

Brettanomyces Monitoring by Analysis of 4-ethylphenol and 4-ethylguaiacol

NEW CAPABILITIES

Brettanomyces yeast are unique in their ability to synthesize the volatile phenols 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG). These compounds are an important part of the *Brettanomyces* character in wine and are important for monitoring *Brettanomyces*.

ETS Laboratories has offered 4-ethylphenol as a tool for monitoring *Brettanomyces* in wine since 1993. New methods and equipment now allow reporting of 4-ethylguaiacol as a standard part of the 4-ethylphenol report.

BRETTANOMYCES AND ETHYL PHENOLS

Hersztyn and Chatonnet demonstrated the strong link between 4-EP in wine and *Brettanomyces*. Subsequent research has shown that *Brettanomyces* is the only common organism in wine capable of producing 4-ethylphenol. As a unique by-product of infection, 4-EP is an excellent indicator of *Brettanomyces* presence and activity.

- 4-EP, in any reportable concentration, indicates the presence of *Brettanomyces*
- the concentration of 4-EP is related to the concentration of *Brettanomyces* and its activity
- increases in 4-EP concentration indicate an active *Brettanomyces* population

Brettanomyces (Dekkera) Yeast



EARLY DETECTION

Analysis of wines for 4-EP enables wineries to identify *Brettanomyces* populations at very low levels, around 2 col/mL. Knowing that *Brettanomyces* is present, modest amounts of SO₂ can be added to arrest development and the wine can be targeted for monitoring.

POPULATION CONTROL

4-EP can be used to monitor the effectiveness of *Brettanomyces* control programs in a wine where *Brettanomyces* is known to exist. First, a baseline concentration of 4-EP is determined. Following treatment, wines with static 4-EP concentrations can be assumed to have inactive populations. Increases indicate continued *Brettanomyces* activity and ineffective treatment.

SIGNIFICANCE OF 4-ETHYLGUAIACOL (4-EG)

4-EG is also a valuable indicator of active *Brettanomyces* populations. As with 4-EP, 4-EG is synthesized from cinnamic acid precursors, but at lower concentrations (the average ratio is 8:1). The aroma profiles of 4-EG and 4-EP are distinctly different. Their combined concentration can be used to estimate the sensory impact of *Brettanomyces*. Relative concentrations of 4-EG and 4-EP influence the character of *Brettanomyces* sensory effects.



DEPENDABLE:

Reliable results on time, every time.

PROMPT:

Turnaround times that meet your production needs.

CONVENIENT:

Heart of the Napa Valley location, extended hours during harvest, 24-hour drop box.

ACCURATE:

International Standard ISO Guide 25 Accredited Quality System.



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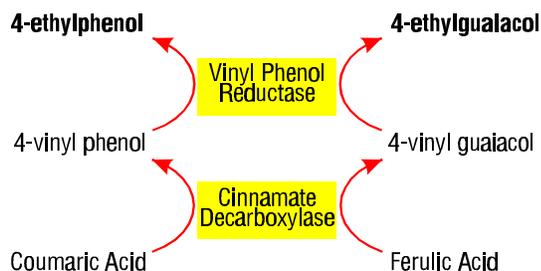
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The biosynthesis of ethyl phenols by *Brettanomyces* is based on the enzymatic conversion of vinyl phenols derived from cinnamic acids.



Brettanomyces Monitoring by Analysis of 4-Ethylphenol and 4-Ethylguaiacol

SENSORY IMPACT

4-EP and 4-EG are both often described as “phenolic” (like phenol). 4-EP is also described as “medicinal” and “band aid”, while 4-EG is more often described as “spicy” and “smoky”.

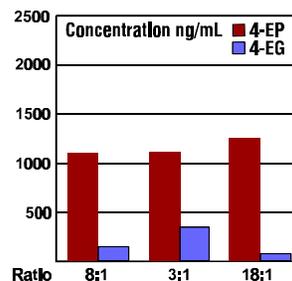
4-EG is present in much lower quantities in red wine than 4-EP, typically about 8 times less. However, it is a more volatile compound with a sensory threshold much lower than 4-EP. In a wine with *Brettanomyces*, both compounds may be well above sensory thresholds.

Perceived Brett character in red wine is influenced by the concentration of both compounds. Variation in the concentration of 4-EG helps to explain why the flavor and intensity of perceived Brett character can be very different with wines having similar 4-EP concentrations.

Analysis for 4-EG in conjunction with 4-EP offers a more complete and superior analytical tool than testing for a single compound. It also offers several advantages over simple colony counts derived from plating wine samples on specialized media.

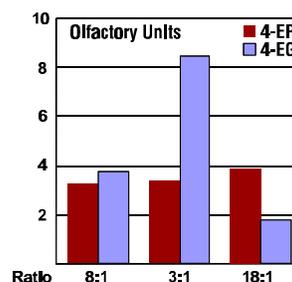
A comparison of ethyl phenol content in selected wines illustrates the importance of their relative concentration.

Graph #1



Graph #1 displays measured concentrations of 4-EP and 4-EG in three wines. In each case, the concentration of 4-EP is fairly consistent. The major difference is the relative concentration of 4-EG. Displayed ratios of 4-EP:4-EG range from 3:1 to 18:1.

Graph #2



Graph #2 presents the same data in terms of the relative sensory impact. “Olfactory Units” are calculated by dividing the concentration by the respective sensory threshold.

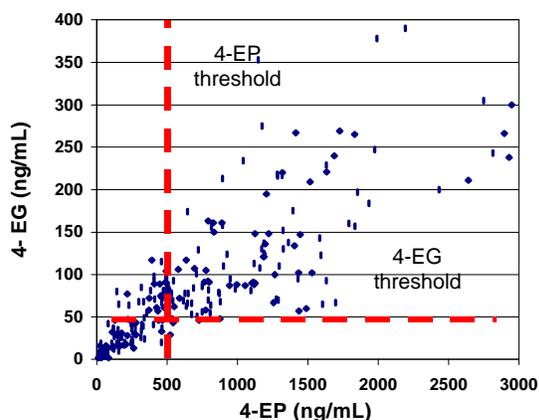
The results clearly illustrate the significance of 4-EG to the overall sensory impact of *Brettanomyces*.

Plating vs. Ethyl Phenol Analysis

Plating on specialized media followed by microscopic evaluation can be used to monitor *Brettanomyces* presence and populations. However, there are several limitations to plating which make chemical analysis an attractive adjunct or alternative.

- *Brettanomyces* yeast are not uniformly distributed in tanks or barrels so representative samples are often difficult to obtain.
- Incubation and confirmation can take 14 days.
- Other yeasts with similar growth characteristics can result in false positives.
- Low cell viability due to SO₂ additions often results in false negatives.

Survey of 4-EP and 4-EG in Red Wines



4-EP and 4-EG as analyzed in 280 red wines. The ratio of 4-EP to 4-EG ranges from a low of 3:1 to a high of 41:1. Dashed lines represent published sensory thresholds for each compound.



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Analytical Details:

Methodology:
Coefficient of Variation:
Min Reportable Quantity:
Equipment Used:
Sample Size Required:
Target Response Time:
Fee Per Sample:

GC/MS with deuterated internal standard and selected ion monitoring
Approximately 3% for 4EP
4 ng/mL (ppb) - typically resultant from approximately 2 colonies/mL.
HP 5973 GC/MS with CTC solid phase micro extraction (SPME)
Representative 30 mL sample
24 hours from receipt of samples
1 Sample: \$60 2-10 Samples: \$48 >10 Samples: \$44

Brettanomyces Monitoring by Analysis of 4-ethylphenol and 4-ethylguaiacol

Q - Is *Brettanomyces* really a problem?

Yes and no. It all depends on your opinion of the sensory impact of *Brettanomyces*. Some winemakers are oblivious to the aromas that result from *Brettanomyces* or feel that they add to complexity. Others want no *Brettanomyces* sensory impact and will do everything possible to control or prevent *Brettanomyces* growth.

Q - How are 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) formed?

4-EP and 4-EG are formed from cinnamic acid precursors in wine. There are several steps in the synthesis pathway. The first steps are common to several wine microorganisms. The last step, the conversion of vinyl phenols to ethyl phenols is unique to *Brettanomyces*. As a unique by-product of *Brettanomyces*, 4-EP is thus an excellent indicator of *Brettanomyces* presence and activity.

Q - Is *Brettanomyces* activity the only source of 4-EP and 4-EG?

In a word – Yes. Neither the work done by Chatonnet in France, nor the validation work done here at ETS, has revealed any reason to suspect another source of significant amounts. Neither 4-EP or 4-EG are normal constituents of red wine. It is quite common to find three and four year old barrel samples without detectable levels of either 4-EP or 4-EG.

Q - How do you analyze for 4-EP and 4-EG?

ETS uses gas chromatography with detection by mass spectroscopy (GC/MS). The compounds are extracted from the sample along with a deuterated internal standard by solid phase micro extraction (SPME). This method can detect either compound at 4 ng/mL, low enough to detect minute populations of *Brettanomyces*.

Q - What is adequate sample for analysis?

Samples should consist of a representative sample of at least 30mL. Ethyl phenols are well distributed within an individual tank or barrel, but sampling must take into consideration the high degree of variation between individual barrels or tanks.

Q - Are there other analytical techniques that I should consider?

Plating on selective media can confirm active *Brettanomyces* populations, but there are several problems inherent in the technique. Unlike the 4-EP, the *Brettanomyces* population is not uniformly distributed within barrels. Sampling is relatively complicated and stirring is often required. Plating is time consuming (plates are cultured a minimum of 12 days), and often result in false negatives.

Frequently Asked Questions

Brettanomyces Analysis by 4-EP and 4-EG...

Q - How can I best apply the analysis in my winery?

There are several applications for 4-EP/4-EG analysis. Two very beneficial uses are:

1) Early detection and control of developing *Brettanomyces* populations.

Analysis of 4-EP allows the winemaker to detect developing populations at very low levels, often less than 2 col/mL. This allows the winemaker to arrest the development of *Brettanomyces* at very early stages with only modest SO₂ additions.

2) Control of established populations.

4-EP can be used to monitor the effectiveness of *Brettanomyces* control programs in a wine where *Brettanomyces* is known to exist. First a baseline concentration is determined. Following treatment, wines with static 4-EP concentrations can be assumed to have inactive populations. Increases in 4-EP indicate continued *Brettanomyces* activity and ineffective treatment.

Q - Can ethyl phenol analysis tell me if there is active *Brettanomyces* in my wine?

When applied properly, yes. In the case of young wines beginning at < 4 ng/mL, any measured 4-EP and 4-EG after time is an indicator of *Brettanomyces* activity. For other wines with unknown ethyl phenol history, two data points are required. An initial number must be first obtained to know the current content of 4-EP and 4-EG. Once this number is known, any increase in concentration indicates active *Brettanomyces*.

Q - What do 4-EP and 4-EG have to do with “Brett Character”?

An active *Brettanomyces* population will create several by-products including 4-EP and 4-EG. These two compounds are responsible for “phenolic” notes associated with the “Brett” character. 4-EP is usually described as “medicinal” or “band aid”, while 4-EG is more often described as “spicy” and “smoky”.

Q - What are the sensory thresholds of 4-EP and 4-EG?

The sensory threshold will vary on the taster and the wine matrix, but generally a wine is described as having a *Brettanomyces* like character when 4-EP reaches between 300 and 600 ng/mL. 4-EG has a lower threshold, somewhere around 50 ng/mL. Contribution of both compounds to the “Brett” character depends on their absolute and relative concentrations.

Q - Do 4-EP and 4-EG always have a constant ratio in wine?

Typically, 4-EP is about 8 times higher in wine than 4-EG. The ratio can vary however. Three hundred red wines recently analyzed at ETS had 4-EP: 4-EG ratios between 3:1 to 22:1. Variation in the 4-EG level can help to explain why the flavor and intensity of perceived “Brett” character can be very different in wines having similar 4-EP levels.

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