# Electronic Nose for Rapid Detection of Food Borne Pathogens in Meats.

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### Summary and Implication

The AromaScan electronic-nose detects volatile chemicals with an array of semi-conducting polymer sensors which enable the user to map aromas in a graphic and digital format. The goal of this research is to explore the use of an electronic nose for rapid detection of food spoilers and pathogens via development of a standard curve of some potential volatile compounds that can be used to develop some specific aroma-labeled substrates similar to the MUG assay for indicator organisms and pathogen detection. The test system was ground pork that was mixed with a diluent, homogenized with a stomacher then incubated at 37°C with 30% RH. The stomacher bag was connected to the AromaScan electronic nose via teflon tubing which as held in a column heater (35°C), through a teflon syringe filter to prevent contamination of the sensors. The AromaScan incubator was held at 35°C and 30% RH. Benzaldehyde was the aroma compound which illustrated the best response as low as 91 ppb. However, to be workable as a rapid method for detection of pathogens this aroma labeled compound must be detectable at part per trillion.

### Introduction

The electronic-nose detects volatile chemicals with an array of semi-conducting polymer sensors which enable the user to map aromas in a graphic and digital format. The department of Food Science and Human Nutrition has an AromaScan electronic nose with 32 sensors. Samples can be evaluated in 10 to 45 minutes. The unit operates a software package referred to as a neural network in which a library of patterns is stored and named for known samples. For example, an aroma pattern for fresh ground beef and one for 1-, 2-, and 3-day old spoiled beef can be stored and named. Therefore, when an unknown sample is presented to the AromaScan 32 sensors the pattern is determined, compared with the library, then declared fresh or spoiled for a specific number of days. The research questions for this grant is: (1) Can the electron nose be trained to identify specific microbial contaminates in different meat products and (2) Can specific rapid assays for specific microorganisms be developed with the use of aroma-labeled substrates aimed at a specific enzymes associated with the indicator or pathogen of interest?

The goal of this research is to explore the use of an electronic nose for rapid detection of food spoilers and pathogens. The specific objectives are to (1) build a library in the AromaScan Electronic Nose software for detection of spoiled foods, specific pathogens, and pathogens in specific meats; and (2) develop a standard curve of some potential volatile compounds that can be used to develop some specific aroma-labeled substrates for indicator organisms and pathogen detection.

### Materials and Methods

*Electronic nose.* An AromaScan electronic nose unit (AromaScan Inc., Crewe, UK) was empolyed. The unit operates with a sensor array containing 32 different polymer sensors. Each sensor has a different void geometry that responds to the specific volatile compounds adsorption and desorption rates at equilibrium. Samples are typically added to sampling bags or bottles, allowed to equilibrate to a specific temperature (i.e., 30°C), then the headspace is flushed into the sensor chamber at a specific flow rate and time. Variability between samples needs to be determined and protocols standardized. Training of the neural network software requires evaluating 25 replicates for each treatment. The computer then stores each electron response pattern for each sensor in its library for future reference and comparisons to unknown samples. Once the neural network has been trained and the library built the identification of contaminated unknowns should take from 15 to 45 minutes.

*Materials.* Benzaldehyde >99%, *p*-hydroxybenzaldehyde, syringaldehyde, and vanillin were purchased from Aldrich Chemical Company, Inc. ( Milwaukee, WI). The meat used was Jimmy Dean Sausage (Jimmy Dean, Inc.), which was purchased from a local supermarket and stored frozen. All frozen sausage samples were thawed at 4°C overnight.

*Sample preparation.* Five grams of meat sample, a specific volume of benzaldehyde (5, 10, 15, 20, 25 µl) and 50 ml of deionized water at room temperature were put in Stomacher 400 closure bags and blended using a stomacher homogenizer (Model #BA6021, distributed by Unique Scientific Apparatus, Tekmar Company, Cincinnati, OH) for 5 seconds. The bags were prefitted with 20 mm red-silicone septa (Wheaton, Millville NJ) placed near the top. Septa was secured to bag by a piece of 3M Corporation mailing tape (5.1 X 5.1 cm) (St. Paul, MN). All contact surface between the bag and septa must be airtight, otherwise some of the air will leak out while the bag is equilibrating in the water bath. The bags were heat sealed using a Micro-seal (Model #MS-1, Dazey Corporation, KS). A 16-gauge hypodermic needle with lurloc (3.8 cm long) was used to fill the bag with air and to take headspace sample for analysis. The needle was connected to the bag fill valve of the Aroma Scan sampling station (Figure 1). The need was puncher through the septa into the sample bags, taking care not to

push through the opposite wall of the bags. The bags were filled with 30% RH air for 60 second (approximately 300 ml). Filled bags were placed in a 37 ± 2°C water bath and the temperature was allowed to equilibrate for 30 minutes.

*Instrument preparation.* Room temperature of the Aroma Scan room was kept at 25 to 30°C. The analyzer part of the machine was warmed for at least 1 hour, and the sampling station was warmed for 15 min. prior to using the conditioned air to fill the sampling bags, and for headspace sampling. Table 1 describes the Aroma Scan conditions optimized to produce the maximum sensitivity.

**Table 1. Optimum Aroma Scan setting for meat sample analysis.**

| | |
|---|---|
| Bag air humidity, % | 30% |
| Reference air humidity, % | 30% |
| Incubator (Aroma Scan) temp., °C | 35 |
| Water bath temperature, °C | 37 |
| Column heater temperature, °C | 35 |
| Room temperature, °C | 25 to 30 |
| Aroma Scan warmed-up time | at least 1 h |
| Aroma Scan sampling station warmed-up | 15 min |

*Sampling.* Stomacher bag with meat, diluent, and ~300 ml of 30% RH air in a 37°C water bath was connected to the Aroma Scan sampling station via Teflon tubing (2 mm in diameter) in a 35°C column heater (Figure 2). A 25-mm, 0.22-μm Teflon syringe filter (Cameo 25F, MSI, Inc., Westborough, MA) was located between the sample bag and the Teflon tube and the hypodermic needle to prevent aerosolized materials from contaminating the Aroma Scan. Table 2 describes the program settings used in the experiment. After each sample a 2% isopropyl alcohol vapor was passed over the sensors to wash or remove volatile buildup.

**Table 2. Aroma Scan data acquisition conditions.**

| | |
|---|---|
| Reference air | 10 sec |
| Sample | 180 sec |
| Wash | 10 sec |
| Reference air | 180 sec |

*Data handling (data manipulation).* The intensity response values obtained from each of the sample bags were exported into spread sheet (MicroSoft Excel) where they were analyzed using scatter graph.

## Results and Discussion

*Aromatic compounds.* Benzaldehyde was the aromatic compound that was detectable by the electronic nose. *p*-Hydroxybenzaldehyde, syringaldehyde, and vanillin were detectable at very high concentrations, and only in the absence of meat product. Therefore, all further analysis was with benzaldehyde.

*Detection levels.* A 5-μl sample of benzaldehyde was the lower limit of detection by the Aroma Scan sensors under these conditions. A disadvantage of this particular setup is the volume of gas needed to flush the environment from the stomacher bag, through the tubing, and into the sensor chamber. Heating the tubing to 35°C ensured no change in relative humidity of the air sample during headspace analysis.

For these proposed applications of the electron nose to detect food pathogens via aroma-labeled substrates, parts per trillion of aromatic compound would need to be detectable. Table 3 presents the concentrations of benzyaldehdyed detectable by the Aroma Scan in the presence of ground pork. Figure 2 demonstrates the different sensor values during each analysis. Each curve represents a replicate of three samples. A μl sample (18 ppb) might be detectable if a smaller volume of air could be used to flush the headspace for analysis.

**Table 3. Concentration of benzyaldehyde detectable by the Aroma Scan electronic nose.**

| Aromatic compound sample volume (μl) | Concentration of aromatic compound in stomacher bag (ppb) |
|---|---|
| 5 | 91 |
| 10 | 119 |
| 15 | 273 |
| 20 | 364 |
| 25 | 454 |

[a] Calculations are based on 50 ml of diluent with 5 g of ground pork homogenized in a stomacker for 5 min, which is equivalent to 55 ml in total volume.

*Bottom line:* Based on the information collected several unknowns are still present.

First, instead of having an array of potential aroma compounds to evaluate we have identified the best sensors for benzaldehyde (Sensor No. 3, 11, 16, 17, 21, 22, and 23).

Second, until the benzaldehdye glucuronide is chemically synthesized we do not know if it will be hydrolyzed by the enzyme, _-D-glucuronidase (GUD) releasing the benzaldehdye for detection by the Aroma Scan.

Third, the aroma labeled substrate itself might be detected by the electronic nose preventing differences from being measured, which would limit the concentration of substrate that can be added to the detection procedure.

Fourth, this benzaldehdye glucuronide also must be able to enter the cell, be hydrolyzed, and then leave the cell to be detected by the Aroma Scan.

Finally, because of these limitations, detection levels in the parts per trillion might be needed.

Therefore, many unknowns still exist. In principal the idea has merit and promise. More research, however, is required before a definitive answer to this question can be provided.

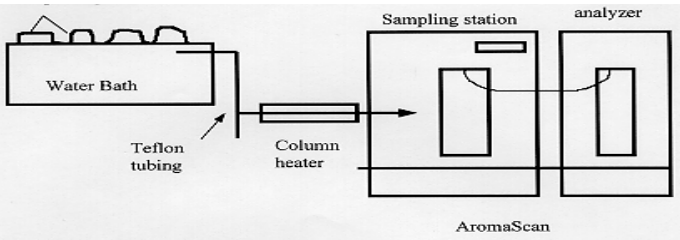


**Figure 1** - Sampling set up

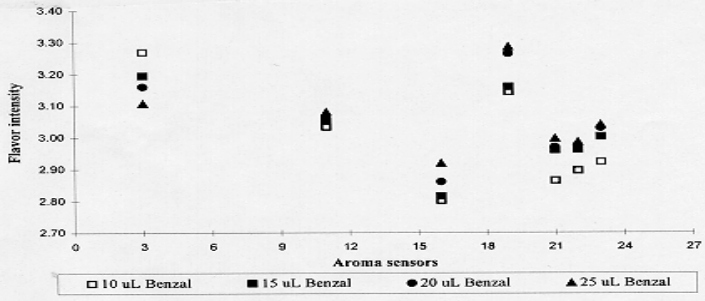


Figure 2. Intensity responses by the Aroma Scan sensors to benzaldehyde conecentration in Jimmy Dean sausage. Note: the averages of three replication with the background flavor subtracted out are presented.