REPORT

Creation and Use of a *Brettanomyces* Aroma Wheel

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Cite this article:
Joseph CML, Albino E and Bisson LF. 2017. Creation and use of a Brettanomyces Aroma Wheel. Catalyst 1:12-20.

Summary

Goals: The ability of the yeast *Brettanomyces* to produce negative aroma attributes from grape phenolic precursors is well known. However, this yeast synthesizes a broad array of aroma-active compounds in wines, some of which are valued or deemed positive in certain wine styles or matrices. To better understand the spectrum of positive and negative aroma traits associated with the presence of *Brettanomyces* in wine, we created an aroma wheel to categorize and describe the variety of aroma impacts of this yeast on the basis of analyses of aroma-active compound production from spiked precursor compounds in a model controlled environment.

Key Findings:

- The *Brettanomyces* aroma wheel categorizes many of the aroma descriptors associated with wine spoilage by *Brettanomyces*.
- Terms on the wheel may categorize aroma-active compounds generally associated with microbial infection of both *Brettanomyces* and lactic acid bacteria (LAB).
- There is no evidence for a *Brettanomyces* strain that produces only positive characters in wine.

Impact and Significance: A comprehensive *Brettanomyces* aroma wheel was generated with different strains under controlled product–precursor conditions and tested for broader utility in commercial winemaking. Thirty commercial wines described by wine critics using two or more of the identical or similar terms to those found on the *Brettanomyces* aroma wheel were obtained from a retail outlet. All wines purchased had evidence of presence of *Brettanomyces*, LAB, or both. The inner circle, or general categories of the wheel, were used more commonly by critics in describing wines and the specific terms (outer wheel) less generally used as commercial wine descriptors. The terms presented on the aroma wheel can therefore be used to determine if a wine is likely to have sensory characteristics contributed by *Brettanomyces* or LAB. This appeared to be particularly true for the general categories "savory," "woody," "spicy," "floral," "earthy," "chemical," and "animal." Thus, the wheel can be used to identify wines with a strong, but not necessarily negative, microbial signature.

Key words: *Brettanomyces*, ethylphenol, lactic acid bacteria, odor-active compound, wine aroma

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Manuscript submitted Apr 2016, revised Aug 2016, accepted Aug 2016

doi: 10.5344/catalyst.2016.16003

Overview

The yeast *Brettanomyces* was initially found in beer in the early 1900s by Claussen[1], but much more research has since been conducted on its role as a spoilage agent in wine. *Brettanomyces* was identified as the cause of off-characters in South African white wines by van der Walt and van Kerken[2]. Seminal studies by Heresztyn, Chatonnet et al., and Licker et al.[3] identified some of the primary compounds associated with the *Brettanomyces* taint and investigated the conditions required to produce them. These volatile phenolic taints are derived from grape precursors. The two most commonly formed compounds,

4-ethyl phenol (4EP) and 4-ethyl guaiacol (4EG), are described as "animal," "barnyard," or "sweaty horse" or as "medicinal," "BandAid™," or "smoky." However, more recent work showed that a wider variety of aroma compounds are produced by *Brettanomyces* from different substrates and different chemical conditions in the wine[4]. Some of these characters would also be generally described as negative, such as "rancid" and "burning tires," whereas others are positive, that is, "floral" and enhanced varietal characteristics.

Anecdotal reports of *Brettanomyces* found in certain wineries giving only positive aroma characteristics prompted winemakers to express interest in the characterization of these strains and the identification of *Brettanomyces* strains that could be safely used as inocula. To address this interest, we screened the 99 independent isolates of *Brettanomyces* that existed in the University of California Davis Department of Viticulture and Enology Wine Yeast and Bacteria Culture Collection to assess the spectrum of end products produced[5]. We conducted these studies in a standard synthetic medium supporting the growth of *Brettanomyces* with and without the presence of ethanol[6]. The use of a synthetic medium enabled full control of the type and level of precursors used as supplements and facilitated product analysis by gas chromatography (GC). In addition, olfactometry GC was used to identify the specific aroma-active compounds associated with specific compounds produced by the yeast using panelists. These studies were augmented by analyses of *Brettanomyces* in actual red wines spiked with different compounds. In these analyses, panelists also assessed the aroma-active characters present in these simple growth solutions as well as in the wine studies[5]. In order to facilitate categorization of the aroma descriptors the panelists used, and to distinguish the different strain and substrate effects[5], we created a *Brettanomyces* aroma wheel.

During these studies, winemakers sent wines for analysis that contained high levels of volatile phenols as determined by chemical analysis, sometimes an order of magnitude above the published level of detection[7], but that did not display the common aroma traits associated with these compounds by nose. The wines were sent in hopes that the strains isolated would prove useful as inocula for wine production. The isolated strains were also included in our previous studies to determine if these strains produced masking compounds, either alternative aroma characters made by the yeast or enhanced from the grape. An alternative explanation, however, was that the matrix of the wine, and not the yeast, was responsible for the masking of these characters. Under defined conditions and in different wine matrices, all of these strains could produce sensorially detectable levels of volatile phenols.

A wide variety of *Brettanomyces* strains were examined under different substrate and oxygen conditions and a large number of aroma-active compounds were identified[5] (summarized in Table 1). All *B. bruxellensis* strains studied could make the compounds 4EP and 4EG, which are considered to be the hallmark of *Brettanomyces* spoilage under low-oxygen conditions when the two cinnamic acids, coumaric and ferulic acid, are present. However, in the presence of other substrates under other conditions, such as amino acids under higher oxygen, these strains can also make a wide variety of other aroma-active compounds. From our previous studies, the major metabolic compounds produced are often not aroma active, and those that contribute to the aroma are often produced in very low amounts[5]. However, the major sensorially active compounds are often closely related to the major products produced by *Brettanomyces*[5].

In the course of this work, we identified 44 compounds either as being major volatile compounds or as compounds that were detectable, using humans as aroma detectors in collaboration with the gas chromatogram and a mass spectral detector (Table 1). Twenty-three (52%) of those compounds were dependent on the substrate that was in the medium and the strain that was assayed. Ten compounds (23%) were dependent only on the substrate availability, and another five (11%) were produced only by some of the tested strains, regardless of the substrate. Six (14%) did not depend on either the strain or the substrate used in the test and were produced by *Brettanomyces* under all conditions[5].

The *Brettanomyces* aroma wheel created with the panelists during these studies potentially represented a useful practical tool to assist in defining the broader aroma impacts of *Brettanomyces* in commercial wines. We assessed the validity of this aroma wheel for the analysis of commercial wines in two ways: (1) by purchasing wines that had been described by wine critics in terms consistent with those found on the *Brettanomyces* aroma wheel and evaluating those wines for the presence of *Brettanomyces*, and (2) by using the wheel in an extension program with more than 100 winemakers in attendance, with tastings of wines spiked with various substrates and strains of *Brettanomyces*

and obtaining assessments of aroma characters from the audience. This paper presents the aroma wheel and the validation thereof, using marketplace wines selected on the basis of commercial descriptions matching multiple terms on the wheel.

Major Observations and Interpretations

The completed aroma wheel is presented in Figure 1. We determined these descriptive terms in a synthetic

Table 1 Chemical compounds produced by different strains of *Brettanomyces bruxellensis* and the aromas they produce.

Chemical compound and CAS number	Type of compound	Substrate dependent	Strain dependent	Major product	Aroma*
2-Ethyl-1-hexanol 104-76-7	Ester	Yes	No	Yes	Citrus, floral
2-Methyl-1-butanol 137-32-6	Alcohol	Yes	No	Yes	Canned fruit, plastic
3-Methyl-1-butanol (isoamyl) 123-51-3	Alcohol	No	No	Yes	Banana, whiskey, chemical
4-Ethyl guaiacol 2785-89-9	Phenolic	Yes	No	Yes	Smoky, clove, spice, phenolic
4-Ethyl phenol 123-07-9	Phenolic	Yes	No	Yes	Phenolic, creosote, band-aid
Ethyl 2-methyl butyrate 7452-79-1	Ester	Yes	Yes	Yes	Mint, citrus, green apple
Phenethyl alcohol 60-12-8	Alcohol, ester	No	No	Yes	Floral, rose
1-Decanol 112-30-1	Alcohol	Yes	Yes	Yes	Waxy, floral, orange
1-Octanol 111-87-5	Alcohol	Yes	Yes	Yes	Citrus, waxy, aldehydic, floral
2-Methyl butyric acid 116-53-0	Fatty acid, ester	Yes	Yes	Yes	Blue cheese, rancid
2-Nonanone 821-55-6	Ketone	No	No	Yes	Fruity, soapy, herbaceous
3-Methyl butyric acid (isovaleric) 503-74-2	Fatty acid, ester	Yes	Yes	Yes	Sweaty feet, cheese
Acetic acid 64-19-7	Organic acid	No	Yes	Yes	Vinegar, sour
β-Farnesene 18794-84-8	Terpene	Yes	Yes	Yes	Woody
Butanol 71-36-3	Alcohol	Yes	No	Yes	Alcohol
Decanoic acid 334-48-5	Fatty acid	Yes	No	Yes	Rancid, sour, fatty
Ethyl acetate 141-78-6	Ester	No	Yes	Yes	Pear, apple, nail polish remover
Ethyl decanoate 110-38-3	Ester	Yes	Yes	Yes	Fruity, apple, waxy
Ethyl dodecanoate 106-33-2	Ester	Yes	Yes	Yes	Soapy, rum, clean
Ethyl isobutyrate 97-62-1	Ester	Yes	No	Yes	Fruity, rum
Ethyl octanoate 106-32-1	Ester	No	No	Yes	Fruity, pineapple, apricot
Ethyl tetradecanoate 124-06-1	Ester	Yes	No	Yes	Waxy, violet
Isobutyric acid 79-31-2	Fatty acid	Yes	Yes	Yes	Rancid, cheese
Octanoic acid 124-07-2	Fatty acid	Yes	No	Yes	Rancid, cheesy
Pentanoic acid 109-52-4	Fatty acid	No	No	Yes	Putrid, rancid, sweat, cheese,
Phenethyl acetate 103-45-7	Ester	Yes	Yes	Yes	Floral, rose, honey
Phenethyl propionate 103-52-6	Ester	No	Yes	Yes	Musty, floral, yeasty
Phenylacetaldehyde 122-78-1	Aldehyde	No	Yes	Yes	Floral, honey
2-Methoxy-4-vinylphenol 7786-61-0	Phenolic	Yes	Yes	No	Woody, cedar, roasted nuts
4-Methoxyphenethyl methanol 105-13-5	Alcohol, ester	Yes	Yes	No	Floral, balsamic, fruit, anise
Amyl-octanoate 638-25-5	Fatty acid	Yes	Yes	No	Wine, elderflower, orris
Bisabolene 495-62-5	Terpene	Yes	Yes	No	Woody, citrus, tropical fruit, green banana
Butyric acid 107-92-6	Fatty acid	Yes	Yes	No	Fruity, cheesy, acetic
Ethyl butyrate 105-54-4	Ester	Yes	Yes	No	Tutti-frutti, pineapple, cognac
Ethyl isovalerate 108-64-5	Ester	Yes	No	No	Fruity, esters, sharp, pineapple
Ethyl valerate 539-82-2	Ester	Yes	Yes	No	Tropical fruit, strawberry, pineapple
Heptanoic acid 11-14-08	Fatty acid	Yes	Yes	No	Fatty, animal
Isoamyl alcohol 125-51-3	Alcohol	No	No	No	Fruity, banana, whiskey
Nonanal 124-19-6	Aldehyde	Yes	Yes	No	Citrus, waxy, melon, aldehydic
Ocimene 502-99-8	Terpene	Yes	Yes	No	Fruity, floral, wet cloth
Octyl butyrate 110-39-4	Fatty acid, ester	Yes	Yes	No	Fruity, oily, fresh or green, earthy
Pentyl formate 638-49-3	Ester	Yes	Yes	No	Fruity, unripe banana, earthy
Phenethyl formate 104-62-1	Ester	Yes	Yes	No	Floral, green, watercress, hyacinth
Undecanoic acid 112-37-8	Fatty acid	No	Yes	No	Creamy, fatty coconut

*Descriptors derived from The Good Scents Company website: http://www.thegoodscentscompany.com/.

environment to define specific product–precursor relationships and confirm production of odor-active compounds by pure cultures of *Brettanomyces*. During discussions with panelists evaluating the synthetic samples, we organized the outer wheel descriptors into the inner wheel broader categories. For example, the smoky character often associated with *Brettanomyces* presence in wine was split into two terms—a smoked meat character under "savory" and a burned beans character under "rotten and putrid," but the smoky character was not considered "spicy." Wine-matrix effects will determine how these characters are perceived in wines, often modulating the more extreme characteristics. The "dairy" characteristics were quite strong in the pure-culture synthetic media for multiple strains of *Brettanomyces*, but are rarely noted for *Brettanomyces* infection in commercial wine-production conditions. The thresholds of detection in synthetic media will obviously differ from those in wines, and detection of the same level of a given compound will vary across different wines. These descriptors may be used as a guide for the types of descriptive terms that indicate microbial impact on wine aroma.

To test the validity of the *Brettanomyces* aroma wheel, we took advantage of the wine descriptions posted by an online wine retailer, K&L Wine Merchants (klwines.com). This site publishes wine descriptions and reviews from a number of sources in a searchable format for wines available for purchase. We searched using specific terms on the wheel and identified 30 wines that were described with multiple terms on the wheel as candidates for assessing the presence of *Brettanomyces*. We also noted whether these wines were described with terms related to those on the aroma wheel. None of these wines were described using the "fermentation," dairy, rotten/putrid, or "veggie" descriptors associated with *Brettanomyces* spoilage, and none of these aroma traits were detected in our own sensory analyses of these wines. We were more interested in determining whether other *Brettanomyces*-associated traits in our research would signal the presence of this yeast in commercial wines. We also noted if wines were described as having been filtered or had any other pertinent processing information (i.e., biodynamic, organic, no added sulfites), but the processing of the selected wines was variable, and none of the reported winemaking procedures was strongly correlated with terms on the wheel. Given what is known about the widespread infections of wine with *Brettanomyces*, this observation was not surprising. In some cases, we also selected common terms used by wine critics, such as "Asian spice," as this descriptor seemed a hybrid of our tamarind and savory terms when the panel evaluated these wines. We then purchased the 30 wines from a retailer to test for the presence of *Brettanomyces* (Table 2).

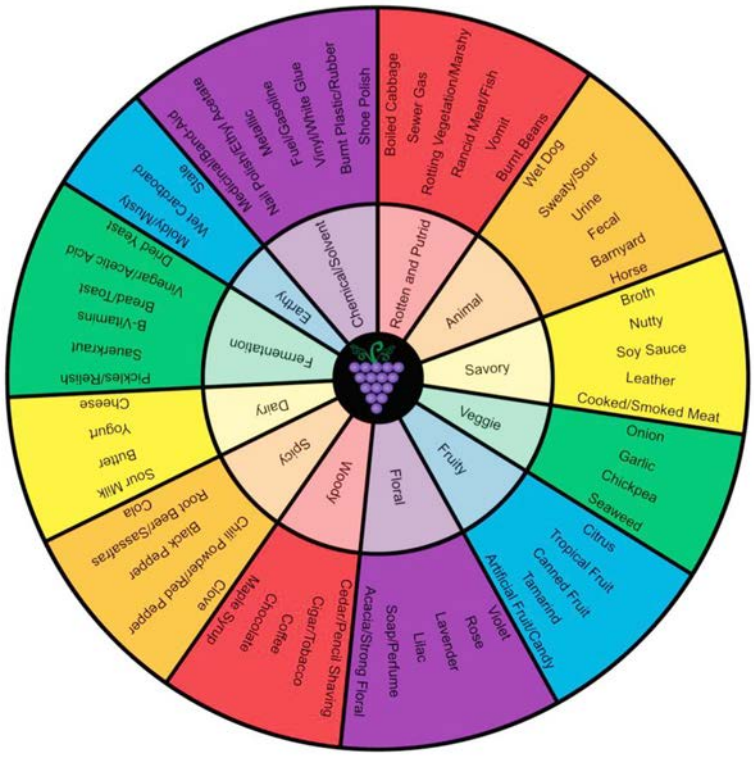

Figure 1 The completed aroma wheel using descriptors for *Brettanomyces* in synthetic wine and determined by a trained panel of judges. Twelve general categories are broken down to between three and seven more specific terms. The reverse of the wheel (not shown) shows photomicrographs of *B. bruxellensis* and summarizes some of the information given here.

We used four different methods to determine the presence of *Brettanomyces* in these wines: (1) microscopic examination, (2) plating on selective media for live organisms, (3) quantitative or real-time polymerase chain reaction (qPCR) to check for *Brettanomyces* DNA, and (4) chemical analysis to detect the signature compounds 4EP and 4EG produced by *Brettanomyces*. The initial microscopic and plating examination on Wallerstein Laboratory Differential nutrient medium with cycloheximide (WLD) for *Brettanomyces* and de Man, Rogosa, and Sharpe medium (MRS) with nystatin for bacteria[8] revealed that 21 of the wines had viable LAB, and another six had viable *Brettanomyces*. Of those 27, one (number 14) eventually proved to have both viable *Brettanomyces* and LAB. A number of these wines, that is, those that had not initially yielded microbial isolates, were also tested with qPCR for *Brettanomyces* and with HPLC for 4EG and 4EP. The qPCR and HPLC analyses were performed by the analytical wine lab at Treasury Wine Estates in Napa,

Table 2 Wines selected for the descriptors used on the *Brettanomyces* Aroma Wheel.

Sample No.	Vintage	Variety	Plating/ microscopy	Brett qPCR cells/mL	4EP[a] ng/mL	4EG[a] ng/mL	Microbes[a] present	Descriptors	Descriptor categories
1	1996	Red Bordeaux blend, France	*Pediococcus*	<10	11.8	6.5	LAB	Smoke and earth	Earthy, savory
2	2004	Red Bordeaux blend, France	*Lactobacillus, Pediococcus,*	6900	13.7	6.9	LAB, Brett	Cedar, tobacco, underbrush, smoke/ burning embers, toast, cigar smoke, asian spices	Savory, spicy, woody
3	2005	Red, Rioja, Spain	NG[b]	1300	12.5	7.0	Brett	Umami, soy sauce, minerality	Savory, earthy
4	2006	Sangiovese Grosso, Italy	*Lactobacillus*	2200	13.6	7.8	LAB, Brett	Cooked meat, tar, smoke, new leather, forest floor, and root beer	Savory, chemical, spicy, earthy
5	2007	Sangiovese, Italy	*Pediococcus, Lactobacillus*	1600	12.5	7.5	LAB, Brett	Musky, black truffle, roses, spice, tobacco, ginger, cola, leather, and game	Earthy, floral, spicy, woody, savory, animal
6	2007	Red Bordeaux blend, Australia	*Brettanomyces*	13,000	12.9	7.2	Brett	Spice box, violets, pencil lead, leather, warm earth, and sweet tobacco	Spicy, floral, woody, savory, earthy,
7	2008	Tempranillo, Spain	*Lactobacillus*	1600	12.7	7.3	LAB, Brett	Beef jerky, meaty, spicy, savory	Savory, spicy
8	2008	Red Bordeaux blend, France	*Lactobacillus*	<10	10.8	6.8	LAB	Smoke, pencil shavings	Savory, woody
9	2009	Rhone blend, France	*Lactobacillus*	<10	10.5	7.0	LAB	Mineral, spicy, black tea, roasted mesquite, graphite, charcoal, truffle, leather, and earth	Earthy, spicy, savory
10	2009	Cabernet Sauvignon, France	*Pediococcus, Lactobacillus*				LAB	Scorched earth/burning embers/charcoal, pencil shavings, rose, gardenia and violet, oriental spice, flinty mineral, graphite, tobacco, and crushed stone	Savory, earthy, woody, floral, spicy
11	2009	Pinot noir, California	*Pediococcus*				LAB	Asian spices, meat stock, soy, mineral, umami, incense, and cola	Spicy, savory, earthy
12	2009	Charbono, California	*Pediococcus*				LAB	Root beer, minerality	Spicy, earthy,
13	2009	Grenache-Mataro-Shiraz blend, Australia	*Lactobacillus*				LAB	Spicecake, funk, and earth plus a little tar	Spicy, earthy, chemical
14	2010	Grenache-Syrah-Mourvedre blend, France	*Lactobacillus, Brettanomyces*	<10	1529	450	LAB, Brett	Spices, smoked meat, acacia flowers, graphite, scorched earth	Spicy, savory, floral, earthy

(continued on page 17)

CA. The results of these analyses indicated that three additional wines also had *Brettanomyces*. One of the wines (number 18) had elevated 4EP and 4EG levels, indicating probable *Brettanomyces* contamination at some point. The other two (numbers 3 and 21) had *Brettanomyces* DNA as determined by qPCR. In summary, 57% of the wines had active LAB contamination, and 27% showed evidence of *Brettanomyces* contamination. Some of the wines (16%) showed evidence of both *Brettanomyces* and LAB. In other words, 100% of the wines chosen using the descriptors from the *Brettanomyces* aroma wheel tested positive for microbial spoilage organisms.

Although these terms were developed using pure cultures of *Brettanomyces*, many of the wines showed evidence of LAB but not of *Brettanomyces*. This unexpected result may mean that both of these classes of organisms can produce the same aroma compounds from amino acid precursors as has previously been reported for the mousy trait derived from lysine[9]. This is also consistent with our observation of the dairy traits found with *Brettanomyces*. However, these were commercial wines, and we cannot rule out the possibility that *Brettanomyces* was present at some point during the lifespan of the wines that tested negative for this yeast in our study.

Table 2 (*continued*) Wines selected for the descriptors used on the *Brettanomyces* Aroma Wheel.

Sample No.	Vintage	Variety	Plating/ microscopy	Brett qPCR cells/mL	4EP[a] ng/mL	4EG[a] ng/mL	Microbes[a] present	Descriptors	Descriptor categories
15	2011	Syrah, France	*Lactobacillus*, *Pediococcus*				LAB	Smoky, meatiness, singed vanilla, tobacco, violet, and cola	Savory, woody, spicy, floral
16	2003	Syrah, California	*Pediococcus*				LAB	Smoked meat, loamy soil, violets, lavender	Savory, floral, earthy
17	2010	Cabernet franc, France	*Brettanomyces*	7	342	96	Brett	Spicy, savory herbs	Spicy, savory
18	2009	Red Bordeaux blend, France	NG	<10	335	77	Brett	Mineral, cedar, violets, and lavender	Earthy, floral woody
19	2007	Pinot noir, California	*Pediococcus*	<10	9	6	LAB	Funky/wild, game, earth, forest floor, loam, truffles, spice, graphite	Animal, earthy, spicy
20	1999	Pinot noir, California	*Lactobacillus*				LAB	Umami, violets, forest floor, chinese five spice, and mushroom	Savory, earthy, floral, spicy
21	2001	Tempranillo-Granache blend, Spain	NG	1200	12.4	7.2	Brett	Singed plum, balsamic, sandalwood, potpourri	Savory, woody, spicy, floral
22	2004	Tempranillo, Spain	*Lactobacillus*				LAB	Leather, cinnamon, ox-blood, smoked meat	Savory, spicy, animal
23	2007	Cabernet franc, New Zealand	*Brettanomyces*				Brett	Cooked meats, wild game, violets, herbs, spice, and mineral	Savory, animal, spicy, earthy, floral
24	2009	Red Bordeaux blend, France	*Brettanomyces*	<10	768	124	Brett	Black tea, earth, mocha, spice, tobacco, and mineral	Earthy, woody, spicy
25	2009	Mourvedre-Syrah-Grenache blend, California	*Lactobacillus*				LAB	Exotic asian spices, sandalwood, crushed rock, mineral, and cedar	Spicy, woody, earthy
26	2009	Syrah, France	*Lactobacillus*				LAB	Mineral, smoky, spice	Earthy, savory, spicy
27	2009	Cabernet franc, France	*Brettanomyces*	11,000	869	313	Brett	Gravel and sand	Earthy
28	2010	Garnacha, Spain	*Lactobacillus*				LAB	Roasted herbs, spice, and woodsmoke	Savory, spicy, woody
29	2010	Mourvedre-Grenache Rhone blend, France	*Lactobacillus*				LAB	Rose, lavender, asian spices, forest floor, and truffle, tarry	Floral, spicy, earthy, chemical
30	2010	Merlot-Cabernet Sauvignon blend	*Pediococcus*				LAB	Earth, tobacco, and roasted cedar	Earthy, woody, savory

[a]4EP, 4-ethyl phenol; 4EG, 4-ethyl guaiacol; LAB, lactic acid bacteria; Brett, *Brettanomyces*.
[b]NG = No growth.
Blank spaces indicate not determined.

Broader Impact

We analyzed these data to look for correlations between the presence of LAB or *Brettanomyces* and the presence of general descriptor categories (Figure 2). This analysis was less than ideal since the backgrounds for these wines differed in each case. The variability in microbial population was not the only difference among the wines. Moreover, the absence of a particular class of organisms does not necessarily mean that members of that class were never present during the life of the wine and merely indicates that they cannot be detected in the final commercial product. Another issue with any analysis is the number of samples in each category: 17 wines had LAB alone, eight had *Brettanomyces* alone, and five had both *Brettanomyces* and LAB. Despite these issues, we could see some general trends in the data.

The categories spicy, savory, and earthy accounted for most of the classes of descriptors used for the wines. The woody and floral categories were used less commonly, and only rarely were the animal and "chemical" classifications found. There were no descriptors in the fermentation, dairy, rotten/putrid, veggie, or "fruity" categories. Some of this may reflect the commercially unacceptable nature of wines with these aroma characteristics, as would be the case for rotten/putrid, whereas other aroma-active characters may have been masked in the wine. The absence of fruity may be more a result of the selection procedure we used, as those descriptors were too commonly used to be valuable as selection criteria. The division of the aroma categories by type of microbial contamination typically followed the number of wines with each contamination profile: LAB (57%), *Brettanomyces* (27%), or both (16%).

Half of the wines with *Brettanomyces* and 40% of the wines with both LAB and *Brettanomyces* had floral descriptors, and only one-third of the wines with LAB had only floral descriptors (Figure 2). A similar trend was seen for the woody descriptor. High numbers of wines with the savory and spicy descriptors had either *Brettanomyces* or LAB (63 to 77%, respectively), but 100% of the wines with both LAB and *Brettanomyces* had these two types of descriptors. The earthy descriptor was also highly prevalent in all the wines; it was found in 75% of wines with *Brettanomyces*, 77% of wines with LAB, and 60% of wines with both.

There are a few specific descriptors that seem to occur exclusively with LAB, but none that occurred exclusively with *Brettanomyces*. One descriptor that stood out for LAB was Asian spice, which also includes Oriental spice and Chinese five spice. Overall, 35% of the wines with LAB were described as having that character. A few other descriptors occurred less often, but still were exclusively associated with wines that had LAB aromas, such as graphite, truffle, and tar. The low number of samples and the variability in processing prevent us from drawing any definitive conclusions about the specific descriptors and their metabolic origins among the community of microbes present. Further analysis of a greater number of wines could yield more specific descriptors from the outer wheel that are associated with specific types of microbial contamination.

The *Brettanomyces* aroma wheel was generated as a consequence of multiple analyses of the growth of this yeast in a synthetic matrix with and without supplementation with specific precursor compounds. *Brettanomyces* can generate an array of aroma compounds in addition to the well-known aromatic phenol derivatives. The wheel was developed in consultation with two panels and was validated by selecting a set of commercial wines that were described using

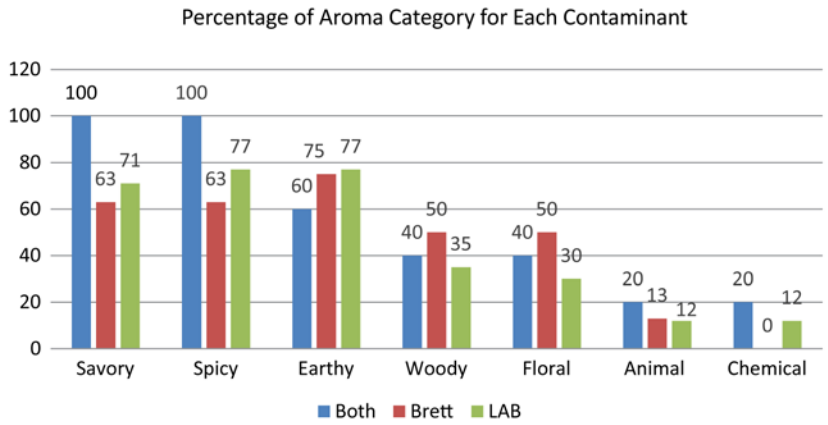

Figure 2 The percentage of the wines with a given microbial contaminant (*Brettanomyces*, lactic acid bacteria [LAB], or both) that were described as having a specific aroma characteristic. For example, 100% of wines with both *Brettanomyces* and LAB and 77% of wines with LAB alone were described as spicy. The information was compiled from Table 2.

multiple terms on the wheel. Although the presence of *Brettanomyces* was confirmed in only 43% of these wines, the remainder contained LAB, *Lactobacillus*, and *Pediococcus*, suggesting that LAB can produce similar compounds in wine or, alternatively, that *Brettanomyces* had been present in these wines at some point but was no longer detectable at the time of the evaluation. The *Brettanomyces* aroma wheel represents a useful tool for assessing the contribution of microbial aroma-active compounds to the aroma profile of wines.

Experimental Design

The work presented here builds on a series of previously published studies that generated an array of terms for the description of synthetic media inoculated with different strains of *Brettanomyces* with and without supplementation with specific precursor compounds[5]. Those publications contain more detailed descriptions of the sensory analyses conducted, and we summarize them here. A set of 99 *Brettanomyces* strains were evaluated for aroma characteristics in minimal media, either unsupplemented or supplemented with phenylalanine, tyrosine, or tryptophan. Aroma terms for the 99 strains were aggregated, for a total of 2646 observations and 90 unique terms. Judges met as a group afterward to clarify terminology in order to aid in descriptor classification, i.e., "coconut oil" referred to a rancid oil smell, and should be categorized as "rotten/putrid" rather than "fruity" or "spicy." Panel discussions were used to group terms into 1 of 13 classes of related descriptors. Panelists agreed on the overall groupings of their terms, and judges were encouraged to use their own terms. There was no communication among the panelists during the initial sample analyses and no effort was made to force agreement among the individual panelists in the terms used. All samples received randomized three digit codes as assigned by a random-number generator (www.randomizer.org). Strains were presented in groups of three or four (i.e., as 12 to 16 samples), in a randomized order for each set. Five judges were recruited for the study, all of whom had previous experience with *Brettanomyces* aroma evaluation. Judges were asked to provide a list of descriptors for each sample; to rate the aroma profile present as "positive," "negative," or "a mix of positive and negative;" and to apply an overall intensity rating on a scale of one through nine.

Descriptors that were used by more than a single panelist during the analyses of both synthetic and actual wine samples were compiled for consideration in the final wheel for discussions with the panelists. The groupings were changed from 13 to 12 by the inclusion of the "acetic" term of the first panel under "fermentation." More general descriptors for certain aroma characters were also chosen after the discussion. For example, fuel and gasoline were combined into a single term, and "boiled cabbage" was used for "pot stickers," "kimchee," and "stuffed cabbage." The more specific terms (on the outer wheel) were then categorized into more general terms, such as fruity, floral, rotten and putrid, or animal (on the inner wheel).

The first version of the wheel was tested during an extension program focused on identifying the impacts of *Brettanomyces* in wine. Commercial wines deliberately made with *Brettanomyces* were evaluated during this program as a series of Merlot wines that we had produced through inoculation with different *Brettanomyces* strains. Attendees were asked to evaluate the wines, write descriptors, and then consult the *Brettanomyces* aroma wheel to determine if the descriptors were present. Some terms were modified slightly, but no new terms were suggested, and none were suggested for removal.

Acknowledgments: The authors thank Treasury Wine Estates, Tom Collins, and Josh Miles for qPCR and HPLC wine analysis.

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