Electronic Noses: What Are They and What Are Their Potential Uses in Meat Science

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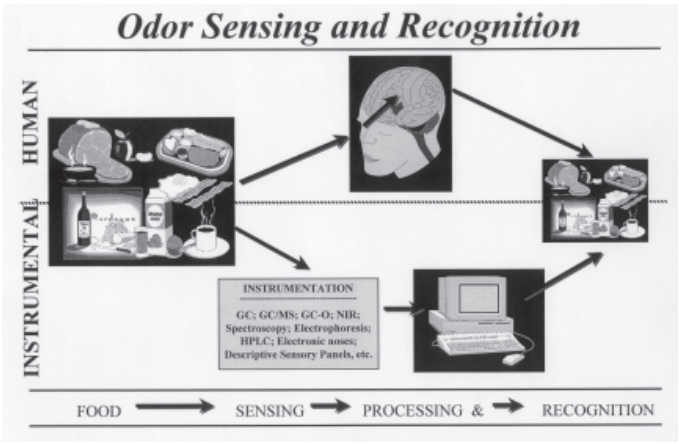
Introduction

The food industry is always in search of rapid means of monitoring the flavor quality of their products. Monitoring food flavor is typically based on analysis of the hundreds of different odorous molecules that comprise the product's specific odor. However, while humans can typically detect odors in the parts per trillion (ppt) range, attempts to instrumentally detect the complex odors at levels that may be below the detection limits of conventional analytical techniques, are very expensive, and are not always possible. Furthermore, most of the instrumental techniques can objectively discriminate odors, but in most cases the sample must be separated into its individual components.

The human system of receptors senses food primarily by reception and transmission along odor and taste sensing neurons (sensors), and to various degrees, by other visual, auditory, and tactile (mouth feel, sound, texture, etc.) cues. The data are sensed by the human neuronal endings and relayed via neurons and synapses to the brain where all of the inputs are processed ultimately to generate a word or words that describe the object (e.g. "this is an apple" or "this is a steak"; **Figure 1**). Unlike humans who possess multiple types of receptors, most instrumental methods of analysis such as gas chromatographs (GC), mass spectrometers (MS), GC-olfactometry (GC-O), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), near infrared (NIR), etc., contain only a single sensor/detector or are designed to examine only one chemical class such as proteins, lipids, carbohydrates, and so on. On the other hand, electronic nose (E-nose) instrumentation (discussed below) contains multiple sensors that measure one characteristic such as, volatile components. Thus, E-nose instruments do not measure taste; rather they assess the bouquet (mixture) of volatiles emitted from and comprising the food commodity under investigation.

FIGURE 1.



In recent years, there has been a major surge in the development of E-nose technology such as, instruments with arrays of gas sensors. If an array of nonspecific sensors could be compiled to rival the human olfactory system, then the sample would not need to be separated and could be monitored analytically as a whole (Gardner and Bartlett, 1992). Furthermore, analysis would prove rapid, nondestructive, and continuous. These multisensor devices are coupled to statistical neural network data processing packages designed to simulate the way the human brain interprets the interaction of multiple sensory inputs. According to Bartlett et al. (1997), "*the electronic nose system parallels the human olfactory system in the following manner: Each chemical sensor represents a group of olfactory receptors and produces a time-dependent electrical signal in response to the odor.*" E-nose technology has encouraged a wide dissemination of this instrumentation within the food and fragrance industries where it is primarily used for quality control and rapid surveys of products. Analysis time using an E-nose is often only a few minutes, making it a valuable alternative to the longer, time-intensive gas chromatographic (GC) techniques. This paper will report upon the potential of electronic nose technology in the meat industry including the detection of microbial spoilage, rancidity onset in ground beef, and the differences among some dry-cured

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hams and between Spanish "Serrano" dry-cured ham processed for two different lengths of time.

What Is an Electronic Nose?

An electronic nose **(Figure 2)** is an array based detector of different gas sensors (see **Table 1** for comparison of the types of commercially available electronic nose instrumentation) for objective front-line screening of odors, taints and volatiles. The sensors register aroma through the interaction of headspace volatiles (**Figure 3**). The sensor responses are processed to give aroma fingerprints from which the unknown may be compared to a standard. Therefore, the E-nose objectively compares standard product with current batches and can graphically illustrate how similar or different they are. Quality control information in the form of quality factors (QFs) can be obtained from the data as Euclidian distances or Malanobis numbers defining distances between centers and geometric centers of data clusters, respectively. These QFs can help save money by monitoring processes.

Several companies (**Table 2**) have developed electronic sensors to detect groups of volatile compounds. Excellent reviews of current technology may be found in Gardner and Bartlett's text (1992) and Nagle *et al.,* (1998). Characteristics of the current types of detectors may be seen in Table 1 which highlights many of their similarities and differences. Major differences to be noted are those dealing with detection temperature which affects sample integrity, humidity control, certain metabolic poisons, interaction mechanisms, and reported sensitivity. Basically, in all E-nose systems the volatiles are swept across an array of sensors, each developed to respond to a different class of compounds. As current is passed over the sensors, the adsorption and desorption of the volatiles cause a change in the current similar to the thermal conductivity detectors used in gas chromatography. The data are handled by various statistical procedures and mapping techniques (Gardner and Hines, 1997).

What Are the Potential Uses of Electronic Noses in the Meat Industry?

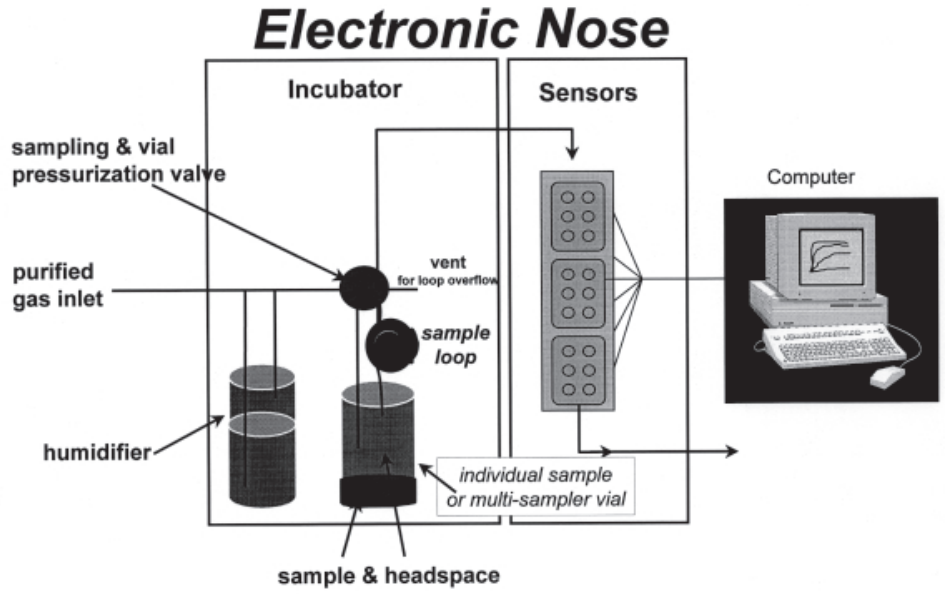
The most obvious applications of an E-nose in the meat industry is for food quality monitoring during processing, shelf-life studies, taint detection, possible species detection, and as a complementary technique to sensory panels to name a few. Indeed, the E-nose has application where ever volatile compounds are produced. One of the first applications of an E-nose studied performance for quality estimation of ground meat (Winquist et al., 1993). Using a combination of MOSFET and MOS sensors, the authors could detect changes in ground beef and pork stored for up to 8 days at 4°C.

Braggins and Frost (1997) studied the odor of raw and cooked ground lamb meat of extended chilled storage in carbon dioxide atmosphere and frozen vacuum packed storage using an array of 18 metal oxide sensors (Alpha MOS Fox 4000). They could reliably discriminate between ground lamb samples of different storage conditions over a period of 4 to 14 weeks.

In a detailed study, Braggins *et al.,* (1999a) successfully measured odor changes in tallow after oxygen abuse and bleaching treatments, rancidity onset in ground beef, and spoilage onset on beef steaks held on retail display using the Fox 4000.

Rancidity onset in ground beef: In an experiment designed to determine if the E-nose was capable of detecting rancidity in meat, ground beef was prepared, vacuum packed in oxy-

FIGURE 2.

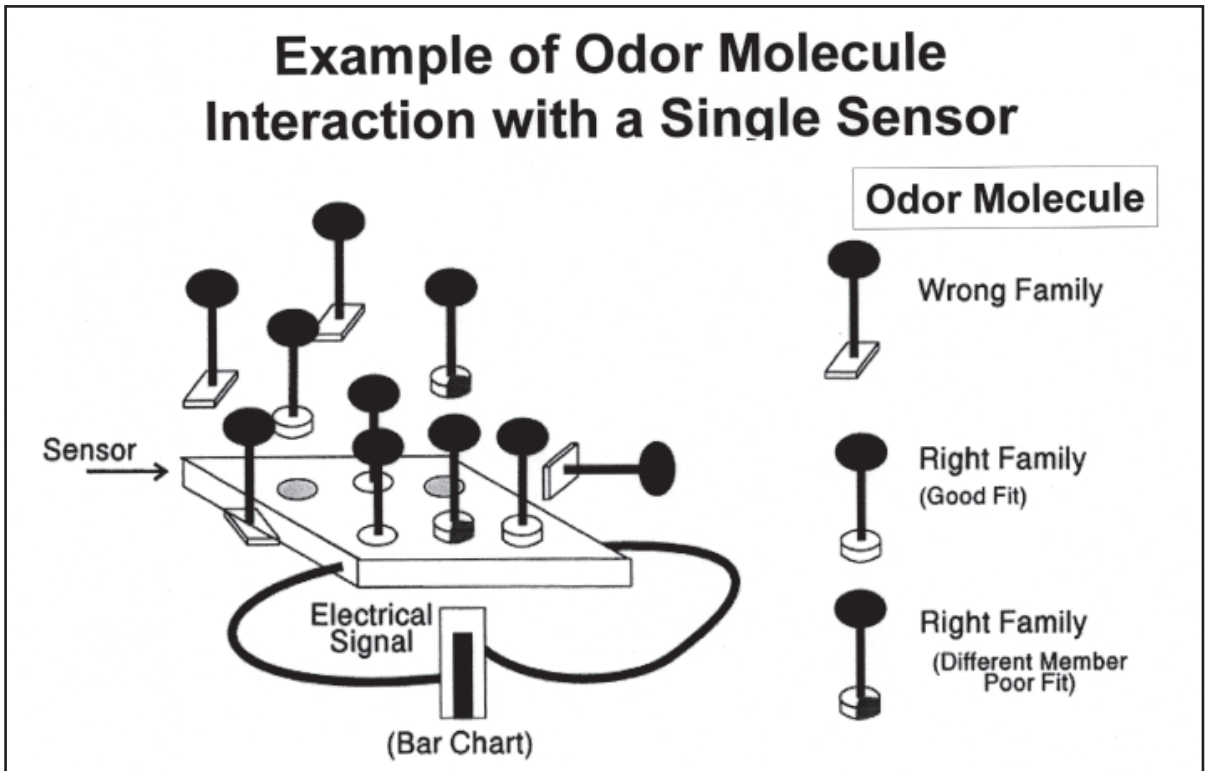


Diagramatic representation of typical electronic nose instrumentation. Variables are the number of sensors, humidity (environment and sample), single or multi-sampler attachments, and computer diagnostic equipment.

TABLE 1. Features of some Electronic Nose devices

Sensor type	Operation principle	Sensitivity	Advantages	Disadvantages
Metal-oxide-silicon field-effect transistor (MOSFET)	Capacitive charge coupling	ppm	Minimal batch-to-batch variation	Susceptible to baseline drift, Operate at high temperatures
Metal oxide (MOS)	Conductivity	5-500 ppm	Inexpensive, less sensititve to humidity	Operate at high temperatures
Conducting polymer (CP)	Conductivity	0.1-100 ppm	Operate at room temperature	Very sensitive to humidity
Surface acoustic wave (SAW)	Piezoelectricity	1pg mass change	Sensitive	Requires complex electronics
Quartz crystal microbalance (QMC)	Change in resonance frequency	1 pg mass change	Minimal batch-to batch	Micrometer scale devices are noisy variation
Mass spectrometer	Mass spectrum	low ppb	Not sensitive to humidity, analytical capability	

FIGURE 3.



Diagramatic representation of how odor molecules interact at the surface of a sensor.

TABLE 2. Commercial electronic noses

Company	*Sensor Technology*	*No. Of Sensors*
Airsense, Schwerin, Germany	Metal oxide seminconductor (MOS)	10
Alpha MOS Toulouse, France	Conducting polymer (CP), MOS, quartz crystal, microbalance (QCM), surface acoustic wave (SAW). Mass Spectrometry (MS)	6-24 800 amu
AromaScan PLC, Crewe, United Kingdom (UK)	CP	32
Bloodhound Sensors Ltd., Leeds, UK	CP	14
Cyrano Sciences, California, USA	CP	32
EEV Ltd. Chelmsford, UK	CP, MOS, QCM, SAW	8-28
Hewlett-Packard Co., California, USA	MS	800 amu
HKR-Sensorysteme, Munich, Germany	QMC	6
Lennartz Electronic, Tubingen, Germany	MOS, QCM	16-40
Nordic Sensor Technologies AB, Linkoping, Sweden	MOSFET, MOS, QCM, Infrared (IR)	22

gen permeable bags, and stored at minus 35°C. Replicate samples were transferred to another freezer set at -4°C at weekly intervals to enhance the onset of rancidity but to prevent spoilage. At the end of 11 weeks, samples were randomly selected from each of the storage times and presented in batches of 6 samples per day to trained sensory panelists and the E-nose (FOX 4000, ALPHA MOS SA, Toulouse, France), in both the raw and cooked state. This took 10 days (two weeks). On each day of testing, two fresh ground beef samples were also tested. The panelists could detect a progressive increase in rancid odor in raw beef, and rancid odor and flavor in cooked ground beef over the 11 weeks of storage at minus 4°C. **Figure 4** shows the canonical correlations analysis of the sensory and the E-nose data for the raw ground beef.

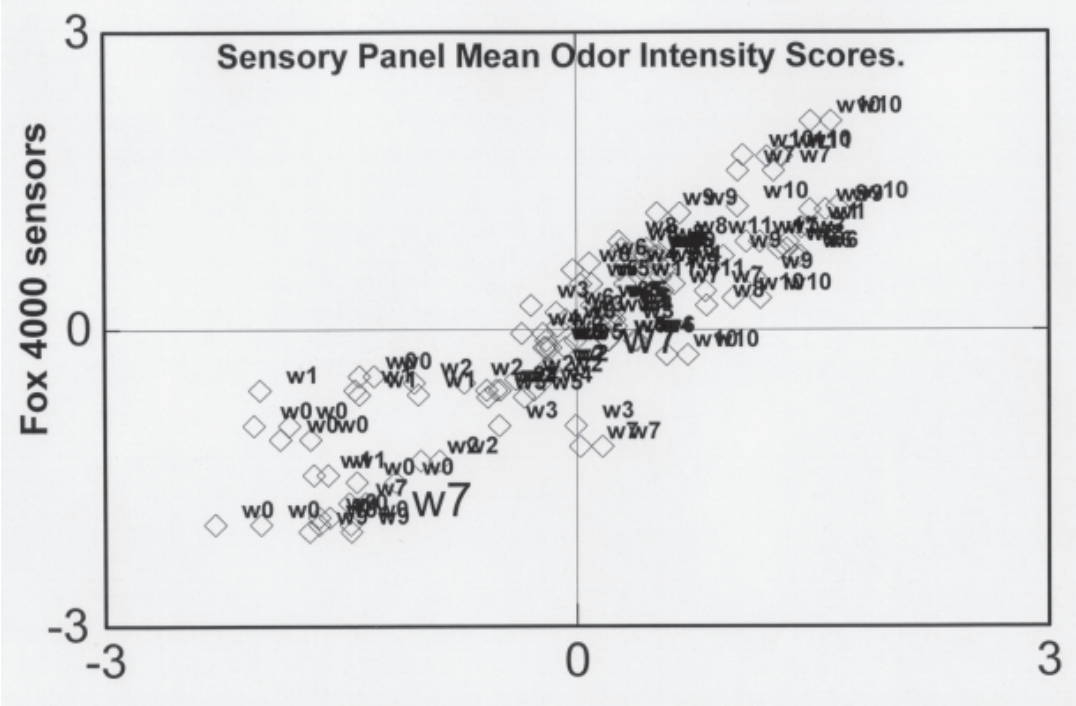
The steady increase of the response from the left to the right for the samples stored at -4°C correlates well with the onset of rancidity over the 11 weeks of storage as observed by the panel.

Mass Spectrometer as an electronic nose:

In a preliminary study, one of us (TJB) investigated the possibility of using direct injection mass spectrometry as a means of quantitatively and qualitatively characterizing rancidity development in cooked beef and lamb stored at 4°C. Ground meat, with about a 20 percent fat content, was cooked to an internal temperature of 75°C and stored in oxygen permeable plastic bags for up to 11 days in the refrigerator. Fresh and rancid meat samples were reheated and a 0.5 g aliquot of rendered fat was removed from the meat and placed into the bottom of 50 ml test tube fitted with a purge tube and outlet tube. Volatile compounds were concentrated dynamically on a Solid Phase Microextraction (SPME) fiber (Sulpelco, Bellefonte, PA) following the method of Braggins *et al.,* (1999b). The SPME fiber was placed in the outlet tube and the fat sample heated to 100°C. The surface of the fat purged with clean nitrogen gas at 30 ml min^{-1}. Volatile compounds were collected on the SPME fiber for 30 minutes.

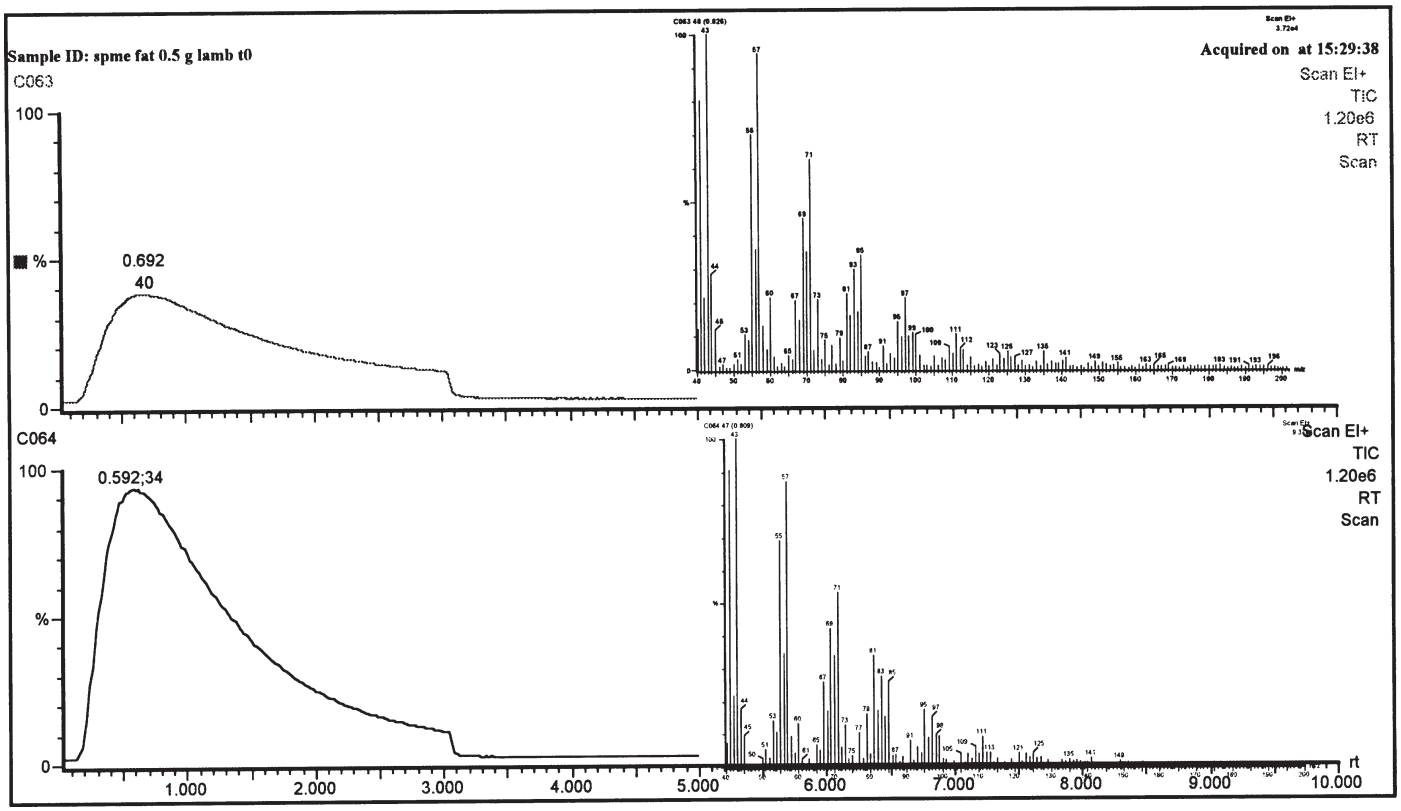
The SPME fiber was then immediately placed in the heated (200°C) injection port of a Masslab gas chromatograph/mass spectrometer fitted with a 2 m length of heated (200°C) deactivated transfer line directly to the mass detector. The injector split ratio was 80:1. The volatile compounds absorbed onto the fiber were desorbed directly into the mass spectrometer for 2 minutes. Total ion counts were recorded over the mass range of 40 to 350 amu to give a broad peak of volatile compounds. The overall intensity and area under the curve gives an indication of the relative intensity of volatile compounds in each sample (**Figure 5**). Differences in spectra derived from each sample gives an indication of qualitative differences. The latter information identifies molecular fragments that best discriminate between fresh and rancid samples. This information can then be used for multiple discriminant analysis. Alternatively, the difference spectra coupled with full gas chromatography/mass spectrometry of the sample, can be used to identify specific compounds that might be present in one sample but not the other (**Figure 6**). The latter technique would be ideal for identifying taints in meat and meat products.

FIGURE 4.



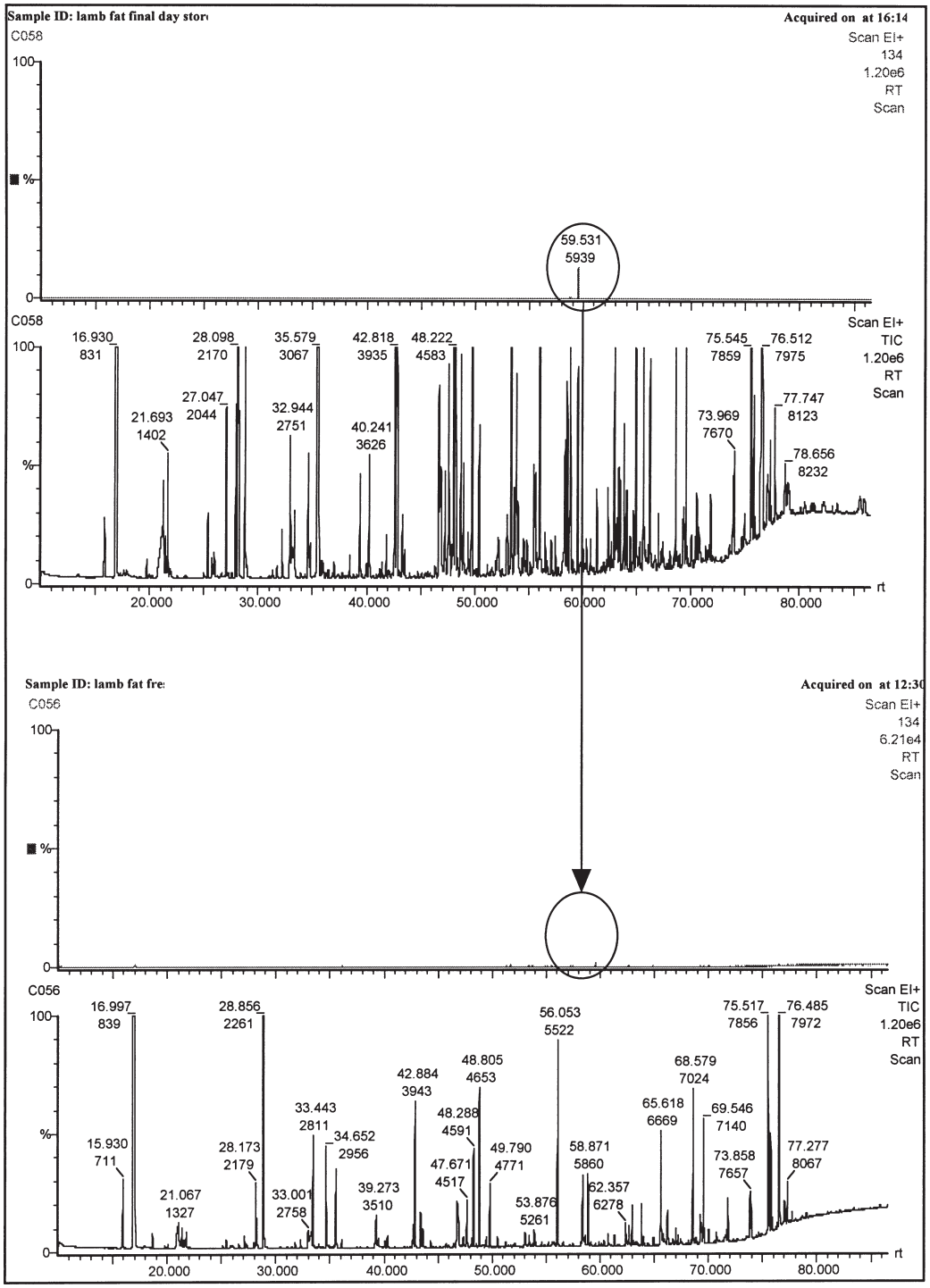
Canonical Correlation plot of the sensory and the electronic nose data for the raw ground beef stored at -4°C for 0 to 11 weeks (w0 to w11).

FIGURE 5.



Total ion counts and corresponding mass spectra of volatile compounds from freshly cooked (top) and rancide cooked ground (bottom) sheep-meat desorbed from a SPME™ fiber directly into the mass spectrometer.

FIGURE 6.



Total ion chromatogram of volatile compounds from rancid cooked (top) and freshly cooked (bottom) ground sheep-meat desorbed from an SPME™ fiber into a gas-chromatorgraphy capillary column. The peak circled is a compound identified as present in the rancid sample but absent in the fresh sample after identifying the indicating fragment ion from the difference spectra obtained by direct SPME/MS (see figure 5.)

Dry-cured Ham and Similar Products:

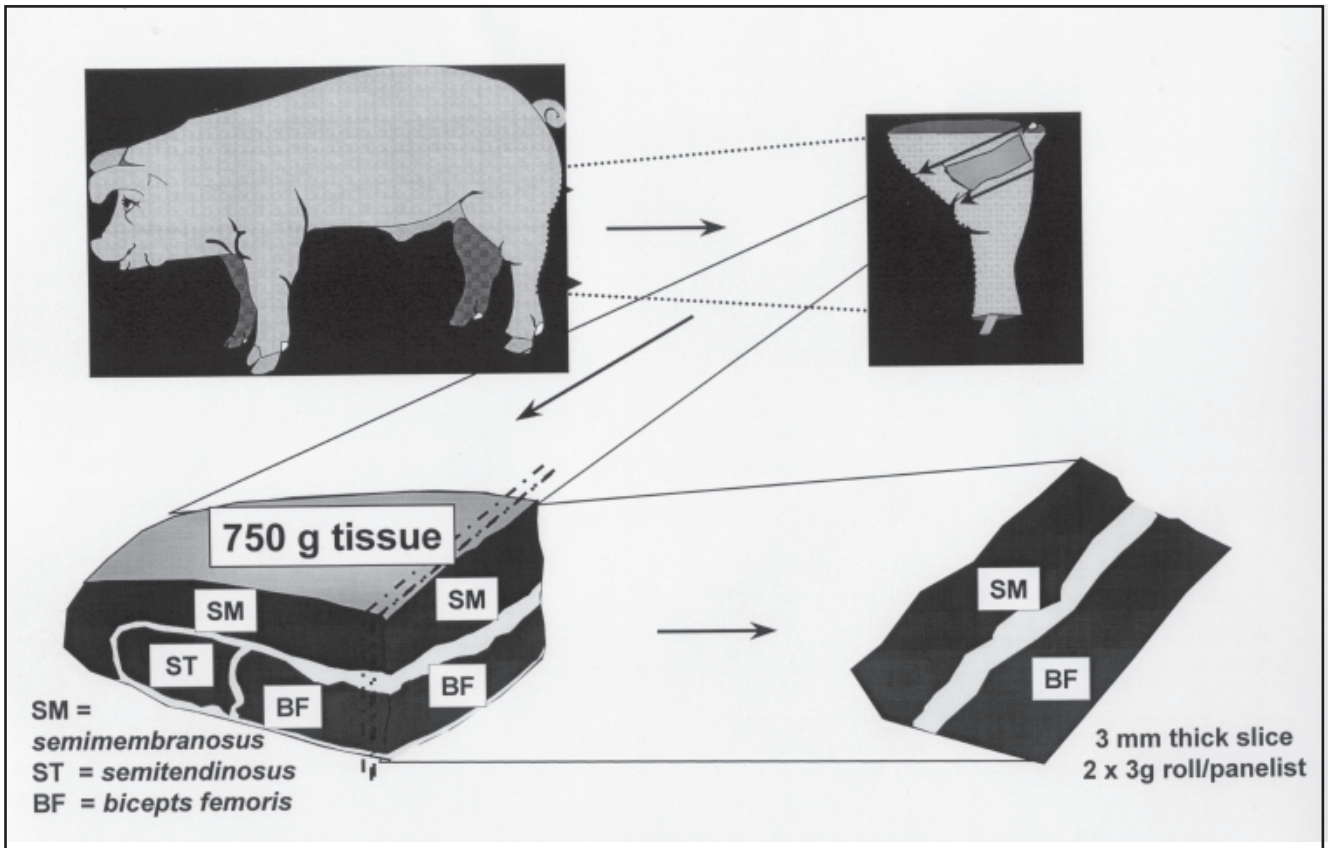
Dry-cured ham is popular in Spain and other European countries not only because of its flavor, but also because it is convenient, ready-to-use, and has a long shelf-life. The salt and nitrate used during the curing process play an important role in development and fixation of the characteristic cured-meat color, in prevention of growth of spoilage and/or pathogenic microflora, and in developing and stabilizing product flavor. The ripening/drying process involves numerous time-temperature interactions that serve as a major source of flavor variability (Toldrá *et al.*, 1997). Some

studies have been performed to demonstrate the relationship of dry-cured ham flavor to chemical data (direct and indirect instrumental methods) and sensory attributes (Berdague *et al.*, 1993; Gou *et al.*, 1995; Flores *et al.*, 1994; Buscailhon *et al.*, 1993; Careri *et al.*, 1993; Hinrichsen and Pedersen, 1995). However, these studies are often too expensive or too time consuming. Therefore, developing a rapid, reliable, and effective method to distinguish differences in dry-cured ham products is desirable. Using an E-nose with 32 sensors (AromaScan™ A32/50S multisampler), we were able to differentiate between Spanish "Serrano" dry-cured hams processed for 7 month (short) and 12 month (long) periods. Since the conducting polymers of the E-nose distinguished between Serrano ham processing times, we examined its ability to distinguish among other processed hams including Prosciutto ham, Country ham, Virginia ham, and Deli ham; the instrument effectively distinguished these products.

Twenty Spanish "Serrano" dry-cured hams were purchased from a factory in Castellon, Spain (Jamones Segorbe). Prosciutto, Country, Virginia (smoked), and Deli (pressed) ham was purchased from local food stores. Short (7 month) and long (12 month) dry-curing method for "Serrano" hams. Ten hams were submitted to a 7 month process (short), consisting of the traditional stages of salting (12 days at 3°C), post-salting (50 days at 4°C) and ripening-drying (1st phase: 60 days at 12°C, 2nd phase: 60 days at 18°C, and 3rd phase: 30 days at 25°C). A second group of 10 hams was ripened for a 7 months process (long) where hams were held, after ripening for 7 months, for an additional 150 days at 15°C. Following these processes, 750 g of tissue were excised from the center of each ham, perpendicular to the femur that included the *biceps femoris*, *semitendinosus*, and *semimembranosus* muscles (**Figure 7**). Samples for analysis were sent within 3 days at 4°C to the Southern Regional Research Center (USDA, New Orleans, LA), following appropriate documentation with the Food Safety Inspection Service (FSIS) and the U.S. Customs Bureau. Ham from each source were finely minced with a new single edged razor blade. Precisely 5 g of the minced ham was placed into 22 milliliters capacity multisampler vials (AromaScan, Hollis, New Hampshire). One ml of deionized water was added to each vial and the vials crimped and sealed with the Teflon side of a septum facing inward. Three samples were analyzed via the E-nose from each ham. The final data point presented on the Sammon's map represents the average of the 3 measurements per ham. Instrumentation available for this research included the AromaScan™ 32S coupled to a Tekmar 50-slot autosampling device. The AromaScan instrumentation included humidity control. The Tekmar autosampler and AromaScan™ instrument were held at room temperature (22°C) in an air-conditioned room to control major humidity fluctuations.

The instrumental method was optimized to the following

FIGURE 7.



Diagramatic representation of preparation of dry-cured ham samples for investigation.

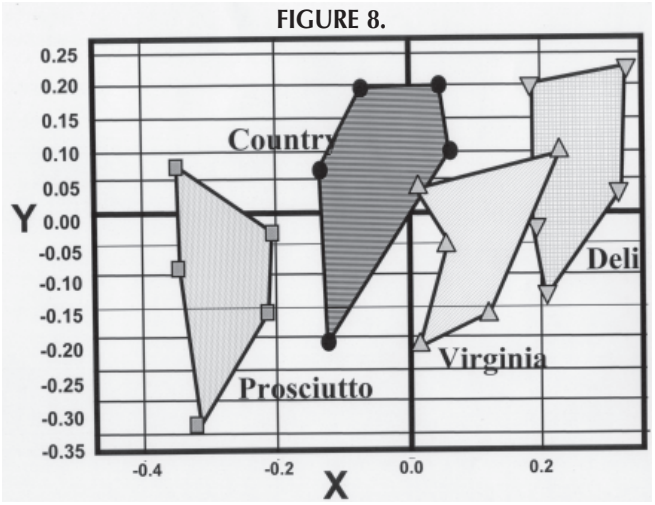
hardware control conditions: *Acquisition method*: Cycles were a Step 1, reference for 27 seconds (the time T), Step 2, sample time of 120 seconds, Step 3, wash time of 30 seconds, and Step 4, reference time of 120 seconds. The graph scale was set to 5, the pattern average to 5, the purge humidity to 45 and the reference humidity to 50. *Multisampler method*: The platen temperature was set to 40°C, the platen equilibration time to 10 seconds, the sample equilibration time to 10 seconds, the vial size was 22 ml. The mixer was set to on with a mix time of 2 seconds and a mix power of 7. There was a 5 minute vial pressure stabilization time, a vial pressure time of 0.50 minutes, and a pressure equalization time of 0.15 minutes. Loop fill time was 0.40 minutes, loop equilibration time 0.20 minutes and an injection time of 1 minute. Sample loop temperature was set to 40°C as was the line temperature, with a sample injection time of 1 minute. The A32S analysis time was set for 6 minutes. Data were handled by the statistical software included with the A32S E-nose.

The nonlinear mapping algorithms of Sammon (1969, 1970) were used to analyze and to plot/map the data obtained from all ham samples. This method was chosen since with an array of multiple sensors, measurements of different gasses or odors produce different patterns that are projected into multi-dimensional space (Hodgins, 1997). Within two- and three-dimensional space, human vision is very good at recognizing almost any relationships present; however, in multidimensional space it is difficult to perceive structural relationship. In order for a human to examine complex multi-dimensional data, a useful approach is to map them from the high-dimensional pattern space in which they are originally presented onto a low-dimensional pattern space as faithfully as possible.

Data obtained from sensory (Flores *et al.*, 1997a) and from instrumental and chemical analysis of volatile (Flores *et al.*, 1997b) and non-volatile (Flores *et al.*, 1997c) components of dry-cured Spanish "Serrano" ham have indicated that there were significant differences in short (7 month) and long (12 month) dry-cured ham. It was felt that the differences observed made it feasible to explore the possibility of utilizing the conducting polymers of the E-nose for surveying the hams and that any differences observed utilizing the E-nose would most likely be related to the flavor differences observed by the other instrumental and sensorial methods of analysis. Sensory differences are observed (Flores *et al.*, 1997a) between the short (7 month) processed and the long (12 month) processed hams. Production techniques have been reported to accelerate the dry-curing process (Marriott *et al.*, 1992). Although the techniques can accelerate the drying stage, it is necessary to determine the optimal conditions to ensure the development of the typical and desirable dry-cured ham flavor. The long drying process method is necessary for the development of the typical dry-cured ham flavor (Flores *et al.*, 1997a). Three factors, cured, pork, and off flavors are present in hams cured for long period (12 months). While the flavor of the short (7 month) dry-cured hams is acceptable, the data clearly indicate that the dry-cured flavor has not fully developed. Thus, the time period between 7 and 12 months at which dry-cured flavor is optimized must be determined if we are to select the most advantageous processing time.

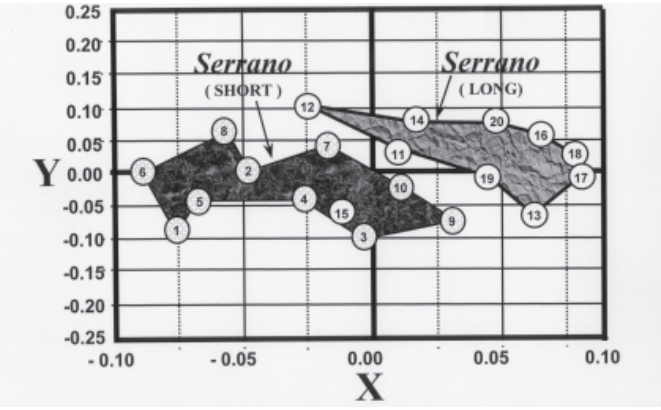
Almost all of the components described by Flores *et al.*, (1997b, 1997c, 1998) have an impact on the final flavor of dry-cured ham. Many of these are volatile in nature and should be capable of interacting with the conducting polymers to give an idea for a flavor profile of the various hams. However, before examining short and long processed "Serrano" hams for differences, it was determined that we (AMS) should explore other varieties of dry-cured and processed hams for differences that could become visible after analysis by the electronic nose. For this purpose, we utilized Prosciutto ham and Country ham which were processed (salted and cured) similar to the Serrano ham, Virginia ham which had been slightly smoked and which represented the flavor of the hams typically sold in the east-central coastal region of the U.S., and Deli ham which was cooked-and-pressed and which represented the typical type of ham sold throughout the U.S.

FIGURE 8.



Sammon plot by AromaScan A32S50 software of data from five different samples of four different processed hams (Prosciutto, Country, Virginia, and Deli).

FIGURE 9.

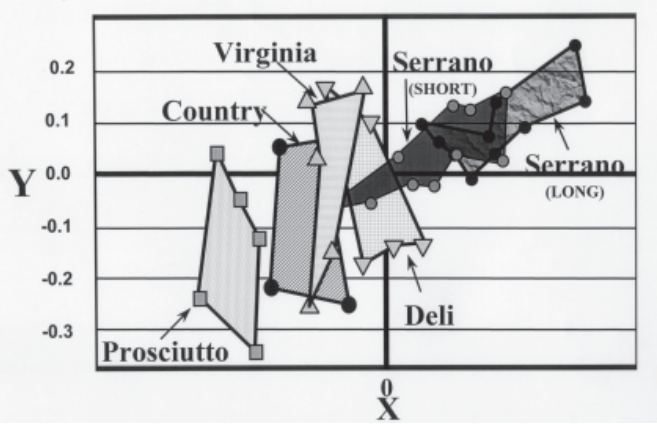


Sammon plot by AromaScan A32S50 software of data from 10 different samples of dry-cured Serrano hams cured for either 7 months (short-cured) or the traditional 12 months (long-cured).

(**Figure 8**).

Figure 8 (above) shows the Sammon (1969, 1970) mapping generated from evaluation of multiple data sets each from five different sample lots (hams purchased at different times) of four types of hams (Prosciutto, Country, Virginia, and Deli). All four ham-types were readily distinguishable as individual cluster groups. **Figure 9** also represents the Sammon's PCA projection and shows a fine separation in the data clusters of long and short processed Serrano hams. At first look, such a clear separation of the data clusters would appear to suggest that E-nose technology is a good rapid technology for assessing the differences in Serrano dry-cured/processed hams. However, when one examines the data from all six groups of hams (**Figure 10**), there is considerable overlap of the clusters suggesting that the hams are not readily distinguishable by E-nose technology. The Sammon's plot of Figure 9 is quite different from the plot of the data for the 4 hams alone (Figure 7), and quite different from the Serrano hams alone (Figure 8). Figure 9 shows a fair amount of overlap in the cluster group for Country, Virginia, and Deli hams and an overlap of about half of the Serrano long and short, cured samples with each other. The Serrano short as seen in Figure 9, showed some samples clustering with the Virginia and Deli hams, while the Serrano long did not overlap with any of the other hams except the Serrano short. When the data set for only the Serrano short-cured and long-cured hams are examined and plotted with Sammon's algorithm (Figure 8), only one of the Serrano long-cured ham samples (#15) showed any overlap with the 10 hams of the Serrano-short processed samples. The question is, "are they or aren't they distinguishable"?

FIGURE 10.



Sammon plot by AromaScan A32S50 software of data from Figures 7 and 8 representing data from five different samples of four different processed hams (Prosciutto, Country, Virginia, and Dela) and data from 10 different samples of dry-cured Serrano hams cured for either 7 months (short-cured) or the traditional 12 months (long cured).

The overlap seen in Figure 9 may be due to a cross-sensitivity as suggested by Horner and Muller (1992). Overlap of data clusters can occur when all samples originate from the same source ... in this case pig ... and thus share many similar volatile components. Multivariate analysis must, therefore, distinguish between the similar volatiles and those that are different among the comparison group. It is entirely reasonable to expect these similarities within a given food system; the "difference-volatiles" are perhaps fewer in number and concentration in the different hams and are masked at the sensor by those volatiles at higher levels. Our experiences thus far with E-nose instrumentation suggests that decreasing the number of similar comparison groups and increasing the number of samples evaluated within a group typically lead to a "tightening" of the clustering and better differentiation.

Conclusion

E-nose technology is proving to be capable of distinguishing between such items as rancidity onset in ground beef, curing-age in dry-cured Serrano ham, different curing methods in other ham products, differences in ground meat stored under different modified atmospheres and more. Much of the E-nose research thus far complement data and results gathered by other methods of analysis such as descriptive sensory panels, capillary electrophoresis, GC, GC-MS, etc. (Braggins and Frost, 1997; Braggins et al., 1999; Flores et al., 1994, 1996, 1997a, b, c, 1998). Perhaps one of the more exciting new techniques combines MS as a qualitative E-nose like tool. It is hoped that with further experimentation and development of E-nose sensors, meat processors will have a rapid, cost effective means of assessing the quality of their product with the final end-point being the production of high quality meat products for both themselves and the consumer.

Acknowledgements

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