Testing the Aroma of Maple Flavored Sausage Using the zNose®

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Electronic Noses

Conventional electronic noses (eNoses) produce a recognizable response pattern using an array of dissimilar but not specific chemical sensors. Electronic noses have interested developers of neural networks and artificial intelligence algorithms for some time, yet physical sensors have limited performance because of overlapping responses and physical instability. eNoses cannot separate or quantify the chemistry of aromas.

A new type of electronic nose, called the zNose®, is based upon ultra-fast gas chromatography, simulates an almost unlimited number of specific virtual chemical sensors, and produces olfactory images based upon aroma chemistry. The zNose® is able to perform analytical measurements of volatile organic vapors and odors in near real time with part per trillion sensitivity. Separation and quantification of the individual chemicals within an odor is performed in seconds. Using a patented solid-state mass-sensitive detector, picogram sensitivity, universal non-polar selectivity, and electronically variable sensitivity is achieved. An integrated vapor preconcentrator coupled with the electronically variable detector, allow the instrument to measure vapor concentrations spanning 6+ orders of magnitude. A portable zNose®, shown in Figure 1, is a useful tool for assessing the quality of aromatic food products such as sausage.

Figure 1- Portable zNose® technology incorporated into a handheld instrument.

How the zNose™ Quantifies the Chemistry of Aromas

A simplified diagram of the zNose™ system shown in Figure 2 consists of two parts. One section uses helium gas, a capillary tube (GC column) and a solid state detector. The other section consists of a heated inlet and pump which samples ambient air. Linking the two sections is a "loop" trap which acts as a preconcentrator when placed in the air section (sample position) and as an injector when placed in the helium section (inject position). Operation is a two step process. Ambient air (aroma) is first sampled and organic vapors collected (preconcentrated) on the trap. After sampling the trap is switched into the helium section where the collected organic compounds are injected into the helium gas. The organic compounds pass through a capillary column with different velocities and thus individual chemicals exit the column at characteristic times. As they exit the column they are detected and quantified by a solid state detector.

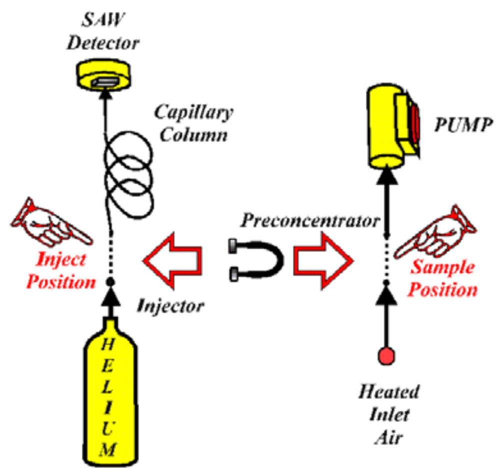

Figure 2- Simplified diagram of the zNose™ showing an air section on the right and a helium section on the left. A loop trap preconcentrates organics from ambient air in the sample position and injects them into the helium section when in the inject position.

An internal high speed gate array microprocessor controls the taking of sensor data which is transferred to a user interface or computer using an RS-232 or USB connection. Aroma chemistry, shown in Figure 3, can be displayed as a sensor spectrum or a polar olfactory image of odor intensity vs retention time. Calibration is accomplished using a single n-alkane vapor standard. A library of retention times of known chemicals indexed to the n-alkane response (Kovats indices) allows for machine independent measurement and compound identification.

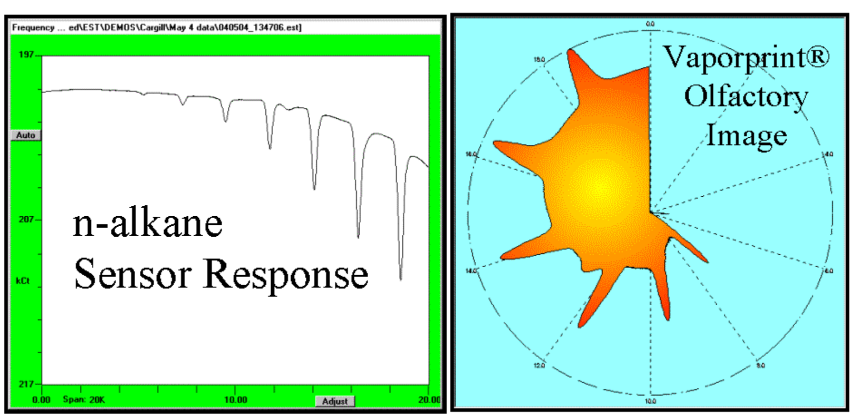

Figure 3- Sensor response to n-alkane vapor standard, here C6-C14, can be displayed as sensor output vs time or its polar equivalent olfactory image.

Chemical Analysis (Chromatography)

The time derivative of the sensor spectrum (Figure 3) yields the spectrum of column flux, commonly referred to as a chromatogram. The chromatogram response (Figure 4) of n-alkane vapors (C6 to C14) provides an accurate measure of retention times. Graphically defined regions shown as red bands calibrate the system and provides a reference time base against which subsequent chemical responses are compared or indexed. As an example, a response midway between C10 and C11 would have an retention time index of 1050.

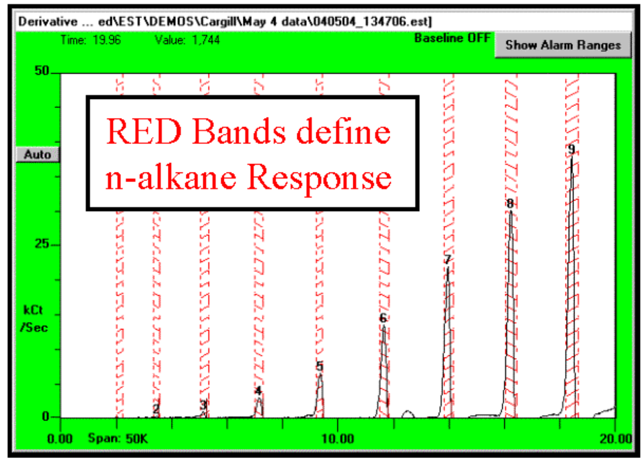

Figure 4- Chromatogram of n-alkane vapors C6 to C14).

Maple Flavorings

The four most common compounds used for maple flavoring (Figure 5) are cyclotene, ethyl cyclotene, Sotolon, and maple furanone. Cyclotene or 3-Methyl-2-cyclopenten-2-ol-l-one ($C_6H_8O_2$) in conjunction with fenugreek solid extract, "cyclotene" is an integral part of nearly all Maple flavors. Most consider its flavor is close to the syrup obtained from a sugar maple. Its odor detection threshold (in water) is 300 ppb.

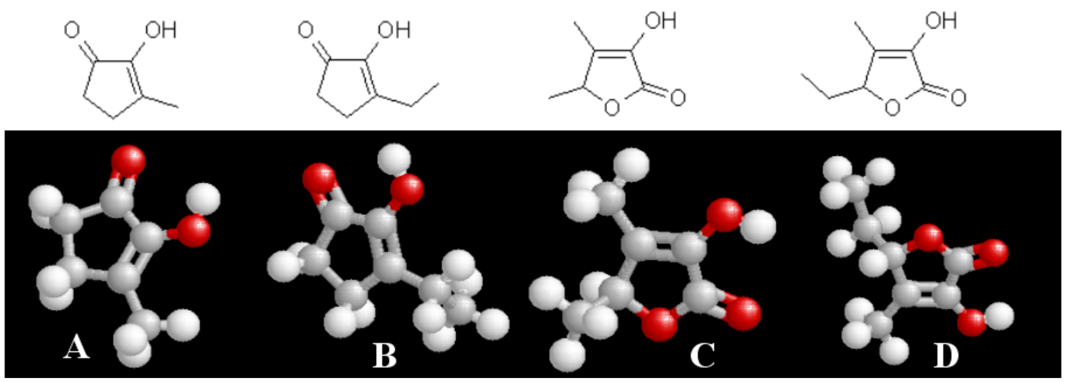

Figure 5- Cyclotene (A), Ethyl cyclotene (B), and Sotolon (C), and Maple Furanone (D) produce strong maple notes.

3-Ethyl-2-hydroxy-2-cyclopenten-1-one or Ethyl cyclotene ($C_7H_{10}O_2$) is the ethyl homolog of cyclotene and possesses a similar odor and taste. It has an odor detection threshold (in water) of 150 ppb with a very strong, caramellic-maple odor and taste.

Sotolon or 4,5-Dimethyl-3-hydroxy-2(5H)-furanone ($C_6H_8O_3$) also known as "Caramel furanone", is the key organoleptic principal of roasted Fenugreek seed. The taste and aroma of brown sugar is mainly due to sotolon (which is the main flavor principal in sugar molasses). However, Sotolon is an extremely powerful aroma chemical for imparting caramel-maple notes. Sotolon has a detection threshold of 0.001 ppb, nearly 30,000 time more powerful than cyclotene with an odor detection threshold (in water) of 0.001 ppb.

Maple furanone or 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone ($C_7H_{10}O_3$) is truly one of the most outstanding of modern flavor materials. This material is much more powerful and lasting than Methyl or Ethyl cyclopentenolone and possesses more of a maple note than Sotolon. With a detection threshold of 0.00001 ppb maple furanone is nearly 3,000,000 times more powerful than cyclotene and in fact is one of the most powerful flavor chemicals known to man. It has an odor detection threshold (in water) of 0.00001 ppb and a powerful maple-caramel aroma and taste.

Tesing Maple Flavored Sausage Samples

Three different samples (1,2,and 3) of maple flavored sausage (Figure 6) were supplied in plastic bags. The samples had each been stored for a different amount of time and hence differences in aroma chemistry might be attributed to a finite shelf life for this type of food product. The aroma associated with each sausage sample was tested by analyzing headspace vapors in the as-supplied plastic bag using a needle probe as shown in Figure 7. Samples were first tested as delivered (frozen) and then after thawing and opening the sausage package to expose sausage meat to the ambient air within the bag. All tests were with samples at room temperature.

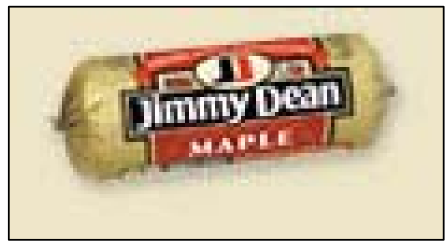

Figure 6- Maple flavored Sausages.

A second type of test involved removing a small amount of sausage meat and placing in a septa-sealed 40-milliliter vial as shown in Figure 8.

Figure 8- Testing samples in septa-sealed 40-milliliter vials.

Figure 7- Sampling headspace vapors

Testing Frozen Sausage Samples

A representative number of replicate chromatograms using headspace vapors from plastic bags containing two of the frozen sausage samples are shown in Figure 9. For comparison and indexing (Kovats) a chromatogram from an n-alkane vapor standard is shown in red. A relatively volatile compound (unidentified) with an index of approximately 740 dominated the odor chemistry due to its relatively high concentration. Because the sausage had not been opened these chemical odors may come from storing the sausage in a contaminated freezer or from the plastic bag itself.

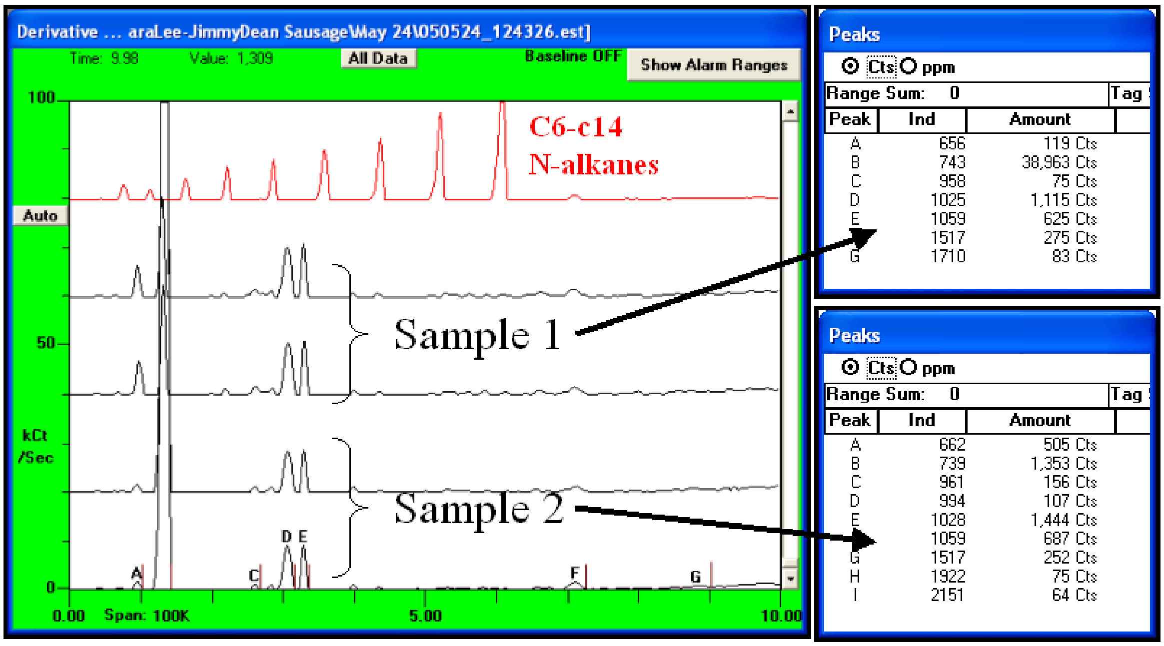

Figure 9- Vertically offset chromatogram analyses of headspace vapors from frozen sausage samples compared to n-alkane vapors. 10° detector, 30 sec sample, 10ps2a1b method, 165° valve 200° inlet.

Testing Opened Sausage Packages in Plastic Bag

Opening the internal wrapper allowed aroma from the sausage to be released into the plastic bag and dramatically altered the headspace chemistry as shown in figure 10. In this figure over 21 different chemical compounds, labeled A thru T, are detected and their retention time indexed to that of the n-alkanes. Using a library of Kovats indices allows zNose software to identify one maple flavoring compound, sotolone, which is indicated by a red hatched band on the chromatogram. A Vaporprint® olfactory image is also shown overlaid on the chromatogram. The Vaporprint® image is a visual pattern which can be used by experienced operators to quickly identify odor variations.

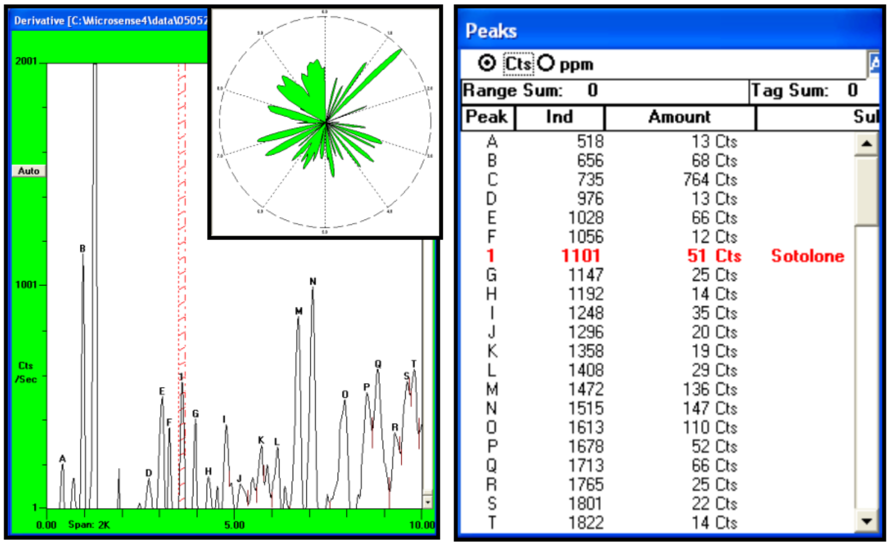

Figure 10- Chromatogram of headspace vapors after opening sausage no 1 wrapper.

Comparing Sausage Odor Chromatograms

Vertically offset chromatograms in triplicate from all three sausage samples are shown in Figure 11 for comparison. There are clear variations in the most volatile compounds (peaks A-C) as well as differing amounts of maple sotolone (peak G) between the sausage samples.

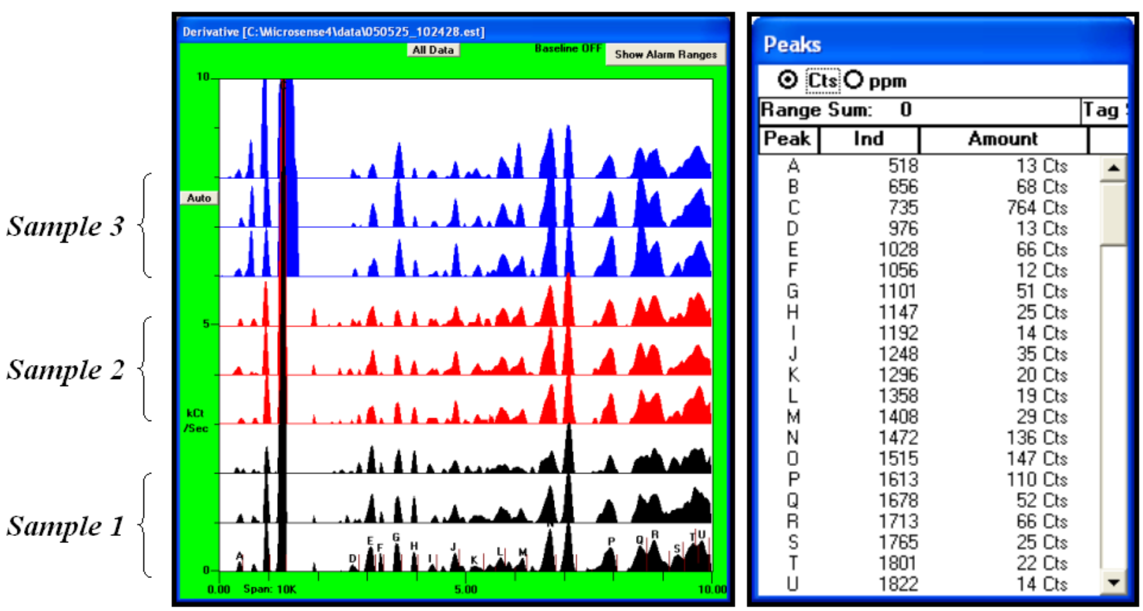

Figure 11- Vertically offset chromatograms from sausage samples in plastic bags.

Very Volatile Organics in Sausage Samples

Using a slower GC method with a 2°C/second column ramp allows the zNose® to examine the very volatile organics within the sample bags. Vertically offset chromatograms under these conditions are shown in Figure 12. These compounds were most prevalent in testing of headspace vapors from the plastic bags and may be due to storing in a the samples contaminated freezer. Often this type of contamination or odor can be found in melt water from the freezer or from the surface of frozen packages.

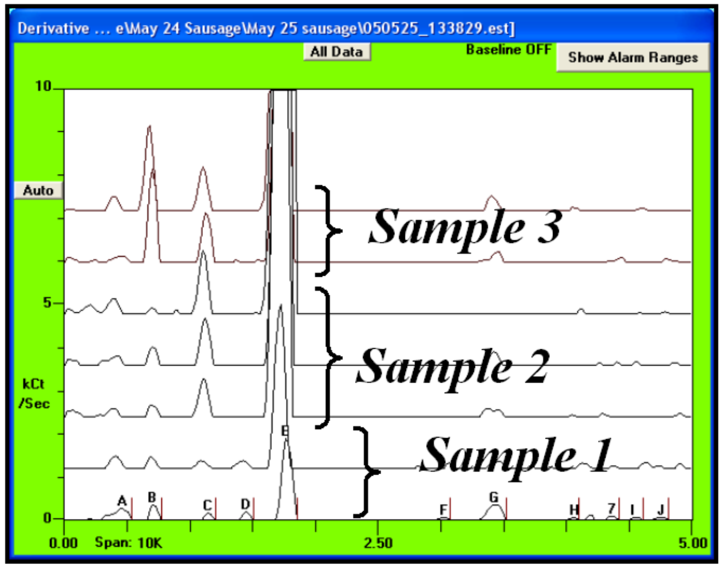

Figure 12- Vertically offset chromatograms from sausage samples in plastic bags. (30° detector, 5 sec sample, 2ps2a1b method, 165° valve, 200° inlet)

Variations in headspace chemistry from different samples of sausage meat removed from packaging materials and placed in clean odor-free septa-sealed vials is shown in Figure 13. Here sausage meat from sample 1 and 3 is compared. Most noticeable is the reduction in maple aroma due to the flavoring compound sotolone. Sample 3 appears to have ¼ the aroma intensity of sample 1. Variations in aroma intensity could be attributed to loss during storage.

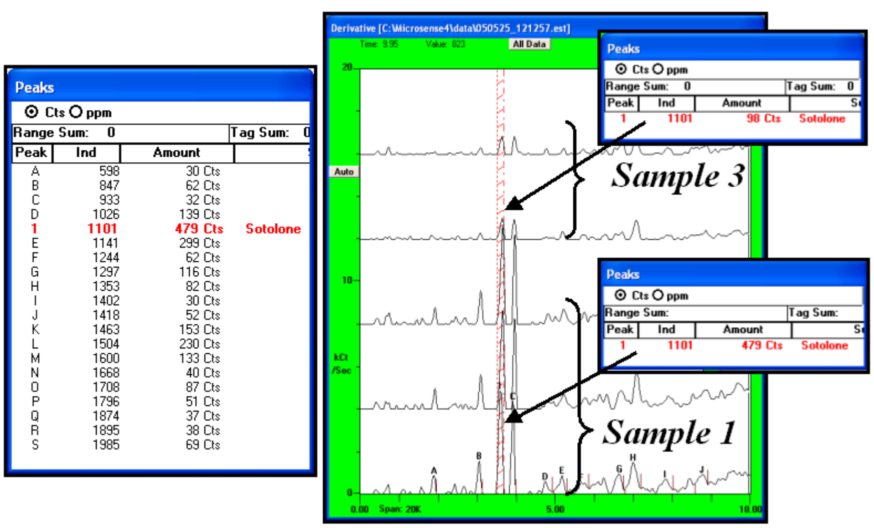

Figure 13- Vertically offset chromatograms from sausage samples1 & 3 in septa sealed vials. 30° detector, 10 sec sample, 10ps2a1b method, 165° valve, 200° inlet

Summary

Chemical profiling aroma associated with maple flavored sausage can be performed in an efficient and timely manner using a new electronic nose called the zNose®. Unlike conventional eNoses based upon sensor arrays, the zNose® utilizes ultra-fast gas chromatography and is able to separate and quantify the individual chemicals within an aroma in less than 10 seconds. The zNose® is best used in concert with human sensory panels where it is able to link aroma chemistry with human preferences. Good chemistry can only be separated from bad by human sensors, however, the ability to work with panels provides a cost effective link with production engineers and can quickly locate the chemical origin of aromatic problems. One example application treated in this report is the variation in maple-flavored sausage aroma vs storage time or shelf-life. Poor aroma quality might be due to loss of aroma producing chemicals such as sotolone or to contamination from environmental storate condiditons such as a contaminated freezer compartment. Indexing of retention times for target compounds using an n-alkane odor standard provides a convenient method of identification and eleminates the complexity of using multiple standards.

Dynamic headspace analysis using ultra-high speed gas chromatography can be coupled with sensory data to affect an objective method of classifying quality of aromatic products of many kinds The chemical and sensory data can be subjected to multivariate analysis such as principal component analysis (PCA) and partial least squares (PLS) methods to determine which volatiles are best used to classify quality. Proper choice of samples and use of optimized variables (compounds indicating off-odors), as well as preprocessing of chemical data, including scaling, transformation, and normaliation, may be used to assess quality. Samples with discernable mixed odors, i.e. having musty, sour, smoky, or unusual odors can be used together with quantitative chemical analyses. The zNose® provides the speed, portability, precision, and accuracy needed for cost-effective sensory panel measurements. Such measurements, because they are based upon well known chromatographic methods, can easily be validated and corroborated by independent laboratory testing.