New Tools to Limit Wine Spoilage

Interaction of temperature and ethanol may help control *Brettanomyces*

By Melissa Hansen

Once *Brettanomyces bruxellensis* takes hold in a winery, eradication is formidable, often requiring more than one approach to keep the wine-spoilage yeast at bay. Recent findings by Washington State University show that the interaction between storage temperature and alcohol concentration may be a useful tool to manage *Brettanomyces*.

Brett, as it is commonly called in the wine industry, is a wild yeast associated with the spoilage of red wine. Unlike other yeasts such as *Saccharomyces*, which converts sugars to alcohol and carbon dioxide, Brett is very difficult to control. Though some wineries prefer the earthy and gamey aromas imparted by small amounts of Brett in red wine and believe it adds complexity and aging ability to young wines, a little can quickly turn into too much. When concentrations exceed sensory thresholds, Brett can result in wine with "barnyard" aromas that smell like a wet dog, horse stables, sweaty horse blankets, wet wool or sweaty shoes.

Dr. Charles Edwards, a professor at Washington State University in Pullman, focuses his research on problem alcoholic fermentations and spoilage organisms of concern to winemakers. His most recent study, supported by the Washington state wine industry and the Washington State Wine Commission, investigates factors that impact Brett's growth and ability to survive under various conditions.

Edwards' research includes taking oak barrels apart to learn the depth of penetration and survivability of Brett in oak staves under various conditions and sanitation treatments such as steam, and examining Brett survivability in the winery waste/pomace. The overall goal of his research is to develop effective Brett control measures for wineries.

KEY POINTS

Cold storage temperature is critical to prevent Brett spoilage.

Alcohol content is a key factor in a wine's susceptiblity to Brett.

Different strains of Brett can behave and react differently to control methods.

Brian Carter, winemaker and owner of Brian Carter Cellars, says alcohol, sulfur dioxide and cellar temperature are the three best tools against Brett contamination.

Edwards' work with graduate student Taylor Oswald was recently published in the *American Journal of Enology & Viticulture* (68:2 2017). His report, "Interactions between Storage Temperature and Ethanol that Affect Growth of *Brettanomyces bruxellensis* in Merlot Wine," is summarized below.

Temperature and ethanol (alcohol) concentration are two factors known to affect the growth of *B. bruxellensis* in synthetic media and wine. Previous research found that the optimum temperature for growth of *B. bruxellensis* was from 77° to 83° F in synthetic media.[4] Growth was found to stop above 95° F.[3]

Growth in wine and synthetic media have been observed at temperatures as low as 50° F. Researchers also have found that *B. bruxellensis* can tolerate ethanol concentrations as high as 15% (v/v),[1] but few studies have looked at the interactions among control methods as a way to limit or manage spoilage by *B. bruxellensis*. For example, the interactions between sulfur dioxide and dimethyl dicarbonate and sulfur dioxide and temperature can affect yeast viability.[2,6] Additionally, interactive impacts have been studied at pH of 3.4, 3.7 or 4.0; alcohol at 10%, 12.5% or 15%, and free sulfur dioxide of 0, 30 or 50 mg/L on *B. bruxellensis*.[5]

"Our study looked for a relationship between alcohol concentrations and storage temperatures," Edwards says. "We wanted to see if winemakers could use ethanol (alcohol) present in the wine and the temperature of storage together as another barrier to manage Brett."

The WSU researchers found that the interaction between storage temperature and alcohol concentration is important and can help wineries limit and lower the risk of Brett infections. The combination of storage temperatures below 54° F and alcohol concentrations of 14% or more resulted in a decline of Brett populations below the detection limit for up to 100 days.

Washington state winemaker Brian Carter of Brian Carter Cellars believes the research findings are useful Brett-management tools. Although he uses temperature to help control Brett in his winery in Woodinville, Wash., this information reinforces his practices. "Alcohol levels, temperature and sulfur dioxide are the three most important defenses to use against Brett," he says.

Although many small wineries do not have multiple barrel and storage rooms, Carter says the synergistic interactions between alcohol level and storage temperature can still be applied. "Armed with this information, winemakers should avoid situations where lower alcohol wines are kept at higher temperatures for extended periods of time. This would be particularly true during malolactic fermentation, when free sulfur dioxide levels are low or non-existent."

Carter suggests that winemakers could use the information to make decisions on the basis of wine style. If Brett is an issue in a winery, winemakers could opt to have higher alcohol (15% or more) in their wines as a means to prevent Brett.

Bottom line: Cold storage temperatures can help lower the risk of Brett. Wines with low alcohol have a higher risk of Brett than high-alcohol wines and should be kept in the coldest rooms of the cellar, when possible. Knowledge that high-alcohol wines have lower risk of Brett could potentially be part of a winemaker's decision-making process if Brett is a problem in the winery.

Study methods

The study used two strains of *B. bruxellensis*, F3 and I1a, in Merlot wines obtained from a commercial winery. Total sulfur dioxide was measured and removed. The wines of five alcohol concentrations (12%, 13%, 14%, 15% and 16%) were held at four temperatures (54°, 59°, 64° and 70° F). Volumes of wine were fixed. Variable proportions of ethanol:water mixtures were added to yield wines with different alcohol percentages. After supplementing the wines with glucose, fructose and a yeast extract, pH was adjusted to 3.75. The wines were sterile-filtered and incubated for 24 hours under the four temperature treatments before inoculation with the same amount of the two Brett strains (10^4 cfu/mL, the number of colony forming units per milliliter).

The wines were monitored for 100 days by sampling twice per week for the first four weeks and then once per week thereafter. After 100 days, concentrations of volatile acidity (primarily acetic acid), 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) were quantified. 4-EP is the compound responsible for contributing earthy and rustic sensory characteristics, also described as animal, smoky or medicinal aromas. 4-EG is the compound associated with bacon, spice and clove aromas. Both compounds are associated with the earthy and barnyard odors resulting from Brett-tainted wines.

Temperature and alcohol interaction

A synergistic response from the interaction of storage temperature and alcohol concentration impacted the growth and production of acetic acid, 4-EP and 4-EG in both strains of Brett. The combination of low storage temperatures (54° F or less) and higher alcohol concentrations of 14% or more resulted in populations at levels below the detection limit of 30 cfu/mL for up to 100 days. At the lowest temperature of 54° F or less, neither strain of Brett grew well, regardless of the alcohol level.

However, when temperatures were 64° F or higher, both strains grew well in the wines containing 12%, 13% and 14% alcohol and reached peak populations of 10^6 cfu/mL in those wines in 40 days or less. Growth of Brett was inhibited in the 15% and 16% alcohol wines, and the high alcohol wines also showed longer lag phases at the lower temperatures.

Strain I1a reached a population of 10^6cfu/ml within 20 days of inoculation in the 12% v/v alcohol wine maintained at 64° F, but it required 40 to 70 days to reach the same population in the 13% and 14% alcohol wines maintained at the same temperature. In the low-alcohol wines of 12%, stored at the coldest temperature of 54° F, both strains showed a decrease in culturability, and recovery was slowed or nonexistent. Additionally, growth of the two strains was retarded in wines above 14% alcohol.

Both strains could not be recovered from the 16% alcohol wines regardless of temperature.

Strain differences

The two strains generally displayed similar growth patterns under the various temperature and alcohol treatments, except for the 15% alcohol wines, where strain differences were observed. Here, F3 appeared to be more inhibited and showed less growth and reduced concentrations of acetic acid, 4-EP and 4-EG at the higher temperatures than strain I1a. The F3 strain never reached populations above 10^4cfu/mL in the experiment, even when wines were held at 70° F.

The different responses of the two strains in the 15% alcohol wines reinforces the concept of genetic diversity within the *Brettanomyces* genus. "What that suggests is that you have to be very careful about what strain you have in your winery, because there are situations where they behave differently," Edwards says. "The diversity of *Brettanomyces bruxellensis,* a single species, seems to be quite large. We do not know much about Brett strains from a genetic standpoint or how they behave in different matrices."

What about cellar humidity?

Humidity was not part of Edwards' study. Humidity of a cellar does affect the loss of water and alcohol through the wood. Humidity is often added to barrel rooms to control topping losses, and there is some cooling effect from humidity, but humidification is done in addition to using a refrigeration system for temperature control. From a temperature point of view, temperature affects humidity but not vice versa, Edwards explains.

"Humidity is crucial to maintain during storage because you are trying to balance the ethanol and amount of water passing through the wood," he says. "But this study looked for whether or not there was a relationship that winemakers could use between the amount of ethanol present in the wine and storage temperature."

Wines in the study were not stored in wood, and Edwards did not try to learn if exterior humidity has an effect on the growth of microorganisms inside a wood barrel. If there is any effect from humidity, he believes it would be indirect.

Defenses and barriers

Defense technology is a risk-management concept used in food manufacturing. It is based on the theory that you should not rely on just one approach to limit the growth of spoilage microorganisms but should use a series of barriers or defenses that the organism must overcome to still remain active or in large enough concentrations to be an issue. If enough defenses are used, the organism is eliminated or eradicated. In the case of Brett, the goal is to control levels below sensory thresholds.

Several methods to control *B. bruxellensis* previously evaluated by the Edwards laboratory include the use of sulfur dioxide, dimethyl dicarbonate, chitosan and filtration. These are all helpful barriers that have been shown to be useful to manage Brett.

With today's trend of higher alcohol wines ranging from 14% to 15%, winemakers may be able to take advantage of cellar temperature to head off potential issues with this particular yeast, Edwards notes.

"Each winemaker has to consider all the parameters of a given wine that pertain to Brett, such as history, pH, sulfur dioxide and filtration," he adds. "In short, what is the risk to that particular lot? Then the winemaker has to find a balance that uses enough risk management barriers without breaking the bank."

Keep it cold

For wineries that have multiple cellar rooms, keeping the lowest alcohol wine in the coldest room may be a viable option. For those with one cellar room, Edwards suggests wine be kept at the lowest temperature that is economically feasible. He recognizes that energy costs for refrigeration can be expensive in some wine regions, but where feasible, cold temperatures can be used as another barrier against Brett.

Melissa Hansen, research program manager for the Washington State Wine Commission, works to make viticulture and enology research supported by the Washington wine industry more accessible to the state's growers and winemakers. Hansen was a journalist for nearly 20 years for *Good Fruit Grower*, a Washington-based magazine, and was involved with California's table grape and tree fruit industries for 15 years.

To see acknowledgments and references for this article, go to winesandvines.com and search under Magazine > Features > June 2018.