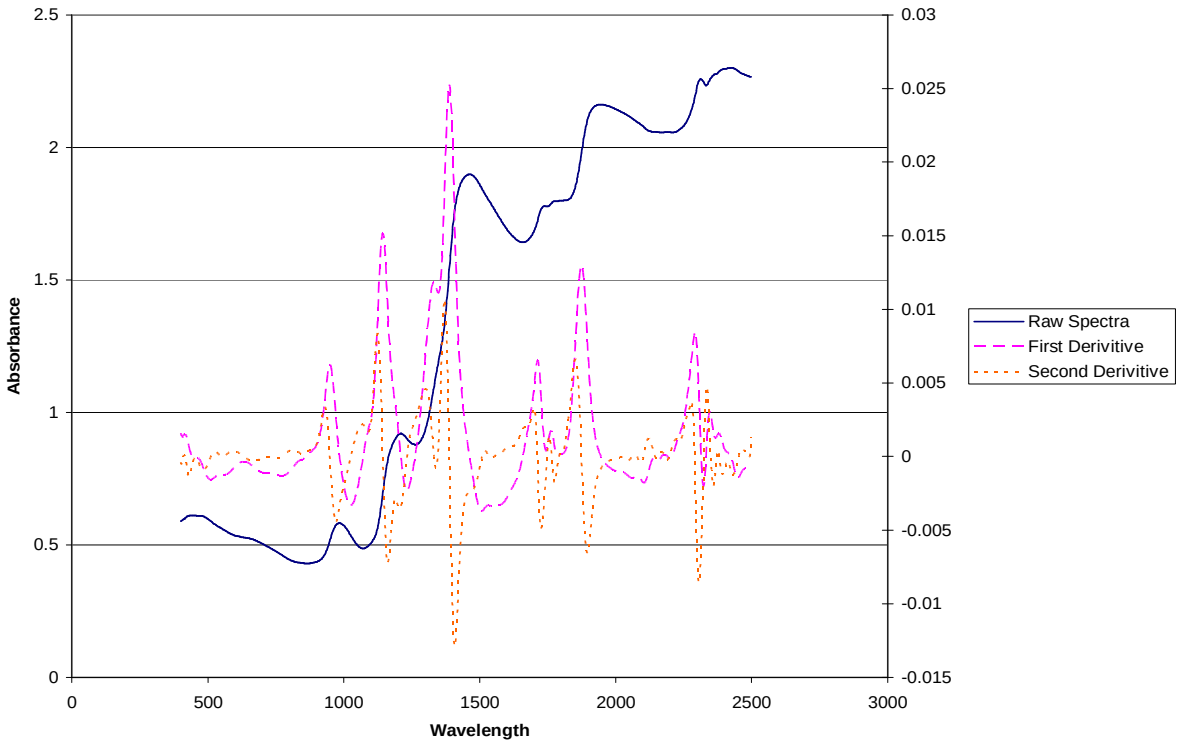



**Figure 5-4: Effect of first and second derivative of a representative spectra**

**Table 5-2: Summary Statistics for VIS/NIR Spectroscopy**

| | Calibration | | Prediction | |
|---|---|---|---|---|
| **VIS Only** | $r^2$ C | SEC | $r^2$ P | SEP |
| Claw | 0.938 | 1.447 | 0.911 | 1.730 |
| Lump | 0.960 | 1.208 | 0.948 | 1.360 |
| **NIR only** | | | | |
| Claw | 0.956 | 1.229 | 0.931 | 1.518 |
| Lump | 0.925 | 1.626 | 0.870 | 2.125 |
| **VIS/NIR combined** | | | | |
| Claw | 0.969 | 1.026 | 0.950 | 1.307 |
| Lump | 0.981 | 0.825 | 0.966 | 1.109 |

Table 5-2 shows the model performance for VIS/NIR spectroscopy. Focusing on the VIS section of the spectra shows that both claw and lump meat go through changes in the visible spectra that can be modeled. It has been shown that visible spectra can be linked to sensory scores in cod (Nilsen and Esaiassen, 2005). While the Nilsen paper states that sensory scores were not directly correlated with time and that this relationship was not linear, a graph in the paper shows a near linear region. As such, a direct relation between time and sensory scores should be applicable for most of the storage period. Since sensory panels use color (sight) as one of the criteria for the sensory score, the fact that the VIS spectra correlated well with sensory scores is not surprising. Since consumer opinion plays such a large role in what is defined as good quality, using the part of the spectrum that is accessible to the consumer should provide a good correlation.

Lump meat showed slightly lower SEP than claw meat. Lump meat is much lighter than claw meat and this allows for a greater degree of change to be observed. Also maillard reactions are in the brown region that are already saturated in the claw meat and therefore can not be detected as easily as in lump meat.

Examining the NIR spectra should produce more chemical data than examining the visible spectra alone. The NIR portion of Table 5-2 shows that claw meat was modeled to an SEP of approximately 1.5 days and lump meat was modeled to an SEP of about 2 days. This tends to the conclusion that both samples are going through significant chemical changes.

Claw meat showed a better SEP than lump meat, using the NIR data. While lump meat goes through more changes in the visible region, claw meat goes through more chemical changes that can be seen in changes in its NIR spectra. Considering that claw meat

has a much stronger odor and taste than lump meat, this would suggest that there are more extensive changes occurring to its chemical makeup and these changes are being detected by the NIR data. This also agrees with the fact that claw meat was more easily modeled by the e-nose that examines off-gas (chemical) changes in the meat samples.

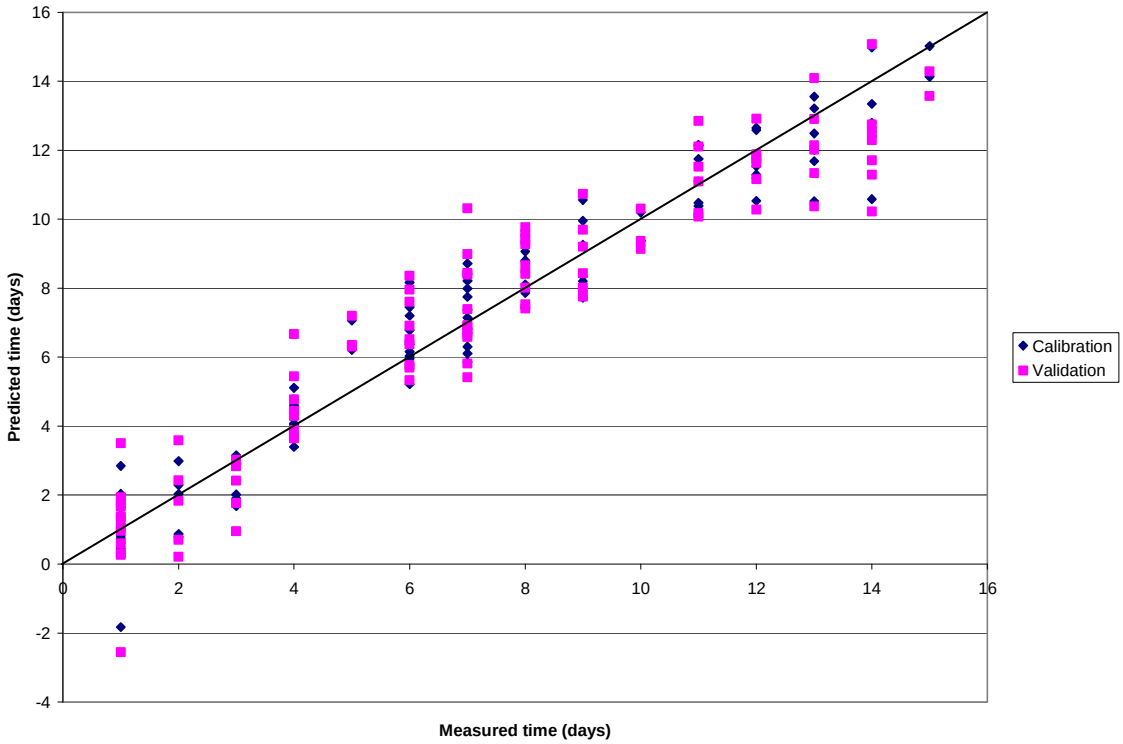



**Figure 5-5: Predicted vs. Measured values for Claw meat using VIS/NIR spectra**

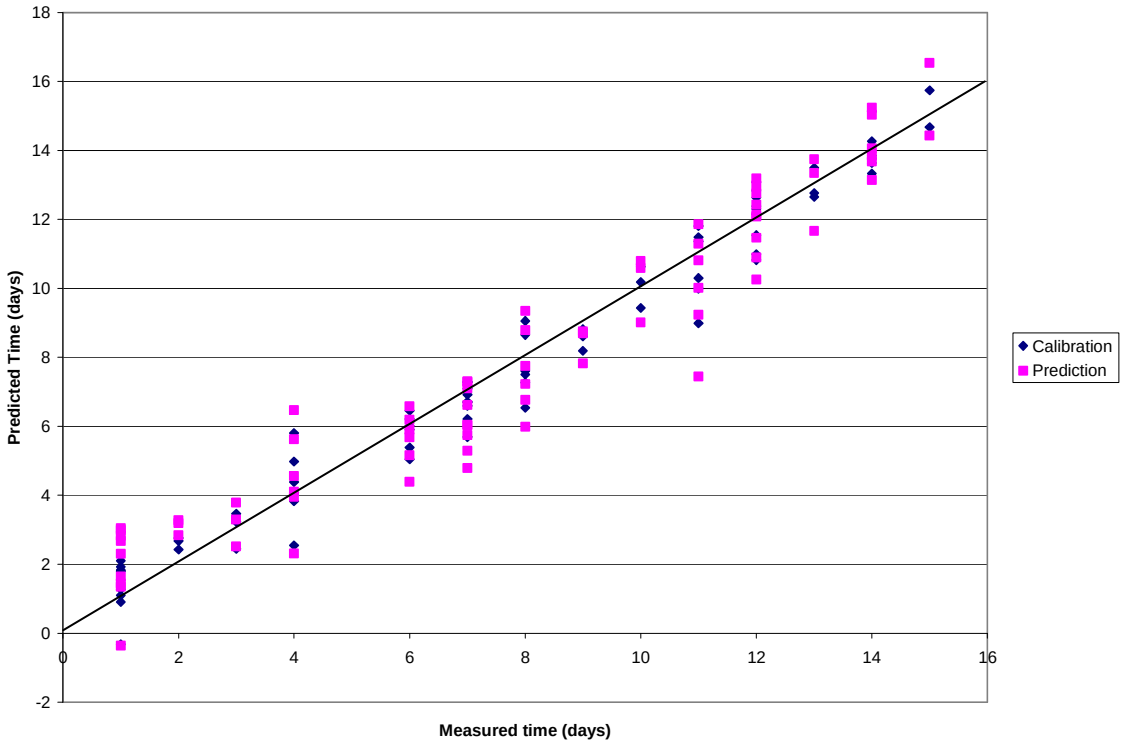



**Figure 5-6: Predicted vs. Measured values for Lump meat using VIS/NIR spectra**

By combining the visible and near-infrared regions of the spectra, more information can be obtained and modeled, which allows for more accurate modeling of the decay process. This can be seen by the VIS/NIR combined section of Table 5-2. Both claw meat and lump meat showed an improvement in SEP of approx 0.2 days. This value is small but not insignificant. This shows that combining the Visible and NIR spectra allowed a more complete model to be generated. Figure 5-5 shows the model performance for claw meat for both calibration and prediction sets. Both groups show a good correlation with very little bias. Figure 5-6 shows similar model performance for lump meat.

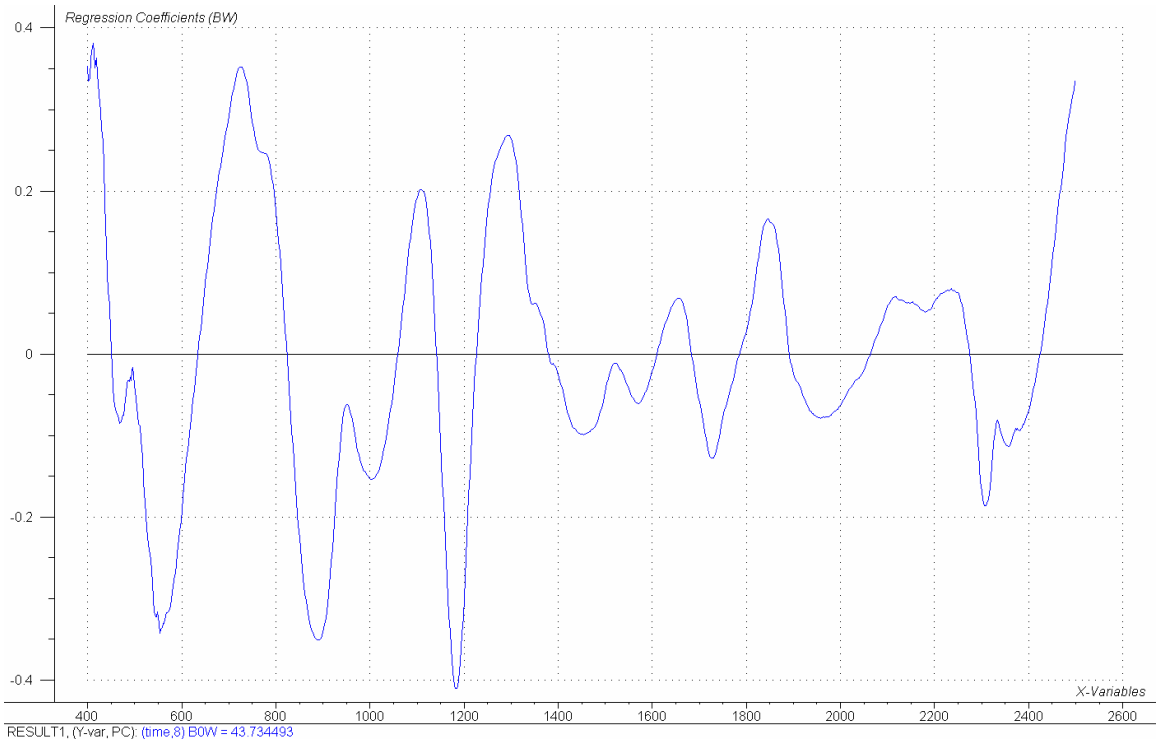



**Figure 5-7: Regression Coefficients for VIS/NIR model**

Figure 5-7 shows the loadings of the model generated using the total spectra recorded. It can be seen that there are contributions made to the model by both the Visible and NIR areas of the spectra. This would lead to the conclusion that both sections of spectra are needed in order to accurately predict storage time.

By using instrumentation to quantify two of the primary senses (smell and sight) used by humans to evaluate food quality, this study was able to show that e-nose and VIS/NIR technologies were able to predict degradation in crab meat over storage time. The physics behind sight are much better understood that those that make up smell. As such VIS/NIR technologies tend to be more mature than those of e-noses. This agrees with the finding of this research that VIS/NIR was able to better predict storage time in crab meat than the e-nose. With increases in the understanding of aroma and with e-nose technologies making

continuous improvements, it is believed that the achievable results with e-noses should increase rapidly.

## 5.6 CONCLUSION

E-nose and VIS/NIR spectroscopy was used to evaluate storage time in both claw and lump crab meat of Atlantic blue crab. This methodology takes the approach of examining the overall storage time rather than specific 'freshness' indicators. For a technology assessment this approach gives a very cost effective, fast and simple approximation of QIM.

While the e-nose shows promise for a quick non-contact method of determining a rough estimate of if a sample is spoiled, the technology still needs refinement in order to be useful in rapid storage time prediction. While e-nose technology was able to model general trends, VIS/NIR was able to model these trends with far greater accuracy. E-nose technologies have the disadvantage of having to extract headspace from a sample that VIS/NIR technology does not have. The VIS/NIR analysis that is developed in this paper can produce a model that can predict the storage time of crab meat to within 1.5 days.

## 5.7 REFERENCES

Bartlett PN, Elliott JM, Gardner JW. 1997. Electronic noses and their application in the food industry. *Food Technol* 51(12): 44-8.

Bene A, Hayman A, Reynard E, Luisier JL, Villettaz JC. 2001. New method for the rapid determination of volatile substances: The SPME-direct method. Part II. Determination of the freshness of fish. *Sensors and Actuators, B: Chemical* 72(3): 204-7.

Deisingh A, Stone D, Thompson M. 2004. Application of electronic noses and tongues in food analysis. *International Journal of Food Science and Technology* 39(587-604.

Delpha C, Lumbreras M, Siadat M. 2001. Discrimination of Forane 134a and carbon dioxide concentrations in an air conditioned atmosphere with an electronic nose: Influence of the relative humidity. *Sensors and Actuators, B: Chemical* 80(1): 59-67.

Delpha C, Siadat M, Lumbreras M. 1999. Relative humidity: An interfering parameter for the characterization of a TGS sensor array. *Proc SPIE Int Soc Opt Eng* 3857(223-30.

Di Natale C, Brunink JAJ, Bungaro F, Davide F, d'Amico A, Paolesse R, Boschi T, Faccio M, Ferri G. 1996. Recognition of fish storage time by a metalloporphyrins-coated QMB sensor array. *Meas Sci Technol* 7(8): 1103-14.

Di Natale C, Macagnano A, Mantini A, Davide F, D'Amico A, Paolesse R, Boschi T, Faccio M. Ferri G. 1997. Advances in food analysis by electronic nose. 1(122-7.

Dodd TH, Hale SA, Blanchard SM. 2004. Electronic nose analysis of tilapia storage. *Transactions of the American Society of Agricultural Engineers* 47(1): 135-40.

Egashira M, Shimizu Y, Takao Y. 1990. Trimethylamine sensor based on semiconductive metal oxides for detection of fish freshness. *Sensors and Actuators, B: Chemical* B1(1-6): 108-12.

Ellis PC, Pivarmk LF, Thiam M, Berger L, Field S, Green D, Hewes D, Lemerise D, Lyttle C, Maciel J, Soper K. 2000. Determination of volatile bases in seafood using the ammonia ion selective electrode: Collaborative study. *J AOAC Int* 83(4): 933-43.

Gao D, Wu S. 1998. Development of artificial olfactory system and its prospects on evaluating flavor of food. *Nongye Jixie Xuebao/Transactions of the Chinese Society of Agricultural Machinery* 29(4): 167-72.

Gupta S, Misra TN. 1997. Manganese phthalocyanine for the detection of fish freshness by its trimethylamine emission. *Sensors and Actuators, B: Chemical* B41(1-3): 199-202.

Gutierrez-Osuna R, Nagle HT. 1999. Method for evaluating data-preprocessing techniques for odor classification with an array of gas sensors. *IEEE Transactions on Systems, Man, and Cybernetics, Part B: Cybernetics* 29(5): 626-32.

Hammond J, Marquis B, Michaels R, Oickle B, Segee B, Vetelino J, Bushway A, Camire ME, Davis-Dentici K. 2002. A semi conducting metal-oxide array for monitoring fish freshness. *Sensors and Actuators, B: Chemical* 84(2-3): 113-22.

Hammond J, Mlsna T, Smith D, Fruhberger B. 1999. Fish freshness sensor. *Proc SPIE Int Soc Opt Eng* 3856(88-96.

Haugen JE, Chanie E, Westad F, Jonsdottir R, Bazzo S, Labreche S, Marcq P, Lundby F, Olafsdottir G. 2006. Rapid control of smoked Atlantic salmon (Salmo salar) quality by electronic nose: Correlation with classical evaluation methods. *Sensors and Actuators, B: Chemical* 116(1-2): 72-7.

Hofmann T, Schieberle P, Krummel C, Freiling A, Bock J, Heinert L, Kohl D. 1997. High resolution gas chromatography/selective odorant measurement by multisensor array (HRGC/SOMSA): A useful approach to standardize multisensor arrays for use in the detection of key food odorants. *Sensors and Actuators, B: Chemical* B41(1-3): 81-7.

Legin A, Rudnitskaya A, Seleznev B, Vlasov Y. Velikzhanin V. 2001. Electronic tongue for recognition of flesh food. 76-81.

Lindsay RC. 1988. Flavor chemistry and seafood quality factors. 1(61-5.

Llobet E, Hines EL, Gardner JW, Bartlett PN, Mottram TT. 1999. Fuzzy ARTMAP based electronic nose data analysis. *Sensors and Actuators, B: Chemical* B61(1-3): 183-90.

MacAgnano A, Careche M, Herrero A, Paolesse R, Martinelli E, Pennazza G, Carmona P, D'Amico A, Di Natale C. 2005. A model to predict fish quality from instrumental features. *Sensors and Actuators, B: Chemical* 111-112(SUPPL): 293-8.

McClure W, Crowell B. 1996. Quantitative information in near-infrared spectra: Part 1. Effects of smoothing and combing. *J Near-Infrared Spectroscopy* 4(129-137.

Mitsubayashi K, Kubotera Y, Yano K, Hashimoto Y, Kon T, Nakakura S, Nishi Y, Endo H. 2004. Trimethylamine biosensor with flavin-containing monooxygenase type 3 (FMO3) for fish-freshness analysis. *Sensors and Actuators, B: Chemical* 103(1-2): 463-7.

Nagle HT, Schiffman SS, Gutierrez-Osuna R. 1998. How and why of electronic noses. *IEEE Spectrum* 35(9): 22-34.

Natale CD, Olafsdottir G, Einarsson S, Martinelli E, Paolesse R, D'Amico A. 2001. Comparison and integration of different electronic noses for freshness evaluation of cod-fish fillets. *Sensors and Actuators, B: Chemical* 77(1-2): 572-8.

Nilsen H, Esaiassen M. 2005. Predicting sensory score of cod (Gadus morhua) from visible spectroscopy. *Food Science and Technology* 38(1): 95-9.

Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschlager J, Tryggvadottir SV, Schubring R, Kroeger M, Heia K, Esaiassen M, Macagnano A, Jorgensen BM. 2004. Multisensor for fish quality determination. *Trends in Food Science and Technology* 15(2): 86-93.

Olafsdottir G, Li X, Lauzon HL, Jonsdottir R. 2002. Precision and application of electronic nose for freshness monitoring of whole redfish (Sebastes marinus) stored in ice and modified atmosphere bulk storage. *J Aquat Food Prod Technol* 11(3-4): 229-49.

Pacquit A, Lau KT. Diamond D. 2004. Development of a colorimetric sensor for monitoring of fish spoilage amines in packaging headspace. 1(365-7.

Roussel S, Forsberg G, Grenier P, Bellon-Maurel V. 1999. Optimization of electronic nose measurements. Part II: Influence of experimental parameters. *J Food Eng* 39(1): 9-15.

Roussel S, Forsberg G, Steinmetz V, Grenier P, Bellon-Maurel V. 1998. Optimisation of electronic nose measurements. Part I: methodology of output feature selection. *J Food Eng* 37(2): 207-22.

Sarry F, Lumbreras M. 1999. Gas composition determination in an air conditioned system using a sensor array: Characterization of three different TGS sensors. *Sensors and Actuators, B: Chemical* 59(2-3): 94-9.

Schweier-Berberich PM, Vaihinger S, Gopel W. 1994. Characterization of food freshness with sensor arrays. *Sensors and Actuators, B: Chemical* B18(1-3 pt 1): 282-90.

Takao Y, Iwanaga Y, Shimizu Y, Egashira M. 1993. Trimethylamine-sensing mechanism of $TiO_2$-based sensors 1. Effects of metal additives on trimethylamine-sensing properties of $TiO_2$ sensors. *Sensors and Actuators, B: Chemical* B10(3): 229-34.

Watanabe KH, Desimone FW, Thiyagarajah A, Hartley WR, Hindrichs AE. 2003. Fish tissue quality in the lower Mississippi River and health risks from fish consumption. *Sci Total Environ* 302(1-3): 109-26.

Zhang Y, Zhang Z, Sugiura N, Maekawa T. 2002. Monitoring of methanogen density using near-infrared spectroscopy. *Biomass Bioenergy* 22(6): 489-95.

Zhao C, Pan Y, Ma L, Tang Z, Zhao G, Wang L. 2001. Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal. *Sensors and Actuators, B: Chemical* 81(2-3): 218-22.