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Sensory and analytical re-evaluation of "Brett character"

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ABSTRACT

Worldwide wine production has been significantly affected by *Brettanomyces bruxellensis* spoilage. This alteration, sometimes referred to as "Brett character", results in the production of several volatile compounds and a large spectrum of flavours and aromas. Ethylphenols (namely 4-ethylphenol and 4-ethylguaiacol) are the best-known markers of this defect with a commonly used aggregate detection threshold of about 400 μg/l. Fifty-one Bordeaux red wines were tasted with the aim of wine profiling for commercial purposes. Ethylphenol concentrations of wines were very poorly correlated to the corresponding tasting notes. Sensory analysis was employed to demonstrate the complexity of "Brett character". A masking effect of isobutyric acid and isovaleric acid on the detection of ethylphenols in wine was proven. This partly explained the poor correspondence between ethylphenol concentrations and presence of "Bretty" descriptors.

1. Introduction

Among all wine spoilage microorganisms the yeast *Brettanomyces bruxellensis* is one of the most feared by winemakers (Loureiro & Malfeito-Ferreira, 2006; Renouf, Lonvaud-Funel, & Coulon, 2007). The development of *B. bruxellensis* may cause "Brett character", which occurs mainly in red wines. Brett character produces a wide spectrum of flavours and aromas that include barnyard-like, mineral, ink, tobacco, leathery, pharmaceutical and smoky descriptors (Boulton, Singleton, Bisson, & Kunkee, 1996; Etievant, 1991). Brett character also entails the suppression of desirable fruity and flowery notes of wine (Gerbaux & Vincent, 2001).

B. bruxellensis spoilage activity is linked to the synthesis of vinyl- and ethylphenols (Heresztyn, 1986). Ethylphenols, and namely 4-ethylphenol and 4-ethylguaiacol, are the best-known markers of the alteration. To date *B. bruxellensis* is the only microbial species whose presence and development in wine has been unmistakably related to the synthesis of ethylphenols. Some lactic acid bacteria (Couto, Campos, Figueiredo, & Hogg, 2006) and the yeast *Pichia guillermondii* (Barata, Nobre, Correia, Malfeito-Ferreira, & Loureiro, 2006) are able to synthesise ethylphenols in culture media but the oenological relevance of these activities was never proven so far.

The aggregate detection threshold for ethylphenols was reported at 426 μg/l (Chatonnet, Dubourdieu, Boidron, & Pons 1992). This value was determined with data from a jury of 20 tasters employing a methodology developed by Boidron, Chatonnet, and Pons (1988). The authors used a Bordeaux red wine that was supplemented with graduated amounts of a 10:1 mixture of 4-ethylphenol and 4-ethylguaiacol. The 10:1 concentration ratio corresponds to the average found in Bordeaux wines. Researchers have subsequently used this threshold value as the "borderline" between spoiled and unspoiled wine. This detection threshold also carries significant economic importance: winemakers have made it the basis for treating wines thought to be at risk of *B. bruxellensis* spoilage.

In 2007, the practical significance of this threshold value was evaluated using a panel of 6 expert judges and 17 Bordeaux red wines of the 2003 vintage. The wines contained from 53 to 1417 μg/l ethylphenols. Quite interestingly some of the wines that presented above-threshold amounts of ethylphenols (up to 668 μg/l) were not described as "Bretty" by any of the tasters (de Revel, personal communication). These results are in accordance with anecdotal reports (Goode, 2005) about scarce correlations between Brett character and ethylphenol contents of wines.

Acetyl-tetrahydropyridine, carboxylic acids and some of their ethyl-esters can also participate to Brett character in wines (Romano, Perello, de Revel, & Lonvaud-Funel, 2008) but their sensory implications are not fully known to date. This work aimed at an analytical and sensory re-evaluation of Brett character on the basis of more refined criteria that take into consideration its complexity. Wines were analysed by gas chromatography–mass spectrometry (GC–MS) and sensorial experiments were carried out to identify products of *B. bruxellensis* metabolism that interact with ethylphenols to mask their aromas.

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2. Materials and methods

2.1. Reagents and standards

4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol and 4-ethylguaiacol, were supplied by Lancaster (Ward Hill, MA). Deuterated 4-ethylphenol was purchased from Cluzeau (Paris, France). Ethanol was from Merck (Darmstadt, Germany). All other standards (carboxylic acids, ethyl-esters and oak wood volatile compounds) and solvents (dichloromethane, diethyl-ether and isohexane) were obtained from Sigma Aldrich (Saint Quentin Fallavier, France). All standards were at least 97% pure, all solvents were HPLC-grade (at least 99.7% pure).

2.2. Wine samples

The wines inoculated with *B. bruxellensis* were 2006 red Bordeaux (pH 3.4–3.5, ethanol 12.4–13.0% (v/v), total reducing sugars 0.8–1.2 g/l, volatile acidity 0.12–0.35 g/l expressed as acetic acid, total phenolics 39–57 expressed as absorbance at 280 nm, total volatile phenols 8–14 μg/l). A 2006 Bordeaux red wine was used for detection threshold calculation (pH 3.5, ethanol 12.2% (v/v), total reducing sugars 2.0 g/l, volatile acidity 0.55 g/l expressed as acetic acid, total phenolics 49 expressed as absorbance at 280 nm, total volatile phenols 13 μg/l). All the other red wines (pH 3.4, ethanol 12.5–13.7% (v/v), total reducing sugars 1.2–2.4 g/l, volatile acidity 0.51–0.84 g/l, 58–98 total phenolics) were commercial samples that originated from wineries throughout the Bordeaux region and belonged to the 2005 vintage.

4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol and 4-ethylguaiacol, isobutyric acid and isovaleric acid pure standards were dissolved at a concentration of 10% w/v in ethanol. When needed these concentrated ethanolic solutions were employed to perform additions to wine.

2.3. Protocol of the experiments with B. bruxellensis

Strain *B. bruxellensis* IOEB L0506 (culture collection of the *Faculté d'Œnologie*, Bordeaux, France) was inoculated in three sterile red wines. Before being inoculated wines were centrifuged twice (15800*g* for 30 min) and sterilized by filtration on a 0.45 μm pore size membrane (Sartorius, Goettingen, Germany). After a pre-adaptation step the microbial strain was inoculated in sterile wine at a concentration of 5×10^3 viable cells/ml (measured by direct epifluorescence method, Divol & Lonvaud-Funel, 2005). Inoculated wines were kept at 25 °C in 2.5 l screw capped bottles with no headspace volume. For each wine a parallel run without inoculation was carried out. After one month all wines were filtered again and submitted to sensory analysis. Prior to sensory analysis non-inoculated blanks were added with the required amounts of volatile phenol pure standards (see further).

2.4. Sensory analysis

Fifty-one red Bordeaux wines of the 2005 vintage were tasted with the aim of wine profiling for commercial purposes. Four professionals of the wine sector evaluated samples both orthonasally and retronasally and a list of freely perceived descriptors was obtained for each wine. Samples were presented to the tasters employing tulip-shaped tasting glasses (ISO 3591:1977).

Triangle tests (ISO 4120:2004) were employed to compare inoculated and non-inoculated wines (three sets of samples in total). The panel consisted of 21 judges belonging to the faculty staff. Samples were presented within tulip-shaped glasses marked by random three-digit codes and were evaluated both orthonasally and retronasally. The inoculated wine samples were taken as examples of "natural" Brett character. Non-inoculated blanks were added with volatile phenol (4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol and 4-ethylguaiacol) pure standards so that their final concentrations matched those of spoiled wines. The samples where the sensory defect was reproduced by mere addition of its markers (i.e. volatile phenols) were taken as example of "artificial" Brett character.

Detection thresholds were calculated following ISO guidelines (ISO 13301:2002). Six sets of three-alternative forced-choice (3-AFC) tests were performed. Each series contained one positive sample supplemented with ascending (17 – 34 – 68 – 137 – 275 – 550 μg/l) concentrations of ethylphenols in a 4-ethylphenol: 4-ethylguaiacol 10:1 concentration ratio. The panel was a subset of the previous one and consisted of 10 expert judges belonging to the laboratory staff. The judges were already trained in the perception of the sensory notes of 4-ethylphenol and 4-ethylguaiacol in wine but they were not informed about the purpose of the test. Samples were evaluated orthonasally and consisted in 4 ml wine aliquots presented within 20 ml screw capped bottles with 2 cm neck diameter. The wine samples were introduced into the bottles and these were capped at least 1 h before the experiment in order to allow for equilibration. All tests were performed in a room equipped with individual tasting booths (ISO 8589:2007).

2.5. Chemical analysis

Ethanol, volatile acidity and total reducing sugars were determined according to the international methods for wine and must analysis published by the *Organisation Internationale de la Vigne et du Vin* (downloadable at http://news.reseau-concept.net/images/oiv/Client/RECUEIL_2007_Vol1.pdf).

Volatile phenols were determined by GC–MS analysis coupled to solid-phase micro-extraction (SPME) on polyacrylate fibers. Samples (10 ml of wine) were placed in 25 ml vials containing NaCl (3.5 g). Deuterated 4-ethylphenol was added as internal standard at a concentration of 100 μg/l. Samples were submitted to GC–MS analysis (Romano et al., 2008). The electron impact (EI) mass detector operated in the selected ion monitoring (SIM) mode and analytes were measured by comparing peak areas of specific ions (4-vinylphenol *m*/*z* 120, 4-vinylguaiacol *m*/*z* 150, 4-ethylphenol *m*/*z* 107, 4-ethylguaiacol *m*/*z* 137) with that of [$^2H_{10}$] 4-ethylphenol (*m*/*z* 113) used as internal standard.

Carboxylic acids and ethyl-esters were quantified by GC–FID analysis of wine liquid extracts. Samples (10 ml of wine) were added with 1 mg/l internal standard (3-octanol) and extracted twice with 2 ml of diethyl-ether:isohexane 1:1 (v/v). The organic fractions were collected and submitted to GC analysis (Bertrand, 1981).

Oak wood volatile compounds (guaiacol, 4-methyl-guaiacol, *o*-cresol, *m*-cresol, *p*-cresol, phenol, 4-propyl-guaiacol, eugenol, isoeugenol, syringol and 4-allyl-syringol) were measured by GC–MS analysis of wine liquid extracts. Samples (10 ml of wine) were added with 1 mg/l internal standard (deuterated 4-ethylphenol) and extracted twice with 2 ml of dichloromethane. The organic fractions were collected and submitted to GC–MS analysis. The mass detector operated in the selected ion monitoring (SIM) mode and analytes were measured by comparing peak areas of specific ions with that of the internal standard. (Bloem, Lonvaud-Funel, & de Revel, 2008).

2.6. Statistical analysis

All chemical analyses were performed at least in duplicate. Experimental data were compared by means of a student *t* test at a 95% confidence level unless otherwise noted. Data treatment