DOI 10.5012/bkcs.2010.31.11.3084

An Array-Based Sensor for Seafood Freshness Assessment†

Anuncia Gonzalez-Martin, Brian Lewis, Marius Raducanu, and Jinseong Kim*

*Lynntech, Inc., College Station, TX 77840 USA. *E-mail: jinseong.kim@lynntech.com*
Received April 17, 2010, Accepted September 8, 2010

This paper describes the development of an automated, hand-held sensor for the fast assessment of seafood freshness. The sensor developed here combined: an array-based chemical sensor, composed of incrementally different conducting polymer elements deposited on a small chip; a highly sensitive, custom-made electronics for the detection of very small signal changes; precise temperature control of the sensor chamber; and an on-board microcontroller for data collection, storage, automation, and analysis. The instrument was used to successfully test seafood samples with different degree of freshness and spoilage. A linear relationship between microbiological count and e-Nose signal for three different fish fillet was developed. Once the linear relationship is included into the hand-held unit software, the e-Nose signal can be used for assessment of seafood freshness without performing the microbiological count technique.

Key Words: Electronic nose, Food safety, Seafood assessment, Array-based sensors

Introduction

Detrimental changes in seafood can occur before or after processing. Many of these changes adversely affect nutrient content, texture, flavor, odor, and color.[1] The assessment of the freshness of food products is important for food safety, wholesomeness, and taste. When needing to assess the degree of seafood spoilage, the industry has traditionally relied on subjective sensory analysis through use of a sniff test, which is also used in most regulations regarding spoiled seafood. Even though highly trained and capable of detecting decomposition or assessing quality, calibrated sensory experts are expensive to maintain and too few in number. More importantly, these experts are not the individuals who routinely make determinations as to the condition of the product at processing and distribution levels. The industry not only has difficulty training staff to be proficient and consistent in sensory assessment, but the need for continuous updating and recalibration, as well as the inevitable turnover in personnel, further complicates the problem. Alternative objective tests for the assessment of seafood freshness are complicated, take too long to produce results, require toxic chemicals and destructive sampling, are difficult to perform outside the laboratory, and/or are expensive in capital, equipment, or skilled labor.

The type of sensor developed here, sometimes called an "electronic nose" or "e-Nose", has been previously investigated by the seafood industry as a means of evaluating fish freshness.[2-8] However, the sensing methods used so far have some limitations: limited ability to have a diverse sensor element array that can effectively recognize the degree of diverse seafood spoilage; low sensitivity; signal processors that are limited by noise and signal resolution; humidity dependence that mask the signal from other analytes, critical for any seafood test; poor reproducibility; and need of special testing procedure (e.g., static or semi-dynamic headspace *vs.* "sniffing"), *etc*. These limitations were overcome by the e-Nose sensor described here. It combines: (i) an array-based chemical sensor, composed of incrementally different conducting polymer elements. The polymerization processes do not require a conductive substrate to deposit polymer films. The interaction of each element with a particular chemical generates an electrical signal, producing a "fingerprint". An e-Nose signal, composed by the contribution of the signals (i.e., changes of resistance) from different sensor elements, is collected and handled by a microprocessor, containing a simple algorithm relating e-Nose signals with seafood freshness. Analysis of the e-Nose signal may be accompanied by the evaluation of the corresponding fingerprint, which are unique for each stage of the seafood freshness. (ii) A specialized signal transduction system that allows the detection of very small signals (i.e., changes of 0.25 Ω in 1 MΩ signals), while avoiding problems related to noise and signal drifting. The signal transduction system is integrated into the microprocessor unit. (iii) The on-board microcomputer, custom-programmed into microprocessors. The operation of the sensor system, including sampling, sensor operation, signal transduction, data collection, freshness assessment, and result display is automated by the use of the microprocessors.

The spoilage of fresh seafood is a bacteriological phenomenon, and the chemical changes that take place are mainly due to bacterial activity.[9] In addition, studies have shown that there is a direct relationship between aerobic plate count and seafood quality (i.e., fresh *vs.* spoilage).[10-12] The aromas of fresh seafood are typified by certain groups of chemicals such as long chain alcohols and carbonyl compounds. However, during the early stages of deterioration, the concentration of these chemicals changes and new types of volatile aroma molecules appear. To detect these changes, the sensor described here used an array of elements of incrementally diverse sensitivity to volatile compounds. This type of sensor can also be used in many other food-processing areas. We have used this sensor for the assessment of freshness of three seafood species: Atlantic Farm-Raised Salmon (*Salmo salar*), blue tilapia (*Tilapia aurea*), and channel catfish (*Ictalurus punctatus*). In our test,

†This paper is dedicated to Professor Hasuck Kim for his outstanding contribution to electrochemistry and analytical chemistry.

no chemicals are required, it is not a destructive test, and it is an easy approach consisting on "sniffing" the seafood sample under study. The e-Nose signal (composed by the contribution of the signals from different sensor elements) was analyzed by a simple algorithm containing a database that allowed relating e-Nose signals to seafood freshness. This approach substantially simplifies data analysis, because traditional pattern recognition techniques used for analyte recognition are not needed.

Experimental Section

Chemicals. Acetonitrile (ACN), tetrahydrofuran (THF), $AgNO_3$, and pyrrole were purchased from Sigma-Aldrich (St. Louis, MO). Carbon black was purchased from Cabot (Boston, MA). A kit containing 44 organic polymers was purchased from Polyscience (Warrington, PA). Ammonia at 200 ppm in air was obtained as a gas cylinder from Matheson Trigas (Houston, TX). Trimethylamine at 200ppm in air was obtained as a gas cylinder from Matheson (LaPorte, TX). Zero air gas was purchased from Botco, (Bryan, TX).

Sensor substrate. The substrate for the sensor chip consisted of an alumina piece (2 cm × 1 cm) containing 16 interdigitated electrodes, IDEs (cf., Figure 1). Each IDE has an area of 1 mm × 1 mm, containing gold-coated 50 μm width lines with a 25 μm spacing. The substrates were fabricated by Zygo TeraOptix Co. (Westborough, MA). The sensor elements were deposited on top of the IDEs by hand-pipetting.

Sensor elements. Two approaches were used to prepare conducting polymer sensor elements. The first approach (described as "composite polymers") is based on mixing organic polymers with conducting carbon black. The second approach (described as "photopolymers") is based on Lynntech's photopolymerization procedure.[13,14] All these sensors were deposited on the substrate by hand-pipetting. Automated deposition could be done using a fast automated dispensing unit.

The basic formulation to prepare photopolymer sensor elements consists of mixing $AgNO_3$, acetonitrile (ACN), and distilled pyrrole (the "pre-polymer formulation"), and exposing an aliquot of the formulation under UV light for short time (usually 10 minutes). Organic polymers (OPs) were added to the pre-polymer formulation to provide diversity to the sensor elements. Polypyrrole is responsible for the electronic conduction in the conducting photopolymer. OPs, and polypyrrole are responsible for interactions with analytes. Mixing ratios were optimized to give a film a resistance value between 0.5 kΩ and 20 kΩ, and have minimal sensitivity to water, while being responsive to chemicals of interest (e.g., ammonia and trimethylamine).

The basic formulation to prepare composite polymer sensor elements consists of an organic polymer (OP), an electron acceptor/dopant (carbon black), and a solvent (ACN or THF).[15,16] The type of OP and the mixing ratio of carbon black to OP(s) were optimized to give a film a resistance value between 0.5 kΩ and 20 kΩ, and to have minimal sensitivity to water, while being responsive to chemicals of interest.

Both composite polymers and photopolymers sensors were screened. A total of 16 photopolymers and 22 composite polymers were tested. Based on the responses to certain chemicals associated with fish decomposition (specifically ammonia and trimethylamine), the polymers shown in Table 1 were chosen as the e-Nose sensor elements.

Sensor chamber. A chamber to hold the sensor chip with minimum dead volume was designed and fabricated. Grade 2 (40KSI-Yield Strength) titanium was used because it has high resistance to corrosive materials. In addition, the chamber components were gold-coated to provide greater corrosion resistance. The instrument also contains a preheating chamber, also made of

Table 1. Organic polymers selected for the e-Nose sensor elements

Photopolymers	Composite polymers
Poly (iso-butyl methacrylate)	Polycaprolactone
Polycaprolactone	Poly (ethylene/vinyl acetate) 82:18
Poly (styrene/maleic anhydride) 50:50	Poly (vinyl acetate)
Poly (4-vinylphenol)	Poly (4-vinylphenol)

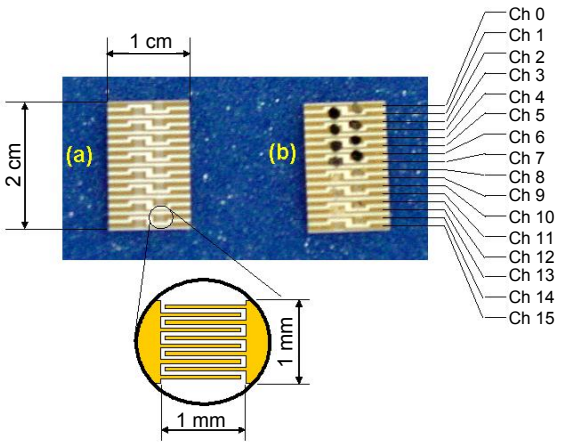


Figure 1. (a) A sensor chip made of alumina and 16 gold-coated IDEs. (b) The sensor chip after 8 photopolymers (Ch 0 - Ch7) and 8 composite polymers (Ch 8 - Ch15) have been deposited.

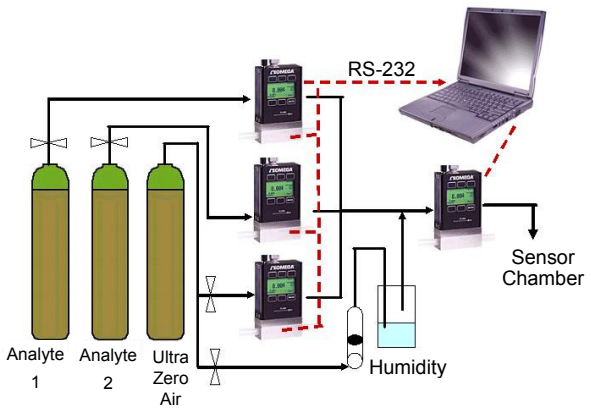


Figure 2. Experimental setup for delivering gas samples to the sensor chamber. The automated mixing system is shown, where 4 mass flow controllers were controlled by a computer.

titanium. It was designed to heat the incoming air before it reaches the sensor array.

Testing system. The testing system for gases consisted of four digital mass flow controllers, one digital flow meter (Omega Engineering, Inc., Stamford, CT), and one manual flow controller (Figure 2). A software program was developed to control the digital flow meter and flow controllers (i.e., flow rates and gas ratios). The program allows an automated/autonomous sequence of exposures and dilution. The operation of the gas handling system was controlled by a laptop computer. One of the mass flow controller was dedicated to zero air gas. Another mass flow controller was dedicated to ammonia gas. Another mass flow controller was dedicated to trimethylamine.

Using the ammonia and trimethylamine gas cylinders with appropriate gas regulators, 1/8" stainless steel tubing and Swagelok® connections, the cylinders were connected to the testing system. Dilutions to the required levels were done by mixing the gases with appropriate levels of air. Trimethylamine was analyzed using an HP5890 (Agilent Technologies, Palo Alto, CA) gas chromatograph (GC). Ammonia was analyzed using ion chromatograph (IC) using a DX-100 Dionex Corporation (Sunnyvale, CA) with a Dionex 4400 integrator. For that, the gas sample containing ammonia was purged (through a diffuser) inside a cylinder containing 10 mL 0.1 M sulfuric acid for 5 minutes at a flow rate of 30 mL/min. This procedure dissolved the ammonia gas into water by converting it into ammonium ions. Known standards were run through the IC before and after unknowns. The average peak areas for the standards were calculated and used as a reference to determine unknown concentrations.

Water vapor was generated as follows: the ultra zero air cylinder was connected to a digital mass flow controller and to a manual flow controller. The second controller went to a 1 L glass flask containing 500 mL of water. The 1 L glass flask was fitted with a rubber stopper with two holes. Two pieces of 1/8" SS tubing were inserted into the stopper. A flow meter was used to verify that no leaks were present in the flask assembly. The apparatus was assembled so that the water vapor could be directed either out into the fume hood or mixed with the air stream from the other gases (Figure 2). A SS tee was placed on the line from the flask to where it was joined with the air stream. The SS line going inside the flask containing water was immersed in the water to produce bubbling. A Hygro-Thermometer Pen (Extech Instruments, Waltham, MA) was used to measure the relative humidity (RH) of the flow coming out of the sensor chamber. Water temperature (and therefore, RH) was controlled by immersing the flask containing water into the reservoir of a Fisher Isotemp Heating Circulator (Fisher Scientific, Pittsburgh, PA). For example, with the water bath at 2 ^{o}C, a RH of about 31% was measured for a mixture of 10 mL/min humidified air, 42 mL/min drier air, and 8 mL/min trimethylamine.

Using the gas delivery system, representative concentrations of ammonia and trimethylamine in air (13, 27, 53, and 77 ppm) were generated and passed over the array at a flow rate of 60 mL/min, with a RH of about 30%. For each test, a baseline was obtained by flowing air over the sensors for at least 10 minutes. Then the flow meters were set to deliver the desired concentration of ammonia or trimethylamine gas for 1 minute at a total flow rate (air + chemical) of 60 mL/min. As the gas flowed over the sensor elements, their resistance values changed. The baseline resistance of the sensor was subtracted from the resistance at 1 minute to determine the e-Nose signal.

Electronics of the hand-held unit. The control system of the hand-held unit is based on the Motorola MC9S12DG256 (the main microcontroller). The board contains the microcontroller, serial port, real time clock, flash memory, 16 current sources, smart battery interface, humidity sensor, air pump control, and two 24 bit 8 channels analog-digital converter (ADC, the sensors reading channels) controlled by the microcontroller through the SPI (Serial Peripheral Interface). The microcontroller generates all the signals to read a 4 × 3 keypad from where the operator can control the prototype system. A 64 × 128 graphical display is used to display the status of the process or to set the parameters of the detection. Also one serial port is available to communicate with a computer. The database structure is flexible allowing adding data for new tests through a serial connection with a computer or directly from the keypad. In this fashion, a new database can be easily used to update or replace the existent one.

An analog PID (Proportional Integral Derivative) controller system was used to control precisely the temperature of the sensor chamber. It was set to control the chamber temperature at (30.0 ± 0.1) ^{o}C. In order to decrease thermal inertia, the mass of the sensor chamber was increased by 10% by adding an additional aluminum plate. Moreover, a preheating chamber was added, and then both chambers (preheating and sensor chambers) were insulated using ceramic cloth. The PID used a microchip 16F876A microcontroller, 5 V/30 mA + 10 V/1 A (heaters).

The instrument also has a Real Time Clock (RTC) to keep the date and time when the instrument is off. It also has a sampling air pump (Schwarzer Precision, Model 135FZ) that delivered 60 mL/min of gas. The pump was integrated to the circuit board on the unit.

The instrument uses a 10.8 V Li Ion Battery (NC2040HD) that allows for about 3 days of continuous use. Table 2 shows a calculation of the estimated power consumption for the hand-held unit. Performance of the unit can be completely carried out by microprocessors, and the energy consumption is 912 mWatt.

The hand-held unit. Figure 3 shows different views of the hand-held sensor. Its dimensions are 7" long, 3.85" wide, and 2" thick. Its weight is 1.38 lbs. The casing was made of ABS (acrylonitrile butadiene styrene) plastic and manufactured at Lynntech with a rapid prototyping machine. The enclosure sealing were tested. LCD (liquid crystal display) screen cover was tested to be water resistant. The control key pad (Digi-key) is a sealed membrane keypad with removable legends. It is splash

Table 2. Estimated power consumption of the hand-held unit

System Component	Duty Cycle (%)	Average Power (mW)
LCD Display	100	42
LED	0.4	0.04
Piezo Beeper	0.4	0.004
Sensor Temp. Control	5.5	100
Electronics	100	400
Pump	95	370
Total		912

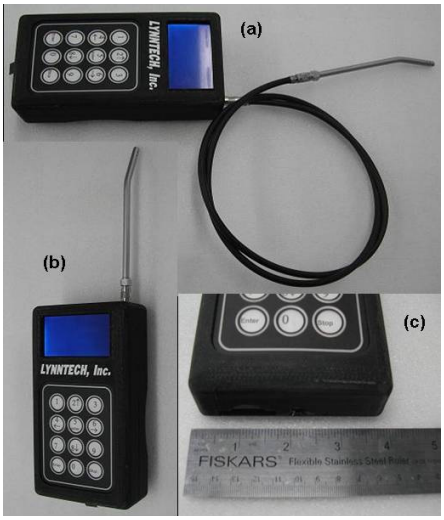


Figure 3. Hand-held sensor. In (a), a flexible wand was between the instrument and the rigid wand shown in (b). (c) Width dimension is shown.

proof, as certified by the manufacture, and has sealed switches and low profile height. The operating temperature is − 30 °C to + 60 °C, as certified by the manufacturer. The LCD display (Blue-white Apollo Display FC51553) has an operating temperature of: 0 °C to + 50 °C, as certified by the manufacturer. At the back of the unit there is a small window which provided access to a removable cartridge made of ABS plastic which houses the titanium sensor chamber with lid. The removable cartridge was engineered to make the process of installing the sensor array easy for the sensor user. The assembled cartridge was designed so that when inserted in its place, the electronic contacts and flow connections were automatically made. No other assembly by the user was required. The prototype also features a sample wand, a stainless steel piece with attached flexible plastic tubing which was easily connected to the sample inlet on the prototype casing (cf., Figure 3). The unit is thermally insulated. The materials used in the casing are all water resistant (i.e., the materials do not degrade when in contact with water), as specified by the manufacturers. The casing is waterproof (i.e., the water doesn't get inside the unit), except at the keypad join.

The prototype is an integrated system that is designed to:

- provide constant source currents for the 16 sensor channels,
- provide signal conditioning,
- read 16 channels with 24-bit accuracy,
- calculate the equivalent resistance for 16 channels,
- display the results,
- provide friendly user interface 12 keys input, display and sound,
- trigger sound or visual alarms,
- store/dump data continuously − up to 3 days.

Once the initialization process was ready, the PID control in the instrument brought the chamber temperature to the set temperature. Once the chamber temperature was at the set point, the end-user could start the data acquisition sequence. First, the type of seafood was selected by the end-user. Then, the data acquisition sequence started by acquiring the baseline for 1 min. The instrument acquired and saved the sensor signals at a rate of one reading per second. After that, the instrument waited for the end-user to indicate when it was time to acquire data related to seafood freshness. Once the end-user pressed the key to start the acquisition of the testing seafood, the instrument sampled for one minute (time can be reduced to a few seconds). All this data was stored in the instrument, and later analyzed with the appropriate software. In the data analysis, the data from the testing seafood was compared to the data from the baseline for each sensor element. This was done by comparing the average of 10 points close to one minute exposure to the seafood to the average of the last 10 points of the baseline, which increased the signal-to-noise ratio. By comparing the data with the database, information on the bacteria count was obtained, and therefore, the degree of spoilage of the tested seafood was known.

Testing seafood with the hand-held unit. Filets of Atlantic farm-raised Salmon (*Salmo salar*), blue tilapia (*Tilapia aurea*), and channel catfish (*Ictalurus punctatus*) (obtained from a local grocery store, or directly shipped overnight in a cooler box from a seafood warehouse) were used for testing. The salmon filets were evenly cut into sections using disposable scalpels and were placed into gallon-sized Ziploc® freezer bags using sterile forceps. The tilapia and catfish filets were stored in the same manner except that due to their smaller surface area, these fish were grouped into pairs. At various time points (0-Control, approximately 6, 12, 24, 48, 72, 100, 145, and 169 hours), e-Nose and microbiological testing (MBT) were performed. After testing was performed using the e-Nose hand-held unit, incisions of approximately 16 cm^2 were made on the surface of the filets (flesh side) using sterile forceps and disposable scalpels to achieve 5 - 10 gram samples for MBT.

About thirty minutes before opening the container of the fish to be sampled, the prototype was turned on, the pump speed was set to be 60 mL/min at the outlet of the sensor chamber, and the temperature of the sensor chamber was set at 30 °C. For the initial test of each seafood experiment carried out when the fish samples arrived at the lab, the box was opened and the inner plastic bag was opened just enough to insert the sampling wand from the hand-held unit, and place it approximately 1/8 of an inch from the fish tissue. The fish odor was sampled in this way for 1 minute. After this initial test, the box was immediately taken to the microbiology lab, where a piece of the fish fillet was aseptically removed from the bag and cut into pieces of similar shape and weight. Then, each fillet piece was placed in a separate, sealed 1 gallon Ziploc® bag and placed in the refrigerator, which was kept cooled between 1° and 5 °C. At this initial time, a surface cut of the fish tissue was taken for microbiology analysis. Then, at each time point, a bag was removed and tested with the prototype as described previously. To generate more data, after testing with the hand-held unit, the bag was resealed and placed in the refrigerator for 10 minutes. Then the bag was tested with the hand-held unit again. This was repeated to have a total of 4 hand-held unit data points at each testing time. After prototype testing, the fish was taken to the microbiology lab for the total plate count analysis. After the surface cuts were made, the fish was thrown away. In this way, the five bags originally stored provided fish samples to test on 5 different days.

For the catfish test, the fillets were divided evenly between

two bags and stored in the refrigerator. Enough fillets were placed in each bag so that multiple surface cuts could be made without cutting the same area twice. At each time point, Bag 1 was removed and opened just enough to insert the sampling wand and place it approximately 1/8 of an inch from the fish tissue. The fish odor was sampled in this way for 1 minute. Bag 1 was then taken to the microbiology lab where surface cuts were made for the total plate count. The fillet was then returned to the bag, and it was resealed and placed back in the refrigerator. After waiting 10 minutes for sensor recovery, this procedure was repeated for Bag 2. This entire procedure for Bag 1 and Bag 2 was repeated at each time point.

Microbial analysis. For microbiological analysis, the filet samples were aseptically transferred using sterile forceps to separate 7.5 × 12 in. filtered Whirl Pak® bags. To the bags, sterile phosphate buffered saline (PBS) was added four times the amount of the weight of the filet sample. The Whirl Pak® bags containing the samples were placed in a Masticator® for two, one-minute cycles to homogenize the samples. The liquefied and blended filet samples were extracted into sterile 50 mL Falcon tubes to be serially diluted into PBS and plated onto Marine Agar 2216, which is used for cultivating heterotrophic marine bacteria. The agar plates were left to incubate at room temperature for two to four days and were then counted to determine the bacterial surface count of the filet samples.

Results and Discussion

Detection of chemicals related to seafood freshness/spoilage by the e-Nose sensor elements. Typically, the aroma of fish passes through a series of changes during storage. These changes include the suppression of the fresh fish aroma, development of sweet fruity aromas that are eventually accompanied by stale and sour aromas, and finally, culminating in spoiled putrid and rotten aromas. There have been several studies of the volatile constituents contributing to the characteristic odors of fish products.[17] Although the mixtures of chemicals in odor are fairly complex, there are distinct families of compounds that are responsible for the aromas of fresh fish. These included C6 aldehydes and alcohols that contribute the green plant-like aromas, for example: 2-hexanal and 1-hexanol. The C9 aldehydes and alcohols that contribute the melon-like aromas were, for example: 2-nonenal, 2,6-nonadienal, 6-nonen-1-ol, and 3,6-nonadien-1-ol. The C8 ketones and alcohols contribute characteristic mushroom-like aromas, for example: 1-octen-3-one, 1,5 octadien-3-one, 1-octen-3-ol, 1,5-octadien-3-ol, 2-octen-1-ol and 2,5-octadien-1-ol. Significantly, decreasing concentrations of some carbonyls (e.g., 1,5 octadien-3-one, and 2,6 nonadienal) and increasing amounts of corresponding alcohols (e.g., 1,5-octadien-3-ol and 3,6-nonadien-1-ol) appeared to signal the change in aroma quality from that of very fresh whitefish to aromas that are suppressed and flat and sweet melon-like. Later stages in the deterioration are associated with the appearance of carbonyls (e.g., acetone and 2-butanone), sulfur (e.g., hydrogen sulfide and methyl mercaptan, dimethyl sulfide) and nitrogen compounds (e.g., ammonia and trimethylamine).

We selected two chemicals related to seafood spoilage (ammonia and trimethylamine) and exposed it to different sensor elements. Using the gas delivery system described in the Experimental Section, concentrations of 13, 27, 53, and 77 ppm ammonia and trimethylamine in air were generated and passed over the array at a flow rate of 60 mL/min, with a RH of about 30%. Figure 4 shows the linearity of the e-Nose signal versus chemical concentration for examples of the e-Nose sensor elements.

Preliminary testing of the hand-held unit. Before beginning the seafood assessment experiments, the temperature control of the hand-held unit and the noise in the 16 sensors (when at 30 °C) was tested. Heating was done by the PID controller with integrated circuit temperature sensors inside the preheating chamber and the sensor chamber. It was thought that this would minimize the noise level and improve the reproducibility of the baseline resistance of the sensors. For the first test, the prototype was operated in a normal room temperature environment and both chambers were heated to 30° C. The prototype was turned on at time zero, and then the PID controller took about 16 minutes to stabilize the sensor chamber at 30 °C. Once stabilized, the actual temperature in the sensor chamber was constant at (30.8 ± 0.1) °C. For the next test, the prototype was turned on and then immediately placed inside a refrigerator at 1 °C. Again, both chambers were heated to 30 °C. As shown in Figure 5b, the sensor chamber temperature was maintained at (30 ± 0.5) °C for more than one hour in the cold environment.

Once the sensor reached the set temperature, the baseline resistance of the sensor elements was very stable, even though the air pump was running so air was constantly flowing over the sensor elements. Also, no significant noise was observed from the operation of the PID controller. The sensor array was composed of eight photopolymer sensors and eight composite sensors, each deposited two times.

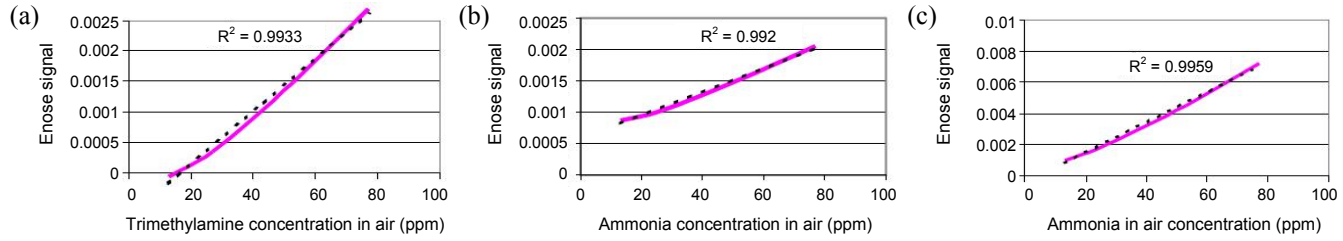


Figure 4. Response of three e-Nose sensor elements - (a) poly(vinyl acetate), (b) poly(caprolactone), and (c) poly(iso-butyl methacrylate) - to either (a) trimethylamine or (b,c) ammonia. The e-Nose signal was obtained from R/R_o-1, where R and R_o are the resistance of a sensor at a certain time and at the initial time, respectively.

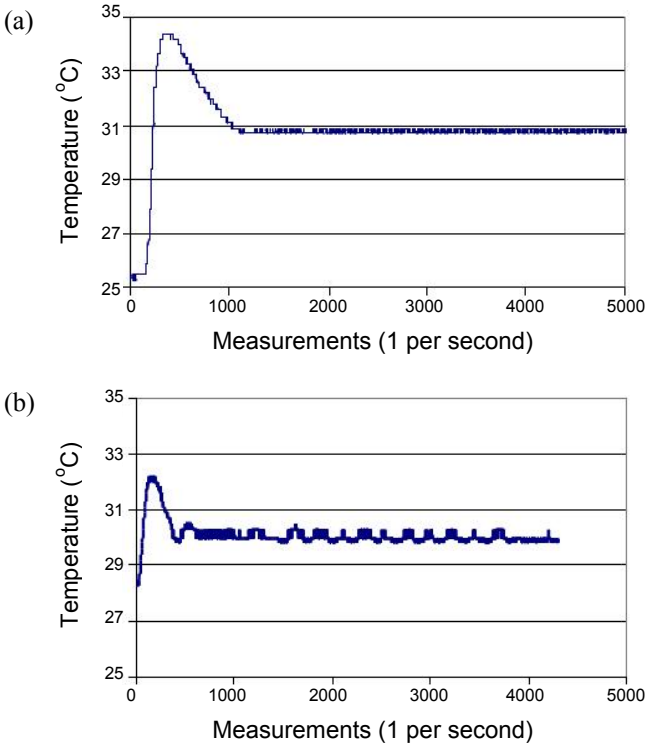


Figure 5. Temperature reading of the prototype sensor chamber. Temperature set point was 30 °C, and the prototype was (a) in a room at 25 °C. (b) in a refrigerator at 1 °C.

Testing of the hand-held unit.

Seafood quality and MBT: The spoilage of fresh seafood is a bacteriological phenomena, and the chemical changes that takes place are mainly due to bacterial activity.[9] The decomposition rate is influenced by the initial number and types of bacteria and the storage conditions such as temperature, humidity, and gaseous atmosphere. One distinct difference between the compositions of seafood as compared to other proteinacious foods such as poultry or meats is the high concentration of low molecular weight organic nitrogen compounds in seafood. The delicate palatability of fish and shellfish originates primarily from these compounds.[18,19] The most common classes of these low molecular weight compounds are free amino acids, short peptides, nucleotides, urea and quaternary ammonium bases. All these readily available compounds serve as substrates for most types of microorganisms which will utilize them as source of energy for growth. The volatile waste products created by this activity is what consumers identify as spoilage or decomposition.

Studies have shown that there is a direct relationship between aerobic plate count and seafood quality (i.e., fresh *vs.* spoilage). For example, it has been reported that fresh fish has initial microbial counts of 10^2 - 10^3 cfu/cm^2 whereas the microbial counts could be 10^4 - 10^6 cfu/g when they arrive at the processing plant.[10] Finne et al. reported that for white (*Penaeus setiferus*) and pink (*Penaeus duorarum*) shrimp, a bacterial count of 10^5 cfu/g represents good but not prime quality,[11] whereas a count above 10^7 cfu/g represents spoiled shrimp (quality confirmed by TVN (total volatile nitrogen) and TMA (trimethylamine)).

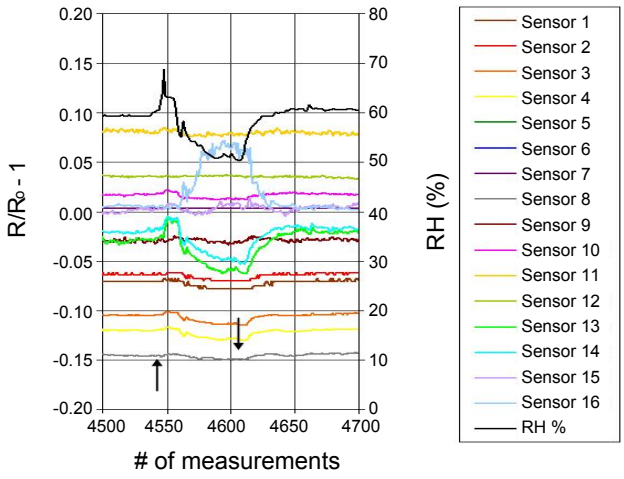


Figure 6. Typical responses of the hand-held prototype unit. Signals at the sixteen sensor elements and humidity sensor were recorded as the prototype unit sniffed headspace vapor of a bag containing Salmon fillets for a minute.

The recommended microbiological limits for seafood according to ICMSF (International Commission on Microbiological Specifications for Foods) for a 3-class sampling plan of fresh and frozen seafood is the following: 5×10^5 cfu/cm^2 of cfu/g separates acceptable counts from marginally acceptable counts, and 10^7 cfu/cm^2 of cfu/g is the boundary between marginally acceptable counts and unacceptable counts.[12]

Although MBT and TVN are analytical tools that can quantitatively indicate the seafood freshness stage, they are time consuming, require trained technicians, do not provide real time information on the seafood freshness, and therefore, are unsuitable as a rapid assessment method.

Thus, if the e-Nose signal can be related to the microbial count, the e-Nose signal can be used as a rapid, real-time method to provide assessment on the seafood freshness/spoilage stage.

Seafood testing. Figure 6 shows the typical responses of the prototype unit with 16 sensing elements and a relative humidity sensor. The responses were obtained from R/R_o-1, where R and R_o are the resistance of a sensor element at a certain time and at the initial time, respectively. Changes can be observed when the hand-held unit "sniffed" the seafood sample. At 4545th measurement (marked with an up-arrow), the prototype started "sniffing" headspace vapor of a bag containing Salmon fillets. The "sniffing" stopped at 4605th measurement (marked with a down-arrow).

For microbiological count (MBT) testing of Salmon fillets, two sets of data were collected: "warm" for which bags of Salmon fillets were left at room temperature for accelerated decay and "cold" for which bags of Salmon fillets were stored in a refrigerator. Figure 7 shows the accelerated decay of the Salmon fillets at room temperature, compared to the delayed decay in a refrigerator. Correlation of the microbiological count (MBT) values with e-Nose signals, where MBT can be used to represent freshness of the Salmon fillets. The correlation coefficient of the MBT values (X) and signals at each e-Nose sensor element (Y) was calculated using the equation:

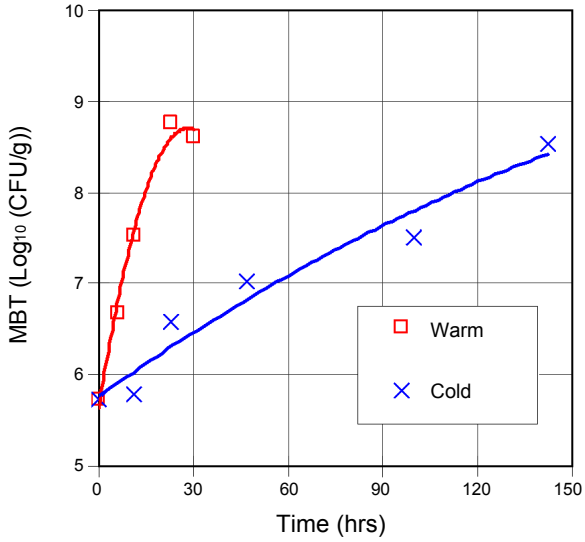


Figure 7. Microbiological count (MBT) values of Salmon fillets stored in "warm" and "cold" conditions.

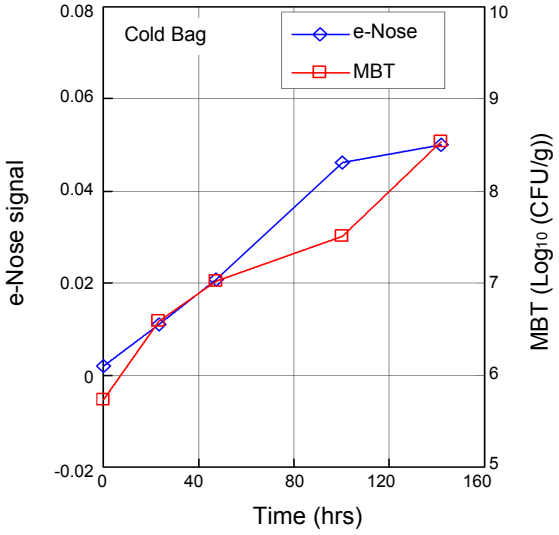


Figure 8. e-Nose signal (Eq. (3)) and the microbiological count (MBT) values for the Salmon sample stored in "cold" conditions as function of time.

$$Correl(X,Y) = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2\sum(y-\bar{y})^2}} \quad (2)$$

Sensor channels with correlation coefficients greater than a threshold value and signals within the range of 0.001 to 0.01 were selected to establish a linear relationship between the MBT and e-Nose signal for each type of seafood tested. For each seafood species, a correlation between the MBT and the e-Nose signal was identified by a mathematical equation. This equation allowed us to predict later MBT values (and therefore, seafood freshness assessment) once the e-Nose values were measured and determined.

Atlantic farm-raised salmon (Salmo salar): For data analysis, signal from sensor elements were considered if their combination could reach a threshold value of at least 0.45, for a good signal to noise ratio. For "cold" Salmon fillets, channels 1, 2, 3, 4, 8, 9, and 10 were used, and the eNose signal was defined as:

$$\text{e-Nose} = -(\text{ch1} + \text{ch2} + \text{ch3} + \text{ch4} + \text{ch8} + \text{ch9} + \text{ch10}) + 0.03 \quad (3)$$

with the resulting correlation coefficient of 0.95 for the linear relationship with the MBT values. Figure 8 shows how close the defined eNose signals for Salmon fillet matched the MBT values. For the "cold" case, using the least square method, we further developed a linear relationship between the microbiological count and eNose signal as:

$$\text{MBT} = 51.1 \times \text{e-Nose} + 5.72 \quad (4)$$

Figure 8 shows the close agreement between MBT and e-Nose values. By the equation (4), the microbiological count method, which is accurate and reliable but time-consuming (and does not provide real-time information), can be substituted by the e-Nose method, which is simple, easy-to-use, and provides real-time information.

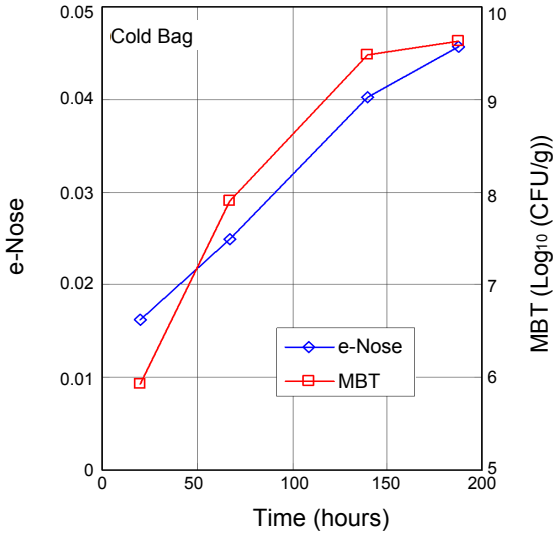


Figure 9. e-Nose signal (Eq. 6) and the microbiological count (MBT) values for the Catfish fillet samples stored in "cold" conditions as function of time.

We later used equation (4) to predict the freshness of Salmon fillet.

Channel catfish (Ictalurus punctatus): A similar approach was taken for Catfish fillet. Here, four channels were selected to calculate the e-Nose signal, which had the correlation coefficient of 0.97 for the linear relationship (see Figure 9) with MBT:

$$\text{MBT} = 123 \times \text{e-Nose} + 4.34 \quad (5)$$

where

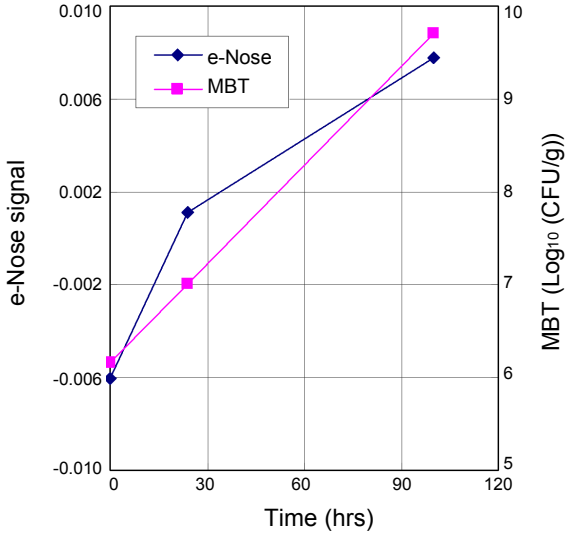


Figure 10. e-Nose signal (Eq. 8) and the microbiological count values for Tilapia fillet samples stored in "cold" conditions as function of time.

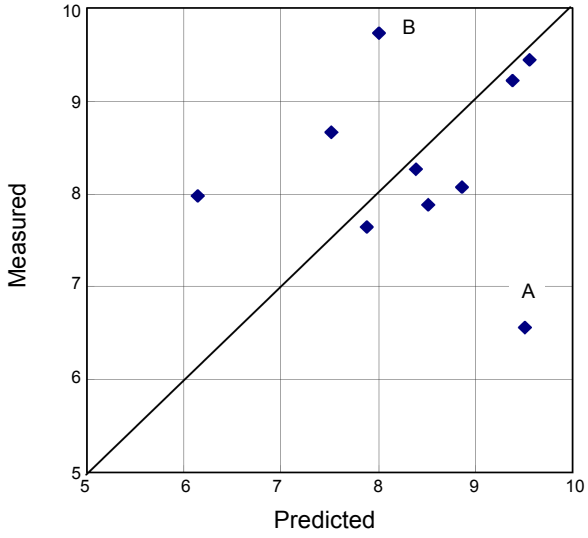


Figure 11. Correlation between MBT values measured and predicted using e-Nose signals for Salmon fillets in "cold" condition. The log number of the MBT values is plotted.

$$\text{e-Nose} = \text{ch8} + \text{ch9} - \text{ch11} + \text{ch12} + 0.03 \quad (6)$$

Blue Tilapia (Tilapia aurea): The third species we tested was Tilapia fillet. Again, a similar approach was taken for Catfish fillet. Here, four channels were selected for the e-Nose signal, which had the correlation coefficient of 0.61 for the linear relationship (see Figure 10) with MBT:

$$\text{MBT} = 250 \times \text{e-Nose} + 7.39 \quad (7)$$

where

$$\text{e-Nose} = -\text{ch7} + \text{ch9} + \text{ch11} + \text{ch12} \quad (8)$$

As a summary, we developed a linear relationship between microbiological count technique and e-Nose approach for three different types of fish fillets. The e-Nose signal components were selected based on the correlation coefficient of MBT to signal at each sensor element. Once the linear relationship is included into the hand-held unit software, the e-Nose signal can asses the freshness of those fish fillet without performing the microbiological count technique.

Seafood freshness assessment.

In house testing: After the linear relationship between e-Nose signal and MBT for Salmon fillet was established, more Salmon fillets were acquired to predict the seafood freshness. This in-house prediction was performed in "cold" condition and MBT was also performed after the e-Nose testing for each Salmon sample. MBT values for the samples were predicted using Equation (4) and the actual MBT values were considered as reference values. As shown in Figure 11, the e-Nose signal predicted MBT values close to the actual MBT measured values except one false-positive (marked as "A" in the Figure) and

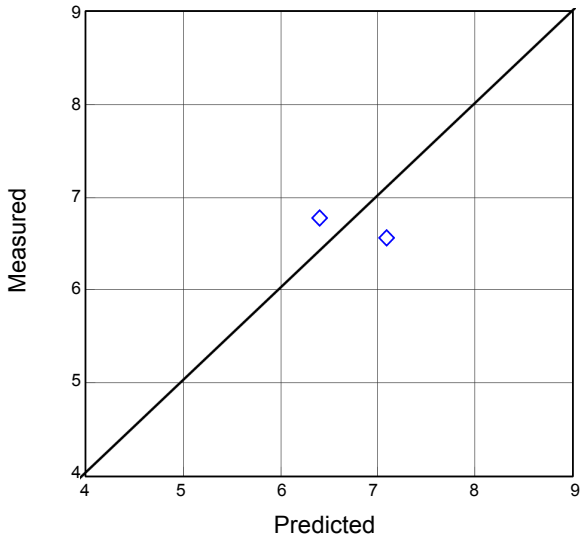


Figure 12. Correlation plot between MBT values measured and predicted using e-Nose signals for Salmon fillets at the seafood distribution center.

one false-negative (marked as "B" in the Figure), for a boundary line of at 10^9 for the MBT value.

Field test: The hand-held unit was taken to the seafood warehouse, where the environment was about 2 °C and 35 - 40% RH. The seafood quality assurance (QA) manager had set aside two boxes of farm raised salmon fillets. These boxes had not been previously opened. Once inside the warehouse, the prototype was turned on and heated to 30 °C. The heating was necessary because the prototype should operate at the same temperature in the laboratory and in the warehouse. The database used for establishing linear relationship had been collected at 30 °C. The data recording function was also started so that all data could be fully analyzed at a later time. The e-Nose heated to the set point in about 5 minutes and was then allowed to run

for another 5 minutes. Then, a box containing the fish was opened − the inner plastic bag was opened just enough to insert the sampling wand to a within approximately 1/8 of an inch from the fish tissue. It was sampled for 1 minute. After waiting 5 minutes for sensor recovery, the next box was tested in the same manner. One test was performed for each of the fish boxes. According to the two warehouse QA managers present, all boxes of fish appeared to be of good quality. One fillet from each box was packed in an ice chest and transported to Lynntech's microbiology lab for analysis and arriving 3.5 hours after the e-Nose test. A surface cut from each fish was made to determine a total plate count, as described in the Experimental Section.

Using equation (4), the MBT values were predicted and compared with actual MBT values (Figure 12). The prediction for Salmon fillets was very close to the actual count. Analysis of data collected by the handheld sensor correctly predicted the MBT count within a 10% error. As a summary, we could successfully demonstrate that the hand-held seafood freshness prototype could be used in the field to quickly predict MBT values and provide seafood freshness assessment.

Conclusion

We have developed an automated, hand-held sensor for the fast assessment of seafood freshness. The sensor combined an array-based chemical sensor, composed of incrementally different conducting polymer elements deposited on a small chip; a highly sensitive, custom-made electronics for the detection of very small signal changes; precise temperature control of the sensor chamber; and an on-board microcontroller for data collection, storage, automation, and analysis. The energy requirement of the hand-held unit is less than 1 Watt. Testing demonstrated the feasibility of using the hand-held e-Nose unit for the assessment of seafood freshness. The instrument was used to successfully test seafood samples with different degree of freshness and spoilage. A linear relationship between microbiological count and e-Nose signal for three different fish fillet (Atlantic farm-raised salmon (*Salmo salar*), blue tilapia (*Tilapia aurea*), and channel catfish (*Ictalurus punctatus*)) was developed. The linear relationship was used to predict the microbial count of salmon fillet samples, which can then be used to assess the samples freshness.

Acknowledgments. This study was supported by the U.S. Department of Agriculture through Small Business Innovative Research (SBIR) Phase II Contract No.: USDA- 2003-33610-14014. We also would like to thank Leigh Justice for her help conducting microbiological testings.

References

1. Marshall, D. L.; Wiese Lehigh, P. L. *Chemtech* **1993 Oct**, 38.
2. Olafsson, R.; Martinsdottir, E.; Olafsdottir, G.; Sigfusson, P. I.; Gardner, J. W. In *Sensors and Sensory Systems for an Electronic Nose*; Garder, J. W., Barlett, P. N., Eds.; Kluwer Academic Publishers: 1992; p 257.
3. Deng, Z.; Stone, D. C.; Thompson, M. *Analyst* **1996**, *121*, 671.
4. Di Natale, C.; Macagnano, A.; Paolesse, R.; Mantini, A.; Tarizzo, E.; D'Amico, A.; Sinesion, F.; Bucarille, F. M.; Moneta, E.; Quagilia, G. B. *Sens. & Actuator* **1998**, *50*, 246.
5. Olafsdottir, G.; Martindosttir, E.; Jonsson, E. H. *J. Agric. Food Chem.* **1997**, *45*, 2654.
6. Ghosh, H.; Sarker, D.; Misra, T. N. *Sens. & Actuator* **1998**, *53*, 58.
7. Du, W.-X.; Lin, C.-M.; Huang, T.; Kim, J.; Marshall, M.; Wei, C.-I. *J. Food Sci.* **2002**, *67*, 307.
8. Chantarachoti, J.; Oliveira, A. C. M.; Himelbloom, B. H.; Crapo, C. A.; Mclachlan, D. G. *J. Food Sci.* **2006**, *71*, S414.
9. Shewan, J. M. In *Handling, Processing and Marketing of Tropical Fish*; Sutcliffe, P., Disney, J., Eds.; Tropical Product Institute: London, 1977; p 51.
10. Acuff, G. R. *Food Bacteriology Lecture Notes*, 10th Revision, Texas A&M University, Department of Animal Science, Food Science & Technology, College Station, TX, 2000.
11. Chang, O.; Cheuk, W. L.; Nickelson, R.; Martin, R.; Finne, G. *J. Food Sci.* **1983**, *48*, 813.
12. Huss, H. H. *Assurance of Seafood Quality*; FAO Fisheries Technical Paper 334, Food and Agriculture Organization of the United Nations, Rome, FAO, 1994.
13. (i) Murphy, O. J.; Hitchens, G. D.; Hodko, D.; Clarke, E. T.; Miller, D. L.; Parker, D. L. *US Patent* #5,545,308, 1996; (ii) Murphy, O. J.; Hitchens, G. D.; Hodko, D.; Clarke, E. T.; Miller, D. L.; Parker, D. L. *US Patent* #5,855,755, 1999; (iii) Murphy, O. J.; Hitchens, G. D.; Hodko, D. *US Patent* #5,919,402, 1999; (iv) Murphy, O. J.; Hitchens, G. D.; Hodko, D.; Clarke, E. T.; Miller, D. L.; Parker, D. L. *US Patent* #5,948,232, 1999; (v) Murphy, O. J.; Hitchens, G. D.; Hodko, D.; Clarke, E. T.; Miller, D. L.; Parker, D. L. *US Patent* #6,210,537, 2001.
14. Gonzalez-Martin, A.; Kim, J.; Jabs, H.; Solar, A.; Rutherford, L. A., Jr.; Westerheim, D. *US Patent* #6,994,777, 2006.
15. Lonergan, M. C.; Severin, E. J.; Dolema, B. J.; Beaber, S. A.; Grubbs, R. H.; Lewis, N. S. *Chem. Mater.* **1996**, *8*, 2298.
16. Freund, M. S.; Lewis, N. S. *Proc. Nat. Acad. Sci. USA* **1995**, *92*, 2652.
17. Josephson, D. B.; Lindsay, R. C.; Stuiber, D. A. *J. Agric. Food Chem.* **1983**, *31*, 326.
18. McCoid, V. R.; Miget, R.; Finne, G. *J. Food Sci.* **1984**, *49*, 326.
19. Papadopoulos, L.; Finne, G. *J. Food Sci.* **1986**, *51*, 812.