COMPARISION OF SENSORY AND ANALYTICAL CHARACTERISTICS OF RED WINES INFECTED BY *BRETTANOMYCES/DEKKERA*

DOMINIKA CERNOHORSKA, MOJMIR BARON
Department of Viticulture and Enology
Mendel University in Brno
Valticka 337, 691 44 Lednice
CZECH REPUBLIC

xcernoh2@node.mendelu.cz

Abstract: The *Brettanomyces/Dekkera* yeast can infect wine, and, thanks to its metabolism, it can produce volatile substances that affect the aroma of wine. Volatile phenols, sensorily active substances formed due to the yeast metabolism, can deliver desirable spicy tones or, on the contrary, very unpleasant wet animal-like odour to the wine. It depends on the concentration of mainly 4-ethylphenol and 4-ethylguaiacol in wine as well as on wine consumers´ ability to perceive these tones. Twenty-four samples of red wines suspected of having increased content of volatile phenols, produced by *Dekkera/Brettanomyces* yeasts, were gathered for this experiment. The content of ethylphenols (4-ethylphenol and 4-ethylguaiacol) in the samples was analysed by GC-MS and ranged from 10 to 3035 µg/l. The samples were then evaluated via sensory analysis carried out by professional tasters. The results were compared and correlated between the concentration of volatile substances and sensory perception of 'Brett' characteristics in wine. Highly probative correlation of the average evaluation of 'Brett' character in analysed wines and the real content of ethylphenols measured by GC-MS is an important outcome of the research. Weak negative dependence was calculated for parameters of sensory evaluation, which confirms generally decreasing evaluation (on the 100-point scale) of samples with a growing 'Brett' character.

Key Words: Brettanomyces, *Dekkera*, red wine, ethylphenols, yeasts, GC-MS, sensory evaluation

INTRODUCTION

The *Brettanomyces/Dekkera* yeast belongs to a group of wild or apiculate yeast. This yeast comes mainly from the surface of the skin of grapes. The yeast does not prevail on the grapes, but it plays an important role in the development of a wine´s aroma (Renouf et al. 2006). Hydroxycinnamic acids are metabolically transformed thanks to this yeast, and enzymes and so-called volatile phenols are formed. These very substances are responsible for the 'Brett' character in red wine. The aroma of such wine is reminiscent of a barnyard, a horse stable, sweaty horse saddle, leather, earth or smoked meat (Eder et al. 2006, Curiel et al. 2010).

The presence of *Brettanomyces* yeast was attributed to inadequate hygiene in cellars, where there is a greater risk of contamination. It was found out later that, despite increased efforts to maintain adequate hygienic conditions, contamination by this specific yeast was still very common (Eder et al. 2006, Oelofse 2008).

Contamination by the *Brettanomyces* yeast may already come from the grape. A large increase in *Brettanomyces* yeast population can be found during maceration or during the very fermentation process. *Brettanomyces bruxellensis* can adapt very well to the beginning of alcohol fermentation. The yeast has also proved to be far more resistant to the conditions of increased alcohol and sugar loss near the end of fermentation than *Saccharomyces cerevisiae* (Suarez et al. 2007, Oelofse 2008). The presence of the *Brettanomyces* yeast during malolactic fermentation is associated with low content of free sulphur dioxide, certain concentrations of residual sugar and the yeast autolysis associated with mild microbial instability. Such technological procedures as storing wine in oak barrels of barrique type also complies with the multiplication of yeast of this species (Renouf et al. 2006, Wedral et al. 2010).

Brettanomyces bruxellensis belongs to the species of yeast which adapts very well to conditions of dry red wine. In certain concentrations, it is resistant to SO_2, to high alcohol content, lack of oxygen and low amount of fermentable sugars (Romano et al. 2008, Coulon et al. 2010).

Increased content of volatile phenols causes the 'Brett' character in red wines. All strains of *Brettanomyces* genus which have so far been discovered are capable of producing volatile phenols. However, *Brettanomyces bruxellensis/Dekkera bruxellensis* is the only species discovered in wine (Agnolucci et al. 2010).

Volatile phenols make a large group of substances that may have a very positive effect in small concentrations due to the production of certain substances, such as vanillin, eugenol, syringaldehyd etc. In this case, very desirable smoky or spicy tones may come into existence in red wine. Also, substances that are not evaluated very positively if they exceed the threshold value of their concentration belong to this group. Such phenols include mainly ethylphenols (4-ethylphenol and 4-ethylguaiacol), but also vinylphenols (4-vinylphenol, 4-vinylguaiacol), which precede ethylphenols formation.

The formation of volatile phenols (ethylphenols) via *Brettanomyces* yeast is a result of the enzymatic transformation of hydroxycinnamic acids, ranking among phenolic substances (Eder et al. 2006, Harris 2011).

The results of a previous experiment focused on the sensory perception of ethylphenols, i.e. substances responsible for the aroma of sweaty horse saddle and barnyard, which show that experts are much more sensitive to the perception of ethyphenols than common wine drinkers.

It has been demonstrated that certain other substances present in wine are able to mask high concentrations of ethylphenols, in particular, isobutyric and isovaleric acid (Romano et al. 2008). If the content of 4-ethylguaiacol exceeds the value of 70–100 µg/l, wine aroma is negatively affected. This is manifested, for example, by the aroma reminiscent of burnt wood. Moreover, concentrations of 4-ethylphenol over 400–600 µg/l are referred to as phenolic aroma, i.e. aroma described as smoked bacon or reminiscent of horse-stable or barnyard (Eder et al. 2006).

MATERIAL AND METHODS

Samples and process

This study gathered a total of 24 samples of red wine suspected of having symptoms related to the change of aroma caused by the activity of *Dekkera/Brettanomyces* yeast and of subsequent formation of volatile phenols. The range of samples comprised nineteen moravian and five foreign wines. A total of five samples from the evaluated collection were labelled as cuvée; the rest were varietal wines. The selection of the wines was based on a questionnaire distributed to wine producers.

To evaluate the samples, two basic methods were used: a sensory analysis and gas chromatography with mass spectrometry(GC-MS) for measuring volatile substances.

Sensory evaluation

The evaluation was carried out using a computerized evaluation system. The samples were evaluated on a scale of 100 points, and the aroma and body profiles of the wines were assessed. As agreed with the tasters, the last part of the aroma profile evaluation included the extent of a 'Brett' character of the wine in question. All the parameters were evaluated on a scale of 1 to 10 based on the level it reached in the particular category.

Samples preparation

Prior to the tasting itself, the samples had been kept at a stable temperature of 12 °C. The bottles were opened about 150 minutes before the tasting to let the wine 'breathe', as it is called. Twenty minutes before serving, the individual samples were decanted in a glass pitcher. When served, the temperature of the wines was about 16 °C.

The jury

The tasters' jury consisted of ten members. The tasters included experts specialising primarily in Moravian wines as well as those with considerable experience in evaluating foreign wines. All of the tasters also own the Certificate of Passing the Selection Process of Specialized Evaluation Experts for Sensory Analysis of Wines in Compliance with the Czech Technical Standard ISO 8586-2.

Analytical evaluation using GC-MS

During the tasting, an amount of wine was withdrawn from each sample. Using a funnel, this amount was poured into 250 ml glass bottles with a screw cap. Samples prepared in this way were, immediately after the tasting, transported to the laboratory of the Department of Viticulture and Enology in Lednice where they were analysed by means of GC-MS.

Table 1 GC-MS Analysis

Instrument:	Shimadzu GC-17A
Autosampler:	AOC-5000
Detektor:	QP-5050A
Software:	Gcsolution

Table 2 Conditions of Separation

Column:	DB-WAX 30 m x 0.25 mm; 0.25 µm of stationary phase (polyethylenglycol)
Injection volume:	1 µl split 1:5
Gas flow (He):	1 ml/min (linear speed of gas 36 cm/s)
Temperature of injection port:	180 °C

RESULTS AND DISCUSSION

Results of the sensory evaluation

The average score of the samples ranged within a span of 11 points, where the sample with the lowest score reached 74.13 points while the top sample score was 85.25 points. Moreover, each sample could be subjectively commented on by the evaluators. In individual evaluations, the score ranged from 67 to 90 points (Table 3). The evaluation of the respective samples varied a great deal among the jury members.

Taking into account the fact that the ‘Brett’’ character in red wines, caused by the activity of the yeast *Brettanomyces/Dekkera*, is described as a wine fault in expert literature, it can be assumed that there would be a certain relation between the score on the 100-point scale and the described extent of its ‘Brett’’ character. The underlying assumption is that the wine that was specifically evaluated as having stronger ‘Brett’’ characteristics would be accordingly lower in score on the 100-point scale system. Its score would then correspond to that of faulty wine. All wines, ranked as being ‘Bretty’ to a greater extent and reaching 6 points or more on the 10-point scale evaluation of ‘Brett’ characteristics, have not obtained more than 80 points out of 100.

Evaluations of certain samples by individual experts very often varied to a great extent. Personal notes that evaluators put down about each sample were important for evaluating the individual perception of ‘Bretty’ characteristics.

Result of the GC-MS analysis

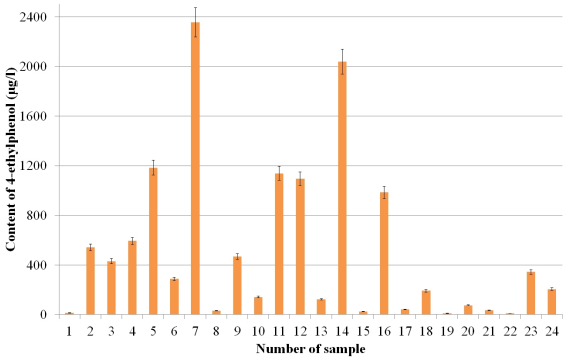
The professional literature provides a limit of volatile phenol perceptibility as 400–600 µg/l for 4-ethylphenol and 33–100 µg/l for 4-ethylguaiacol. Eder (2006) asserts that the ‘Brett’’ character in red wine is perceptible provided that the total of the 4-ethylphenol and 4-ethylguaiacol content is greater than 425 µg/l. There were 11 such samples out of the 24 evaluated ones (Table 3).

Figure 1 below shows which samples exceeded the limit given for the sensory perception threshold. If the lower limit for 4-ethyl phenol is 400 µg/l, there was a total of 10 samples surpassing it. The greatest amount of 4-ethylphenol was measured in sample No. 7, where the perception threshold was exceeded by nearly six times. In sample No. 14, the value was about five times above the limit.

Table 3 Summary Results of Sensory and Analytical Analysis

Number of sample	Sensory evaluation (100 points scale)	Sensory evaluation of Brett character	4-ethylphenol (µg/l)	4-ethylguaiacol (µg/l)	4-ethylphenol + 4-ethylguaiacol (µg/l)	Number of sample	Sensory evaluation (100 points scale)	Sensory evaluation of Brett character	4-ethylphenol (µg/l)	4-ethylguaiacol (µg/l)	4-ethylphenol + 4-ethylguaiacol (µg/l)
1	74.13	4.30	14	0	14	13	81.63	5.00	122	20	142
2	80.88	6.20	542	70	612	14	78.75	7.70	2038	367	2405
3	79.13	3.00	430	45	475	15	81.00	4.50	25	3	28
4	84.13	4.90	593	67	660	16	76.50	7.20	985	269	1254
5	79.13	4.60	1184	129	1313	17	81.50	4.80	42	11	53
6	79.63	4.80	288	35	323	18	80.38	4.70	192	44	236
7	78.00	6.40	2355	680	3035	19	78.63	5.10	10	0	10
8	78.38	5.50	32	12	44	20	83.50	4.30	75	12	87
9	77.38	4.20	469	94	563	21	85.00	3.50	35	8	43
10	81.50	4.20	142	25	167	22	85.25	4.70	9	9	18
11	82.25	5.10	1138	141	1279	23	78.88	6.90	343	312	655
12	76.13	6.50	1096	312	1408	24	81.13	3.70	205	33	238

Figure 1 Values of 4-ethylphenol Content



Figured 2 Values of 4-ethylguaiacol Content

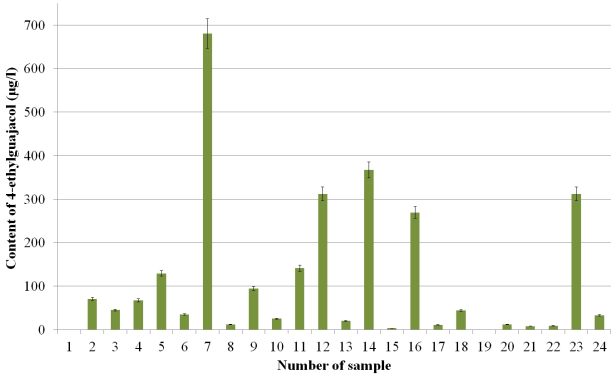


Figure 2 shows the values of 4-ethylguaiacol measured in the individual samples. The minimum perceptibility limit is ascertained to be 33 µg/l. Among the analysed samples, this number was surpassed in a total of 14 cases. In sample No. 7, the content of 4-ethylguaiacol was about twenty times higher than its perception threshold limit.

Highly probative correlation of average evaluation of the 'Brett' character in analysed wines and real content of ethylphenols measured by GC-MS is an important outcome of the research. Weak negative correlation was calculated for parameters of sensory evaluation, which confirms a generally decreasing evaluation (on the 100-point scale) of samples with growing 'Brett' character (Table 3). The connecting lines in figures 3 and 4 confirm the linear dependence which clearly indicates that the more 'Brett'' character is detected in a wine, the greater content of 4-ethylphenol and 4-ethylguaiacol.

Figure 3 Dependence of 'Brett'' Character Perception Perception on the Measured 4-ethylphenol Content

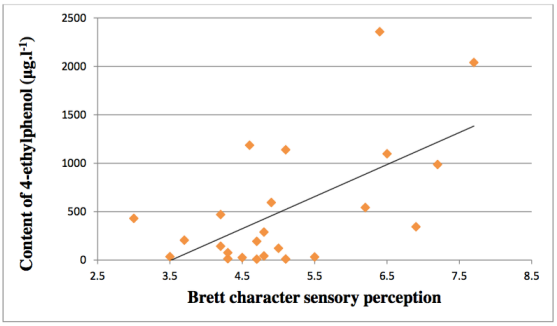
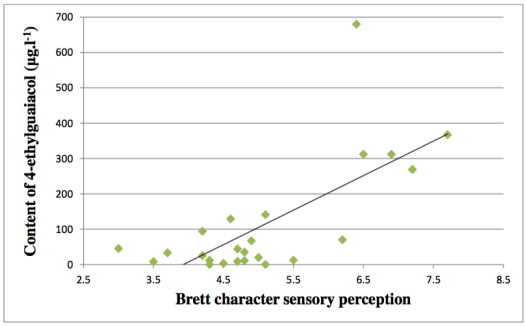


Figure 4 Dependence of 'Brett'' Character on the Measured 4-ethylguaiacol Content



DISCUSSION

After sorting all analysed samples from those with the most intense 'Brett' character to the samples manifesting it the least as well as sorting them from samples with the highest content of ethylphenols to the lowest content, we have found out that samples numbers 7, 12 and 14 were in the top five. Sample number 7, which contains up to seven times greater concentration of ethylphenols than the value stated by Eder (2006) as threshold content of perception, was not evaluated as the most sensorily 'Bretty', but took 5th place. Sample number 14 was regarded as the most 'Bretty'. It has the second-biggest content of ethylphenols 2 405 µg/l.

Bende, et al. (1997) determined that different perceptions of these specific substances in wine can be caused by many factors: personal experience, increased or reduced sensitivity, as well as by the presence of other substances that may affect perception of ethylphenols. Romano, et al. (2008) observed that the perception of ethylphenols in wine can be affected by isobutyric and isovaleric acids, and, by their presence in wine, the 'Brett' character may be masked. For this reason, the analysis of these carboxylic acids in all samples was conducted within our research as well. The correlation of sensory perceptions of ethylphenols and increased concentration of isobutyric and isovaleric acids has not been proved. However, their increased concentration in some samples was recorded.

CONCLUSION

Our research proves that there is a highly probative correlation between the perception of the 'Brett' character in wine and the real content of alcoholic ethylphenols, i.e. substances which are responsible for the 'Brett' aromas in wine. We can thus claim that experts are certainly able to identify manifestations of 'Brett' character in wine. However, experts are far from having the same opinion on the degree of positive or negative perceptions of such tones. The perception threshold of a substance´s presence varies with every person. Also, the border of tolerability of its manifestation differs with every person. A hundred wine tasters may perceive this threshold value in a hundred different ways. This has also been confirmed through sensorial evaluation. Variations in range in total wine quality evaluation of nine samples equalled 15 and more points. Based on the evaluations of 10 tasters who analysed 24 samples with a 'Brett' character, we can claim that the 'Brett' character was sensorily identified in all samples that had an increased content of ethylphenols. The extent to which the influence of these microorganisms is positive cannot be determined on the basis of this research.

According to less number of international samples there was no significant difference between the assessment of domestic and international wines.

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