Monitoring Brettanomyces In Wine Aroma with an Electronic Nose

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The management of Brettanomyces and other yeasts is fast becoming one of the most important winemaking issues. Winemakers need new tools for reliable and early detection of Brettanomyces within their facilities before the wine quality is altered. One such tool is the zNose®, an ultra-fast and portable gas chromatograph. Acting more like an electronic nose or chemical flashlight, this instrument can go anywhere and report the chemical composition and concentration of odors, aromas, and other vapors within seconds. A simple method for detecting 4-ethylphenol and 4-ethylguaiacol in wine is described and results using spiked wines presented.

Brettanomyces yeast

Brettanomyces is the name of a natural species of yeast makes its presence known in red wines after fermentation, while they are aging in the barrel. Winemakers often find this yeast where they least want it: in barrels, production lines, bottled wine and even on the grapes themselves. When left uncontrolled in wineries Brett creeps into wine barrels and *Brettanomyces* affected wines begin to taste "mousey," strangely metallic, or else stinkingly manure-like.

Figure 1

Brettanomyces - a quality or flaw?

Pinot Noirs affected with Brett can deliver an aroma described as sweet cherry perfume and smoky, spicy incense-like on top of a velvety textured flavor. But other Pinot Noirs affected more strongly can have an aroma reminiscent of something half way between sweaty saddle leather and a faint, manure-like earthiness. Some critics claim wines with distinctively leathery, earthy, or even barnyardy aromas and flavors should be praised as having "French-like" qualities. Leather and earth, after all, are nuances commonly found in some of France's most famous reds, such as Chateau Pichon-Lalande from Bordeaux and Chateau de Beaucastel Chateauneuf-du-Pape.

Figure 2- Sensory evaluation of wine aroma.

Aroma of Brett Wines

Aroma is a highly important aspect determining the quality of wine. Primary aromas are those belonging to and characteristic of the variety of grape used for the elaboration of the wine. Descriptive sensory evaluations show an inverse relationship between fruity and bretty flavor perception. The “Brett” aromas in some wines are considered a positive attribute, espe-

cially when present at low concentration. Often however these flavors are considered a defect. The wine's varietal and regional flavor characteristics might be completely masked by these flavors and the wine can be unpleasantly bitter.

The compounds originating from fermentation – the most abundant – are responsible for the fruity and/or flowery aromas of wine and are known as secondary aromas. The compounds giving rise to the tertiary aromas come from the oak wood during the aging process in the cask. Brettanomyces yeasts are unique in their ability to synthesize the volatile phenols 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) during aging and these are an important part of the Brettanomyces character in wine.

Screening Wine Aroma with Electronic Noses

New methods and equipment now allow reporting concentrations of 4-EP and 4-EG in almost real time. Electronic noses based upon ultra-fast gas chromatography now can identify and quantify the presence of these compounds by measuring the concentration of chemicals in the aroma produced by the wine onsite and in the field. One such electronic nose, called the zNose® and shown in Figure 3, it is a battery operated portable instrument, which is able to separate aroma chemicals and quantify their concentrations in mere seconds. With this, winemakers don't have to wait for Brett to grow, as with standard culture plating that can take up to two weeks. This gives them a heads-up for potential problems in the future, so they can take earlier action to control or manage Brett before it becomes a problem." and to adjust treatment levels accordingly.

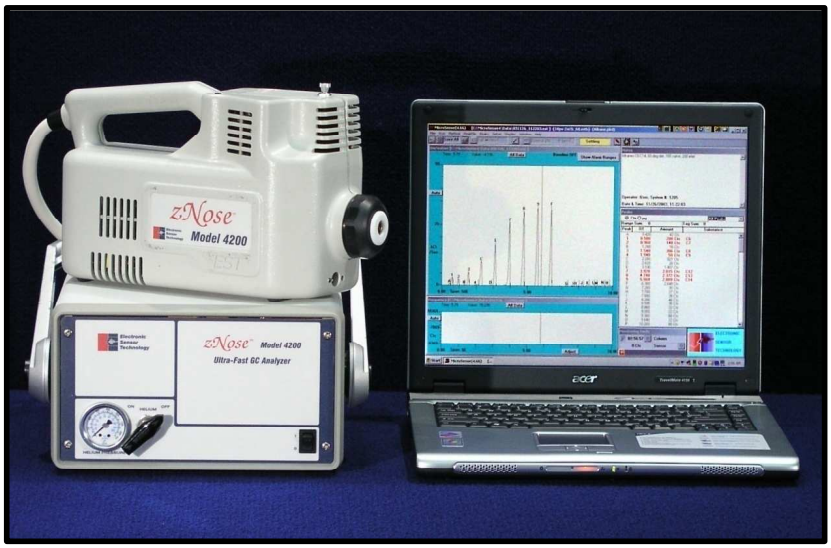

Figure 3- Portable battery operated zNose® gas chromatograph.

The objective is to provide winemakers with a tool to help them control and optimize 'brett' aroma in their wine production. You can still find Brett in some of the best French Bordeaux and domestic cabernet sauvignons. Many people -- winemakers and consumers alike -- think a little 'brett' is a good thing. The ability to quantify and monitor the presence of these chemicals and others in real time is therefore a definite advantage.

Direct Headspace Sampling of Wine

Headspace vapors of any wine can be easily sampled and chemically analyzed in seconds with a handheld electronic nose. Two different approaches are shown in Figure 4. The method on the left simply involves holding an open bottle of wine against the Teflon inlet of the instrument and collecting a sample of headspace vapor using the instrument's internal sample pump. Improved repeatability can be achieved using septa sealed vials and a sampling needle attached to the inlet of the zNose to pierce the septa and collect a sample of the headspace vapors within the vial.

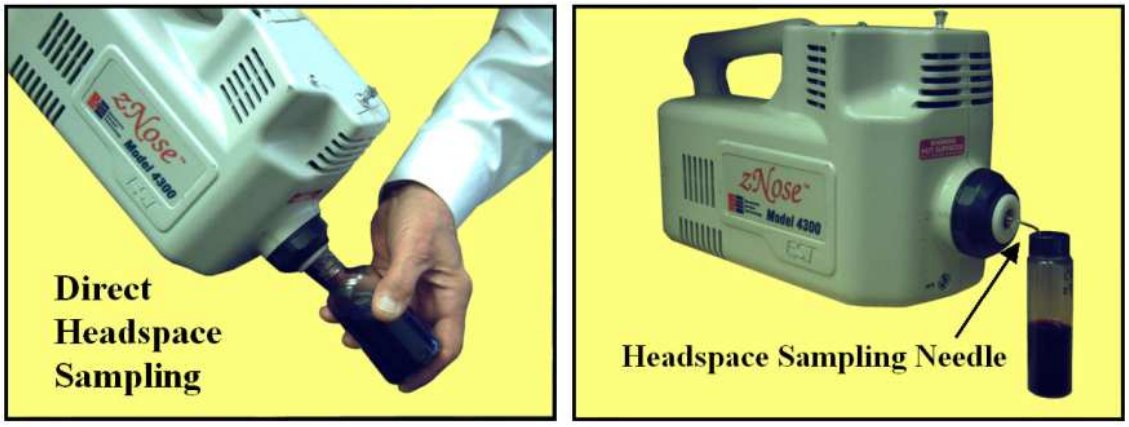

Figure 4- Sampling wine headspace vapors with the zNose.

Wine Aroma Chemistry

The headspace chemistry of two similar wines, a merlot and a cabernet, were evaluated. The zNose® is designed to measure the intensity of vapor chemistry as a function of retention index. Results for a merlot and cabernet wine are shown in Figures 5 and 6 respectively. A polar plot of aroma intensity vs. retention index can be used to create a characteristic polar image of the aroma chemistry.

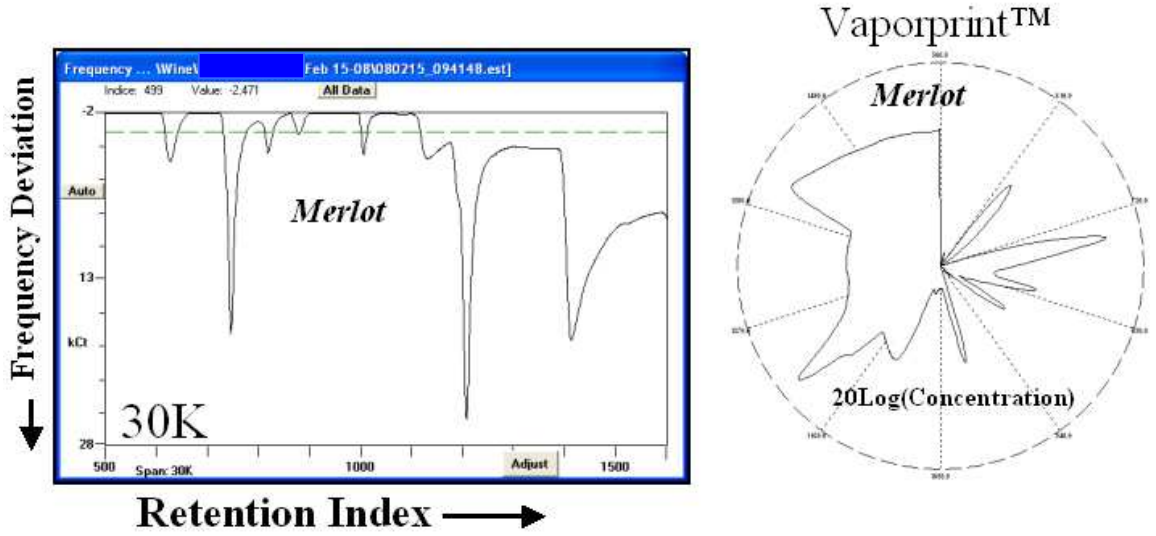

Figure 5- Aroma intensity vs. retention index as measured by the zNose can be used to create olfactory diagrams or Vaporprint™ images. (5ps2a1b method, 20° detector, 10 second sample)

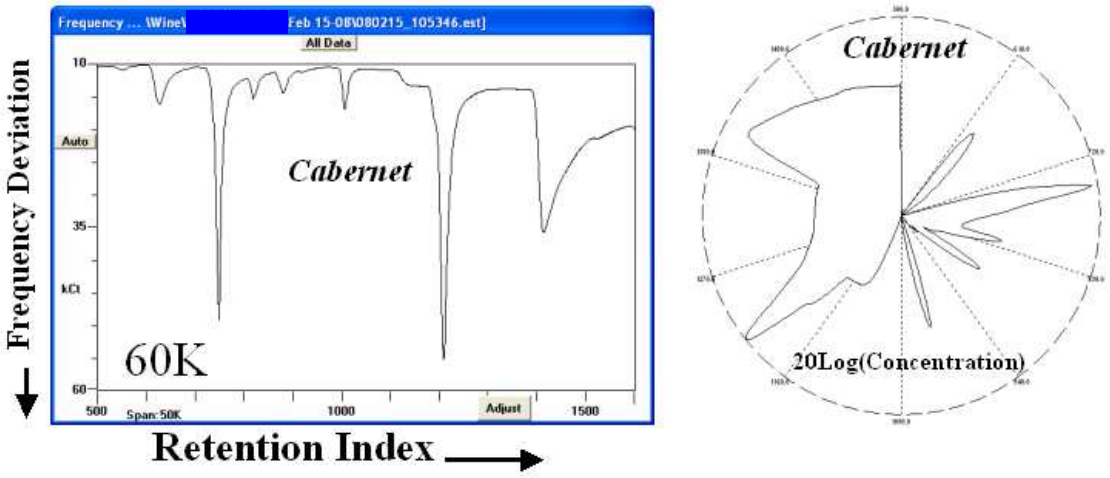

Figure 6- Cabernet wine aroma intensity vs. retention index can be used to create olfactory diagrams or Vaporprint™ images. (5ps2a1b method, 20° detector, 10-second sample)

Taking the derivative of aroma intensity vs retention index results in chromatogram spectra, which effectively separate the individual chemicals within the aroma. In effect, the intensity or concentration of individual chemicals is equal to the integral of the aroma's chromatogram. This is a familiar relationship to all chromatographers. To illustrate shown in Figure 7 is the chromatograms of merlot and cabernet wine aromas superimposed for comparison.

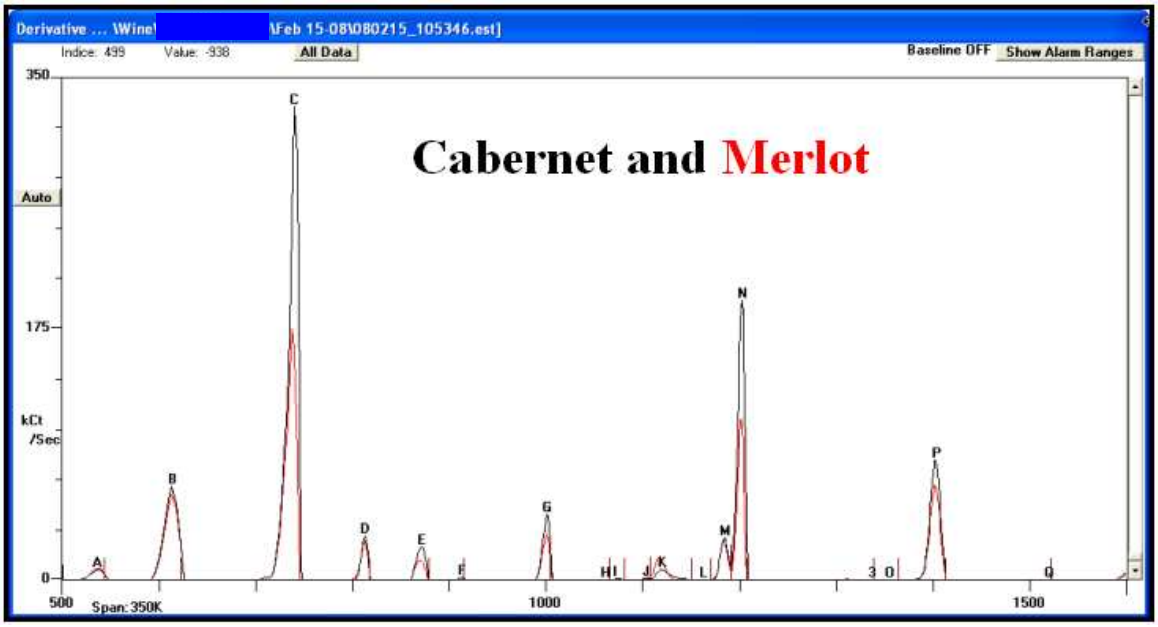

Figure 7- Chromatograms of merlot and cabernet wine overlaid for comparions.

Measurement Precision and Accuracy

Precision is the ability to repeat a measurement result. Precision is measured by calculating the standard deviation of a series of measurements as shown in the offset replicate chromatograms shown in Figure 8. Standard deviations less than 2% are routinely achieved with the zNose®.

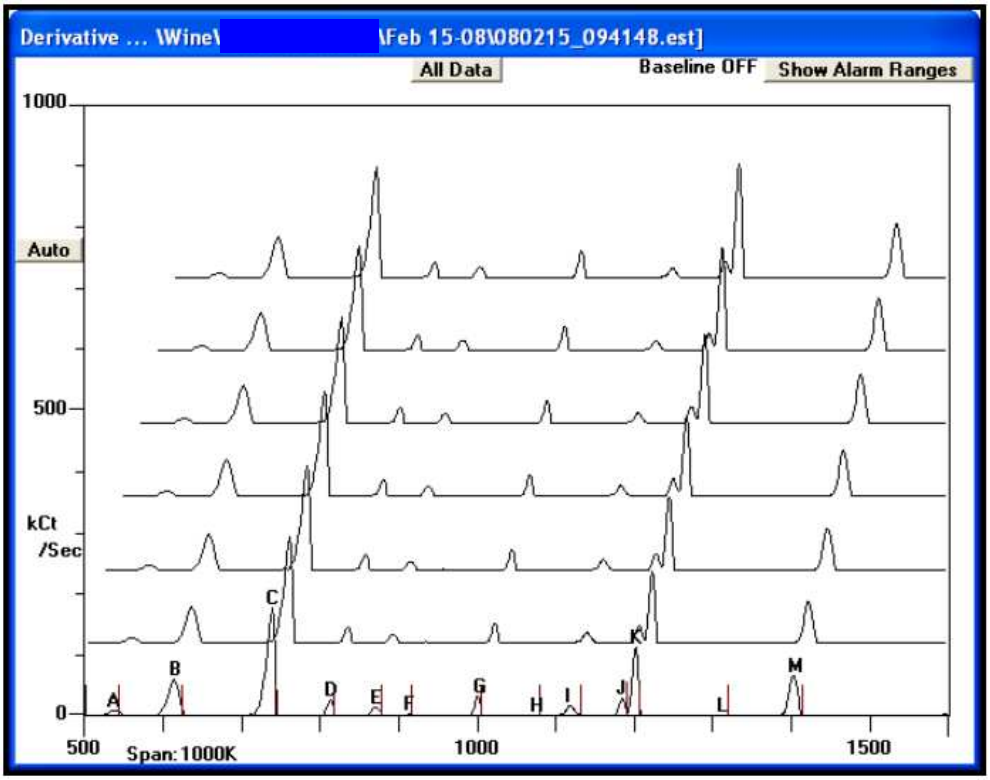

Figure 8- Repeatable results indicate high precision in zNose measurements.

Accuracy is the ability to arrive at the correct answer. For chemical measurements accuracy takes two forms: (1) compound identification and (2) compound concentration. For the zNose compound identification is dependent upon retention index or retention time.

The retention index of an individual compound 'peak' is measured by comparison to the retention times observed using an n-alkane aroma standard. Compounds identification is then determined using an index look-up table or library in software. Retention indices are also published in a NIST database and can be experimentally verified using individual compound standards.

Relative comparison of compound vapor concentration is simply a matter of comparing compound peak areas measured in counts. Absolute compound concentration is achieved by calibrating the instrument with vapor standards of known concentration. Vapor standards can be created by injecting liquid standards into closed containers such as septa sealed bottles or nylon bags equipped with septa. As an example shown in Figure 9 is a merlot chromatogram in which the primary compounds have been identified and their concentration displayed as relative vapor pressure in counts.

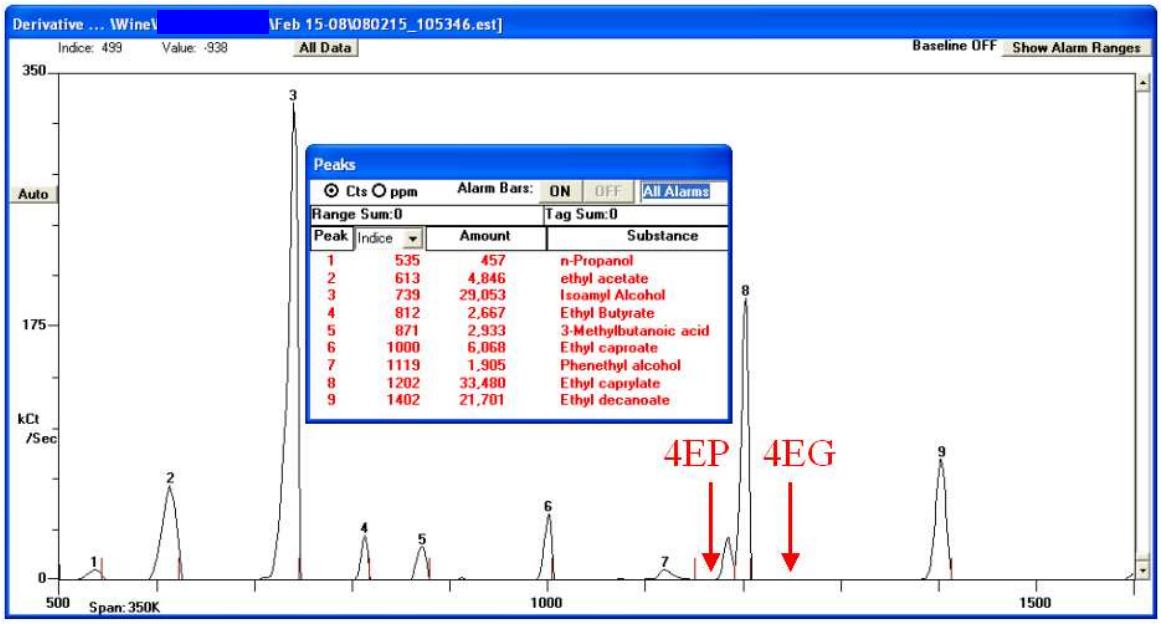

Figure 9- Compounds identification based upon Kovats retention index.

The primary compounds identified in Figure 9 are collectively referred to as the wine matrix and their relatively high concentrations are measured in parts per million. Because the concentration of the wine matrix is high it can be difficult to detect wine flaws such as the presence of 4-ethylphenol and 4-ethylguaiacol. These two compounds have retention indices of 1269 and 1272 as shown by red arrows. They are often used to indicate the presence of Brettanomyces yeasts in the wine.

Expanding the region where these compounds occur (figure 10) in the chromatogram clearly shows their concentrations are well below that of the wine matrix. In this case the relative concentration of 4-EP is 157 counts and 4-EG is 228 counts. Comparison with the counts shown in the table of Figure 8 clearly shows that the concentrations are in the part per billion range -- more than 100 times lower than the wine matrix itself.

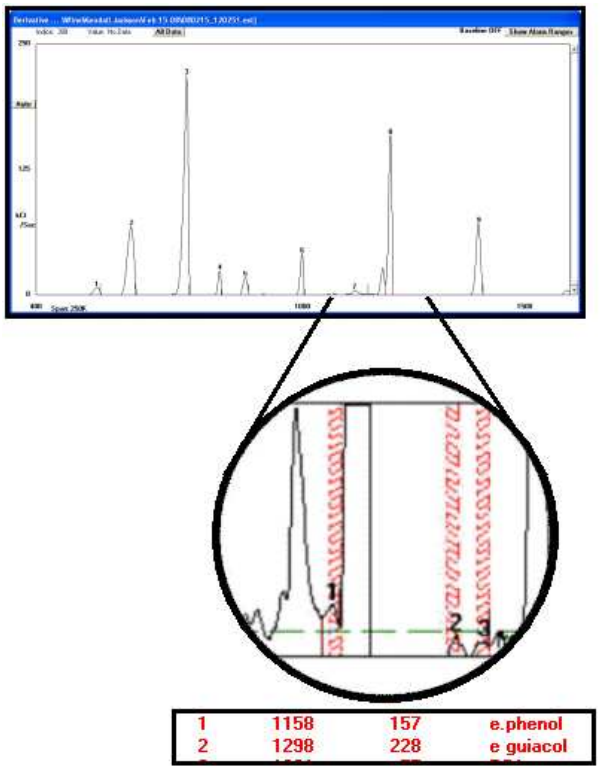

Figure 10- Wine flaws contribute chemicals such as 4-EP and 4-EG in the part per billion range.

The zNose is equipped with a sophisticated array of features, which allow the instrument to be tuned to specific regions of the chromatogram while decreasing or eliminating the effects of others. This is particularly useful for reducing the effects of the wine matrix and making it easier to measure wine flaws in the part-per-billion concentration range. As an example it is possible to use wait states or delays in the column ramping and taking of data. The elimination of high concentration early peaks in the chromatogram by means of wait states is shown in Figure 11.

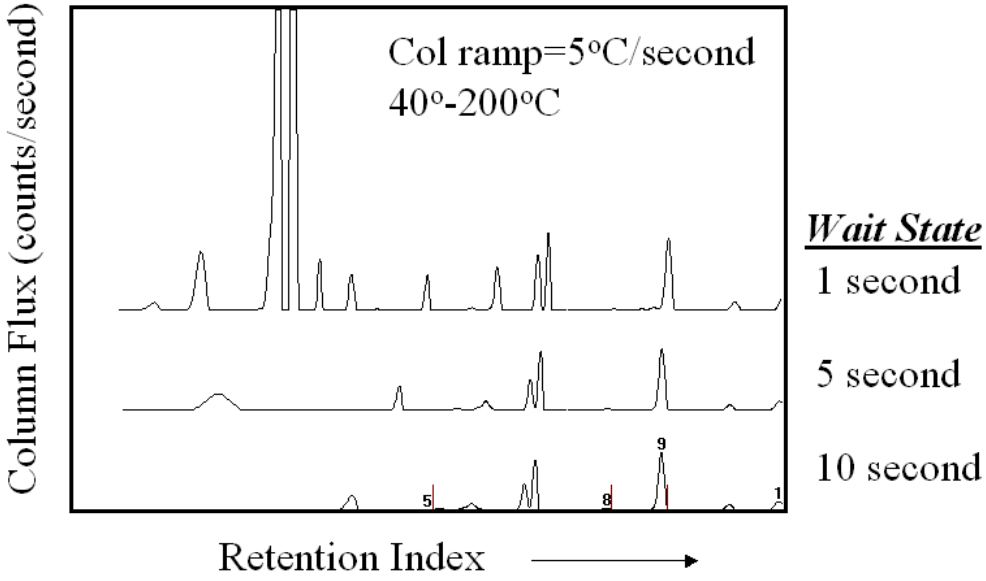

Figure 11- Vertically offset wine chromatograms showing the effects of wait states.

Slowing the column ramping rate and raising the starting temperature increases the resolving power of the measurement as shown in the alkane chromatogram of figure 12.

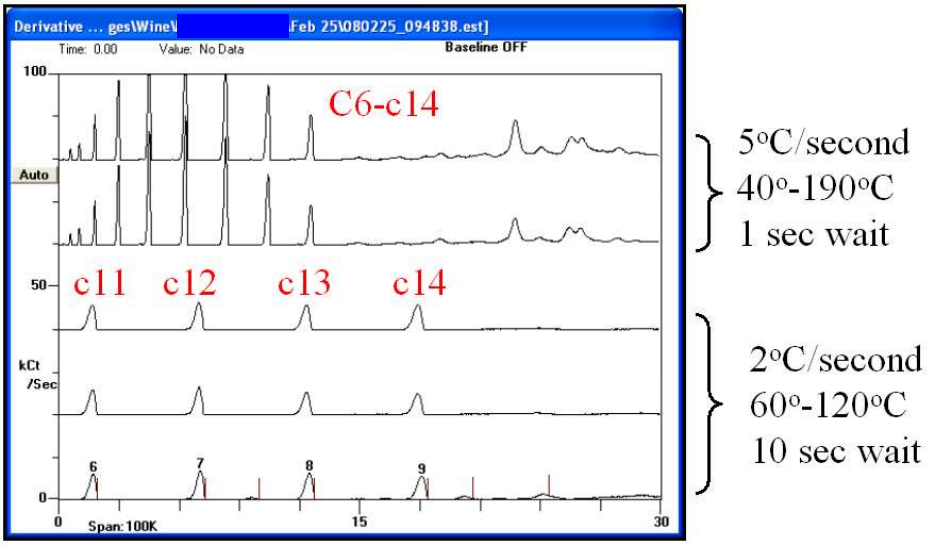

Figure 12- Alkane chromatogram showing effects of increased starting temperature and slower column ramping rate.

The application of the previous method to a headspace vapors from a merlot wine as well as from 4-EP and 4-EG standard is shown in Figure 13. In this figure it can be seen that baseline separation of 4-EP and 4-EG from the wine matrix compounds has been achieved.

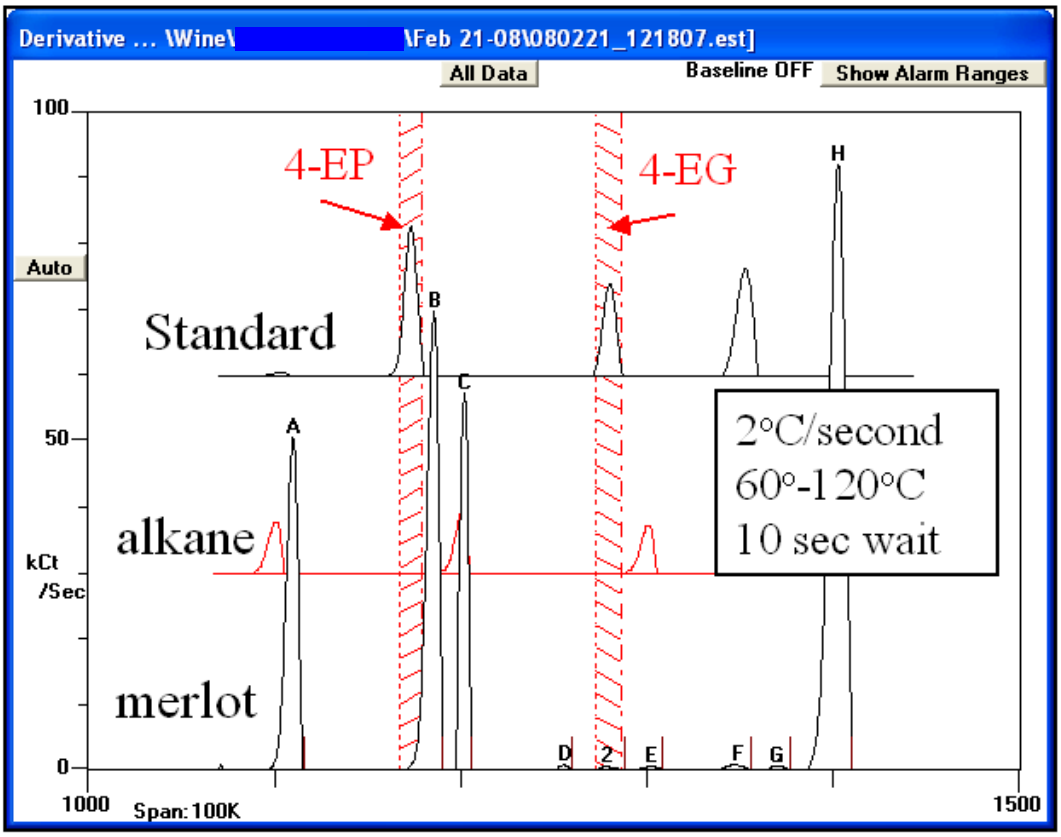

Figure 13

Having achieved this the remaining task was to increase sensitivity of the method such that the ppb is achieved. This was accomplished using another stage of preconcentration with a sample pump and preconcentration tube as shown in Figure 14.

Figure 14

After collecting headspace vapors from the wine sample the desorber tube is attached to the inlet of the zNose, heated to 180°C, and analyzed with the higher resolution method as shown in Figure 15.

Figure 15

Two different wines, merlot and cabernet, were used to test the method. Four spiked samples of each were used containing 500 ppb, 250 ppb, 50 ppb, and no 4-EP and 4-EG. A typical chromatogram comparing headspace vapors from a wine and one from a standard are shown in Figure 16.

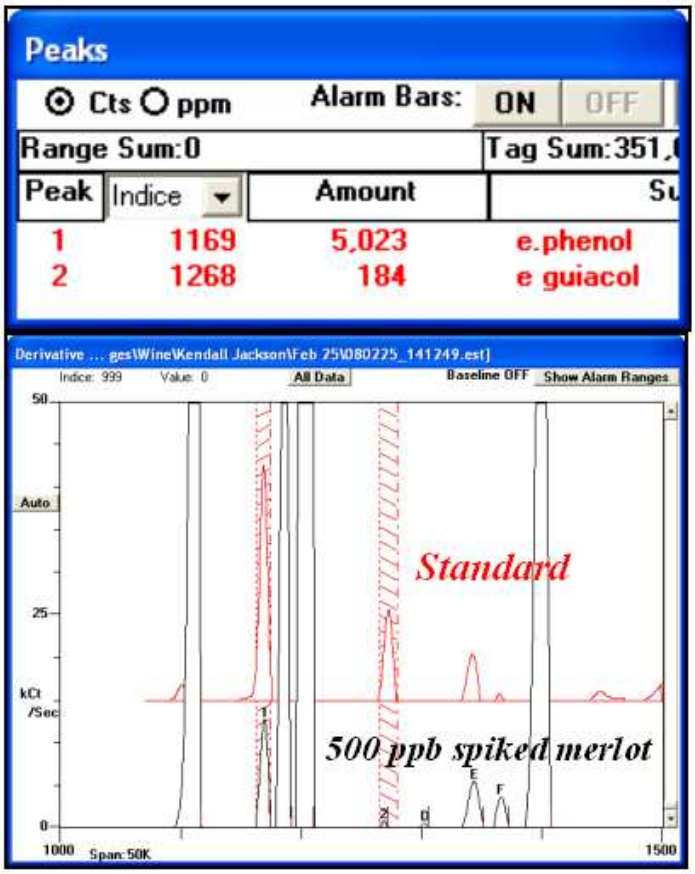

Figure 16

Four overlaid chromatograms (expanded) showing the results for all four merlot samples is shown in Figure 17. It is clear that the unspiked wine sample contained 4-EP.

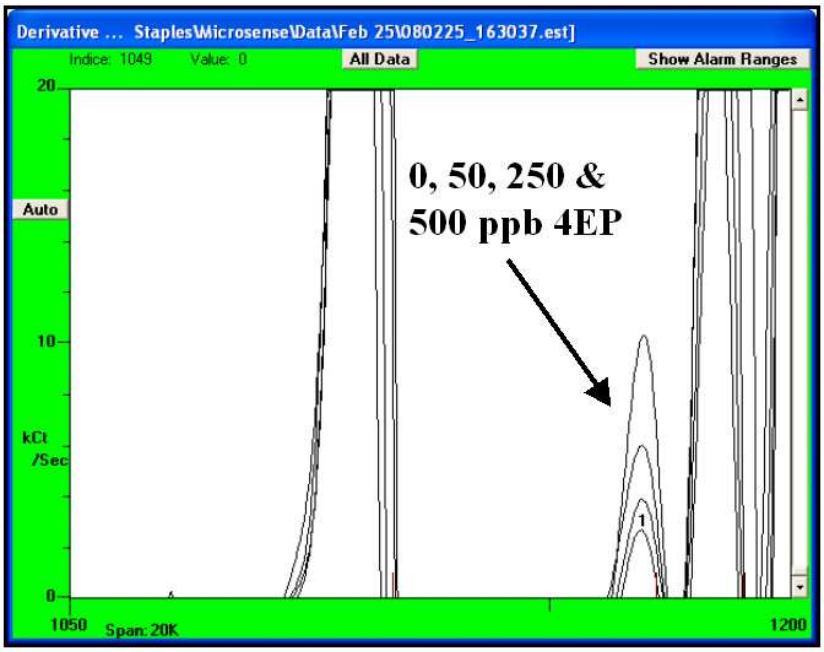

Figure 17

Plotting the resulting concentrations in counts is shown in Figure 18. The inset equation can be used to predict the concentration of the unspiked wine.

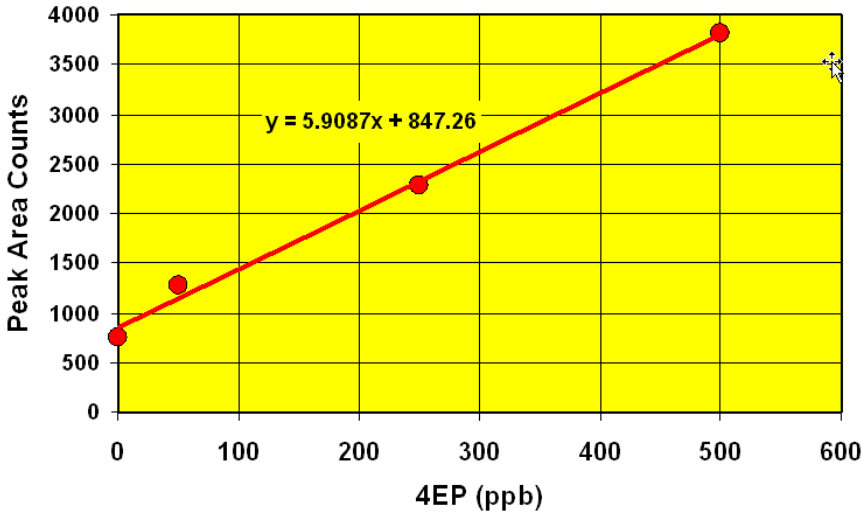

Figure 18

Summary

The management of Brettanomyces and other yeasts is fast becoming one of the most important winemaking issues. Winemakers need new tools for reliable and early detection of Brettanomyces within their facilities before the wine quality is altered. One such tool is the zNose®, an ultra-fast and portable gas chromatograph. Acting more like an electronic nose or chemical flashlight, this instrument can go anywhere and report the chemical composition and concentration of odors, aromas, and other vapors within seconds.

In this paper a simple method for detecting 4-ethylphenol and 4-ethylguaiacol in wine has been described and results using spiked wines presented. The objective of this work has been to provide winemakers with a tool to help them control and optimize 'brett' aroma in their wine production. The ability to detect these compounds in near real time while on-site is a definite advantage. Several applications for this new technology come to mind. For example it is known that there can be large variations in barrel-to-barrel populations of Brettanomyces. Also, often it is necessary to periodically stir barrels before plating. Measured populations can increase - in some cases by 10-fold or more, so populations need to be measured during aging.

Wood cooperage itself is a frequently cited source of Brettanomyces within a winery. Some wineries encourage enologists to destroy Brettanomyces-infected barrels to avoid further contamination within the winery. Another problem is barrel-to-barrel variations within the same lot. The zNose® is an excellent tool for characterizing barrel aromas and could be put to good use in monitoring the health of barrels within a winery.

. The ability to quantify and monitor the presence of wine aroma chemicals and others in real time may find many other applications within a winery. The following uses are suggested:

- To develop strategies that enable winemakers to reduce or eliminate wine spoilage by elucidating the environmental factors.
- To understand the full sensory impacts of compounds formed by yeasts in wine, and elucidate their relative sensory importance.
- To help develop analytical methods for volatile grape and wine aroma and flavor components and their precursors
- To help define the relationship between wine composition and wine flavor.
- To determine the origin, mode of formation and fate of volatile wine aroma and flavor compounds.