


Effects of different vinification technologies and yeasts on qualitative parameters and terpene compounds of Sauvignon Blanc wines

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ABSTRACT

Sauvignon Blanc represents an important grape variety. The wine made from this variety is known to have a wide range of aroma profiles from nettles to tropical fruits. Beside the raw material quality (grapes), the quality of wines can be fundamentally influenced by the technological conditions applied in the wine making process. Yeast and other microorganisms play a key role in the formation of metabolites during alcoholic fermentation. In this study, the effects of autochthonous or selected wine yeasts (*Saccharomyces cerevisiae*) and fermentation temperatures (15 °C and 19 °C) were tested on major monoterpenes contents of wines during the period 2016–2017. The obtained values show that the highest contents of linalool (24.36 µg L⁻¹) and hotrienol (11.84 µg L⁻¹) were determined in wine samples produced with active (selected) wine yeast at lower temperature. Sensory evaluation results indicated that monoterpenes can have a positive effect on the overall sensory quality of Sauvignon Blanc wines, despite the fact that their determined concentrations in the evaluated samples were not higher than their threshold values.

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KEYWORDS

yeast, fermentation, Sauvignon Blanc, *Saccharomyces cerevisiae*, sensory evaluation

1. INTRODUCTION

Sauvignon Blanc is a green-skinned grape variety, which originates from the Bordeaux region in France (MacNeil, 2015). The main process involved in wine production is the alcoholic fermentation with emphasis on yeast as a complex microbiological process (Ribéreau-Gayon et al., 2006a). Fermentation can be spontaneous or controlled. Spontaneous alcoholic fermentation occurs with the action of non-*Saccharomyces* species. These are yeasts that naturally occur on the skin of grapes; primarily *Kloeckera*, *Candida*, *Metschnikowia*, *Hansenula*, *Pichia*, and *Torulaspota* spp. (Ribéreau-Gayon, 2006b). When the level of alcohol reaches approximately 5%, the yeast *Saccharomyces cerevisiae* begins to prevail, because it is more tolerant to higher concentrations of ethanol. The fermentation process will cease at the concentration of alcohol that becomes toxic for the yeasts species (Furdíková and Malík, 2008).

In contemporary viticulture, dried wine yeast species *S. cerevisiae* and *Saccharomyces bayanus* are used in the Czech Republic (Michlovský, 2014). Research on new suitable yeasts is still ongoing, with the aim of discovering yeast strains with unique properties (Furdíková and Malík, 2008). Recent studies have shown that yeast can play an important role in the monoterpene content of wine during fermentation as it contains enzymatic systems to break down terpene glycosides. Thus, some yeast strains can contribute to the floral aroma of wine (Barrajón et al., 2011).

Sauvignon Blanc wines have a typical aroma characterised by green (vegetal, grassy, herbaceous, nettle, green pepper, capsicum, tomato leaf) and tropical (grapefruit gooseberry, and passion fruit) flavours (King and Dickinson, 2000). The main contributors to the characteristic Sauvignon Blanc aroma are thiols (tropical) and pyrazines (green character). Terpenes are often below their odour threshold and probably contribute as odour enhancers to certain aromatic notes (Coetzee and du Toit, 2012).

Terpene compounds are present in grape must in free, volatile form as well as non-volatile sugar conjugates (glycosides) (Gunata et al., 1985). Either enzymatically or through chemical hydrolysis, the volatile terpenes can be released from their bound forms. Enzymatic hydrolysis can take place by β -glucosidases (Hernández-Orte et al., 2008). In Sauvignon Blanc wines, the most important terpenes are the monoterpenes linalool, citronellol, and nerol, all of which contribute to the floral aroma (King and Dickinson, 2000).

Monoterpenes and terpene compounds generally form the axis for sensory expression of wine aroma but are present at low concentrations. Many authors state that during the fermentation of must, some yeast can produce glucosidases (Fleet, 2008) that can induce a two-stage enzymatic hydrolysis. The expression of glucosidases varies among yeast species and strains (Fleet, 2008). Reactions between geraniol, citronellol, nerol, linalool, and α -terpineol and yeast do not imply that monoterpenoids present in wine originate directly from the corresponding terpene through biotransformation (King and Dickinson, 2000). The yeast *S. cerevisiae*



can synthesise monoterpenes in the absence of grape-derived precursors (Carrau et al., 2016). The ability of yeasts to release aromatic compounds from bound odourless precursors is also possible (Hernández-Orte et al., 2008).

The aim of this study was to monitor the combined effect of autochthonous and active selected wine yeast (*S. cerevisiae*) and fermentation temperature conditions on qualitative parameters and amounts of monoterpenes involved in the varietal aroma of Sauvignon Blanc wines.

2. MATERIALS AND METHODS

2.1. Must origin and processing

The Sauvignon Blanc grapes (Winery Červinka, Horní Věstonice, Pod Děvínem, 48°52' 21"N, 16°37' 21"O) were harvested mechanically on 19 September 2016 and 1 September 2017. The grapes were destemmed and pressed using a membrane press allowing a gentle juice extraction (0–2 bar or 0–2 · 10⁵ Pa). The sugar concentration of the pressed juice was determined with a normalised saccharimeter and expressed as °NM (kg of total sugar in 100 L of juice). The result obtained were 21.2 °NM in 2016 and 23 °NM in 2017. After the harvest, the temperature of the juice was 19 °C; later, it spontaneously decreased to 15 °C. The content of titratable acidity was 6.3 g L⁻¹ (2016) and 8.5 g L⁻¹ (2017). The following additives were added to the must: 1 g L⁻¹ tartaric acid, 40 mg L⁻¹ sulphur dioxide, 0.4 g L⁻¹ VitaFerm Ultra F3 (ERBSLÖH Geisenheim GmbH, Germany), and eventually yeast (more below). After 24 h, the must was racked and the sediment was filtered with a lees rotating filter.

2.2. Fermentation procedure and yeast strains

The fermentation process was carried out in stainless tanks of 2000 L volume. For experiments associated with the preparation of Sauvignon Blanc wines, a selected yeast strain was inoculated into the must after rehydration, considering different fermentation temperature regimes. During the process, parameters (fermentation time and temperature) were continuously monitored and controlled (company cooling technology F-control, Hodonín, Czech Republic). Based on the fermentation duration, the different yeasts were compared in terms of their capability to efficiently ferment the grape juice. All must fermentation batches were replicated two times.

2.3. Yeast strains

The commercial *S. cerevisiae* yeast strain Zymaflore VL3 (noted Var.1; Laffort, France) with an excellent capacity for revealing thiol-type varietal aromas was used. These yeasts are intended for the production of full-bodied white wines with a typical varietal aroma. They are suitable for fermentation at temperatures in the range of 15–21 °C. Control variant (noted Var.2) was the must, which was not inoculated by the commercial yeast. The fermentation process was carried out by the natural yeast microflora on the surface of the harvested grapes.

2.4. Estimation of essential analytical parameters after fermentation

Determination of total acidity, volatile acidity, and pH were evaluated following the OIV official methods (OIV, 2015), these parameters were measured by TITROLINE EASY (SI Analytics



GmbH, Mainz, Germany). Residual sugar, acetic acid, and sugar free extract concentrations were estimated using a Fourier-transform infrared (FTIR) spectrometer (ALPHA) with Attenuated Total Reflection (Bruker Optik GmbH, Ettlingen, Germany). Depending on the calibration used, the measured values were evaluated automatically using the Opus Wine Wizard software. For the determination of glycerol, the colorimetric method of Rebelein was used (Rebelein, 1956).

2.5. Extraction of terpenes and GC-MS analysis

The amount of terpenes in the wine was determined by extraction with methyl tert-butyl ether. Twenty mL of wine, 50 μL of solution of internal standard (500 mg L^{-1} nonan-2-ol, 25 g L^{-1} cyclopentanone in ethanol), and 5 mL ammonium sulphate (saturated) were added into a 25 mL volumetric flask. The content of the flask was mixed with 0.75 mL of extraction solvent (1% neohexane in methyl tert-butyl ether). After shaking, the supernatant (hydrophobic part) was transferred into a tube for centrifugation; then the pure hydrophobic part was additionally dried by anhydrous magnesium sulphate (Baroň et al., 2017). The clean extracts were used for analysis by GC-MS.

For the chromatographic separation, a Shimadzu GC-17A system with autosampler (Shimadzu, Kyoto, Japan) was used with the following specifications: column (Agilent DB-WAX, 30 m length, 0.25 mm inner diameter, 0.25 μm film thickness of stationary phase PEG); injection volume: 1 μL ; split ratio injection 1:5; carrier gas: helium; flow: 1 mL min^{-1} ; linear gas velocity: 36 cm s^{-1} ; injector temperature: 180 $^{\circ}\text{C}$. The total time of the separation was 45 min.

The compounds were determined by a MS detector (SCAN mode–interval: 0.25 s, range: 14–264 AMU, voltage 1.5 kV). Compounds were identified by MS spectra and retention times. The peak area was used for quantification using external standard (Instrument: Shimadzu GC-17A; autosampler: AOC-5000; detector QP-5050A; software: GC solution). Terpenes were identified by comparison of the MS spectrum and the retention time with the NIST 107 library (Baroň et al., 2017).

2.6. Sensory evaluation

Altogether seven experts participated in the sensory evaluation. All experts were trained in the employment of scales and descriptors according to ISO 8586-2 (ISO, 2008). Individual wine samples were evaluated by using the UIOE (International Union of Oenologists) 100–point scale system.

3. RESULTS AND DISCUSSION

Figure 1 shows average values of temperature and length of the fermentation process for the different yeast cultures (Var.1, Var.2) and Sauvignon Blanc musts (seasons 2016, 2017). When it comes to temperature, it is very important to avoid major decreases during fermentation. If this occurs, the yeast may suffer a thermal shock and the fermentation will either slow down or cease (Michlovský, 2014). The fermentation of must went on at two temperatures (15 and 19 $^{\circ}\text{C}$), in two years (2016 and 2017), with added (Var.1) and natural (Var. 2) yeasts. From the temperature curves (Fig. 1) it is clear that the fermentation lasted the longest (12 days) at 15 $^{\circ}\text{C}$ and at 19 $^{\circ}\text{C}$ it happened within 9–11 days.



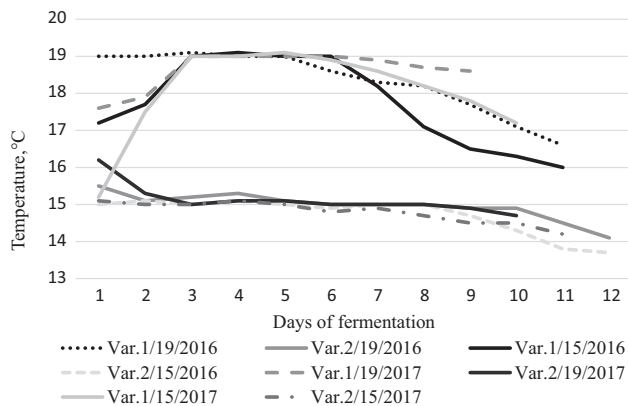


Fig. 1. The course and length of the fermentation process

Several factors can affect the varietal aroma of wine. The environmental factors are mostly climatic and local soil conditions. The factors that affect the wine making process are yeast species, temperature, amount of sulphur dioxide, and filtration (Romano et al., 2003). Temperature has an impact on both the wine making process and the aroma of wine. For better results, low fermentation temperature (15 °C) may be applied by winemakers to promote the production of volatile compounds by yeast and improve the wine aroma profile (Furdíková and Malík, 2008). Essential wine parameters obtained in this study by analytical measurements are shown in Table 1.

The results of the analysis are in accordance with previous findings that the spontaneous fermentation is characterised by increased contents of glycerol, higher alcohols, and volatile acids, as reported by (Průšová et al., 2018). Also (Michlovský, 2014), reported that the formation of glycerol depends on the initial amount of sugar, the nature of the yeast, and the fermentation conditions. Its average content ranges from 6 gL⁻¹ (in white wines) to 10 gL⁻¹ (in red wines). The results of the analysis show that the final glycerol concentration for the measured samples was between 7.65 and 9.07 gL⁻¹, which corresponds to the specified range. Fermentation parameters affect final ethanol and glycerol concentrations (Molina, 2007). The results obtained in this study are in agreement with this finding. While the alcohol content was higher for Var.1 at 15 °C and 19 °C in 2016, in 2017 the content was higher for Var.1 at 15 °C and Var.2 at 19 °C. The higher alcohol content in 2017 is directly related to the higher sugar content of the must. In 2016, the levels of residual sugars were higher for Var.2 at both 15 °C and 19 °C. In 2017, higher levels of residual sugars were observed for both Var.1 and Var.2 at 19 °C. The obtained results show that in most cases spontaneous alcoholic fermentation was not completed at low temperature.

The pH values were in the range of 3.10–3.33, which, as stated by (Štefěcová and Čepička, 2001), is a good prerequisite for the future chemical and biological stability of the wines. The titratable acidity values varied between 5.26 and 7.34 gL⁻¹. The recommended titratable acidity range in white wines is 5–7 gL⁻¹ (expressed as tartaric acid) (Carrau et al., 2016). Regarding acetic acid, its content was always lower (0.32–0.51 gL⁻¹) than the maximum limit defined by



Table 1. Analytical parameters of the produced Sauvignon Blanc wines

Var/temp/year	Alcohol %	Residual sugar (g L ⁻¹)	pH –	Glycerol (g L ⁻¹)	Density (kg L ⁻³)	Titratable acid (g L ⁻¹)	Malic acid (g L ⁻¹)	Lactic acid (g L ⁻¹)	Acetic acid (g L ⁻¹)	Tartaric acid (g L ⁻¹)
Var.1/19/2016	13.90ab	1.74b	3.25ab	8.06e	0.99415a	6.32d	3.47g	0.09a	0.47ef	1.86a
Var.2/19/2016	13.00a	8.61d	3.28ac	7.65b	0.99416a	5.61a	1.86a	1.23e	0.36ab	1.85a
Var.1/15/2016	13.67ab	1.30a	3.27ac	7.88c	0.99272a	5.59a	1.66e	0.77b	0.43de	2.45b
Var.2/15/2016	13.58ab	4.65c	3.33c	8.33d	0.99142a	5.26b	1.90ab	1.37f	0.43cde	1.81a
Var.1/19/2017	13.20ab	45.91h	3.20b	8.34a	1.00673a	5.93c	1.37d	0.40c	0.32a	3.43d
Var.2/19/2017	13.70ab	40.77g	3.10d	9.07g	1.00998a	7.34g	1.15c	0.83b	0.37abc	4.05f
Var.1/15/2017	14.90c	33.92e	3.11d	8.20f	1.00538a	7.01f	1.92b	0.14a	0.41bcd	3.80e
Var.2/15/2017	14.44bc	37.34f	3.26ab	7.94d	1.00321a	6.55e	2.32f	0.53d	0.51f	3.23c

Var: variant; temp: temperature.

In columns, different letters indicate significant differences at $P < 0.05$ by Tukey's-HSD test.



EEC Regulation 2676/90 (1 g acetic acid L⁻¹), which is also the sensory detection threshold (EEC, 1990).

Terpenes belong to an important group of aromatic substances, which are characterised by floral, nutmeg, and fruit aromas. The most important group of varietal aromas in wine are monoterpenes (Průšová et al., 2018). Yeasts can exhibit a specific enzymatic activity to release certain monoterpenes from involatile precursors (Makhotkina et al., 2012). Terpene biosynthesis by industrial yeasts is related to nitrogen metabolism, and high assimilable nitrogen content in juice stimulates monoterpene formation (Carrau et al., 2016). The free terpenes pass during fermentation into the wine in a substantially unchanged form. Their odour thresholds are high in wines, but in order to determine their true contribution to wine aroma, it is necessary to know their cumulative or synergistic effects. The most common terpenes are the monoterpene alcohols, namely linalool, geraniol, nerol, hotrienol, and alpha-terpineol. Amounts of these compounds and their sensory expression determined in this study for the evaluated wine samples are presented in Table 2.

The results show that the highest monoterpene contents were those of linalool, hotrienol, and geraniol. The obtained values also prove that the highest content of terpenes was measured both years (2016 and 2017) for Var.1 (*S. cerevisiae*) with fermentation temperature of 15 °C. Examples of model fermentations associated with the biotransformation of linalool, alpha-terpineol, nerol, and geraniol monoterpenes by the yeast *S. cerevisiae* have been discussed by (Masneuf-Pomarède et al., 2006). The mechanisms of monoterpene biotransformations have not yet been precisely defined. However, the total terpene content in these biotransformations does not seem to increase.

Other fermentation derived compounds that are synthesised by yeast and affect the aroma of wine include esters, higher alcohols, volatile fatty acids, carbonyls, and sulphur containing compounds. Their production is dependent on parameters such as the genetic background of the dominant yeast strain, the availability of precursors (including added yeast nutrients), the fermentation temperature, the exposure to oxygen, as well as fermentation stresses (Swiegers et al., 2005). In previous studies (Polaskova et al., 2008), the highest concentration of linalool was found in the varieties especially in Muscat Ottonel, Sauvignon, Tămăioasă Românească, or Fetească Albă.

Table 2. Monoterpene contents in the produced Sauvignon Blanc wines

Var./temperature/year	Linalool	Hotrienol	Alpha-terpineol µg L ⁻¹	Nerol	Geraniol	Total
Var.1/15/2016	24.36d	11.84c	6.20b	0.56c	9.75c	52.71c
Var.2/15/2016	14.59a	8.94b	7.50c	0.37b	7.12b	38.52b
Var.1/19/2016	16.70b	7.38a	5.77a	n.d.	6.20b	36.05a
Var.2/19/2016	21.51c	8.12b	6.51b	0.08a	1.39a	37.61a
Var.1/15/2017	13.04c	10.14c	5.39c	n.d.	11.99d	40.56d
Var.2/15/2017	12.37b	6.76a	2.93a	n.d.	4.84c	26.90a
Var.1/19/2017	6.63a	7.45b	5.23c	n.d.	0.07a	19.38b
Var.2/19/2017	12.07b	7.54b	4.68b	n.d.	0.26b	24.55c

Values are means. In columns, different letters indicate significant differences at $P < 0.05$ by Tukey's-HSD test; n.d.: not detected.



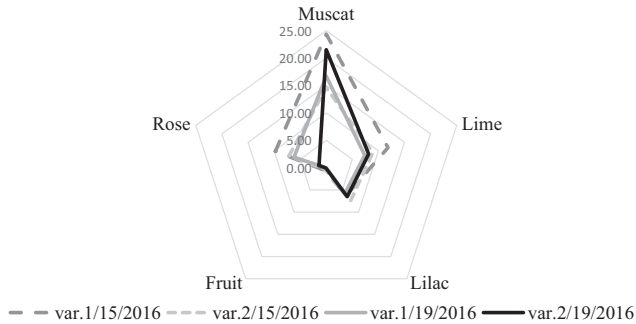


Fig. 2. Spider plot indicating the effect of temperature and yeast on the Sauvignon Blanc aroma profile (2016)

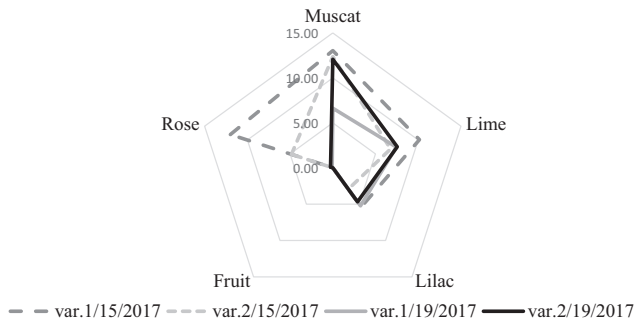


Fig. 3. Spider plot indicating the effect of temperature and yeast on the Sauvignon Blanc aroma profile (2017)

The olfactory impact of monoterpenes, or their compounds, can be synergistic (interacted) with a significant effect on the overall complexity of the wine (Ribéreau-Gayon et al., 2006b).

Figures 2 and 3 show the effect of temperature and yeast on the aroma of the wine samples according to the content of the analysed terpene substances.

Also, Carrau et al. (2016) stated that the corresponding detection thresholds for Sauvignon Blanc are $132 \mu\text{g L}^{-1}$ for geraniol, $100 \mu\text{g L}^{-1}$ for linalool, $400 \mu\text{g L}^{-1}$ for nerol, and $460 \mu\text{g L}^{-1}$ for alpha-terpineol. However, it is clear from our results that the determined concentration levels did not reach the detection threshold for any sample.

Table 3 presents the results of the sensory analysis, which was performed 6 months after the end of fermentation by using the UIOE 100–point scale system.

From a statistical point of view, there was no significant vintage difference for the evaluated variants of the Sauvignon Blanc wines except for Var.1/15. Sauvignon Blanc wines, in which the fermentation took place with the application of natural yeasts (Var.2), at both temperatures (15 and 19 °C) had lower sensory evaluation points. According to experts involved in the sensory evaluation, the evaluated wine samples of Var.1, fermented at the temperature of 15 °C, were more pronounced in terms of the sensory profile, which showed lime, muscat, and rose notes.



Table 3. The results of sensory evaluation in Sauvignon Blanc wines: Variant/Temperature

Year	Var.1/19	Var.2/19	Var.1/15	Var.2/15
2016	84.7ab	82.3a	87.0b	82.3a
2017	84.4a	83.4a	89.0b	84.2a

In lines, different letters indicate significant differences at $P < 0.05$ by Tukey's-HSD test.

4. CONCLUSIONS

This work describes the influence of wine fermentation temperature and selected yeast strain on qualitative parameters and the production of monoterpenes in Sauvignon Blanc wines. Wines with a higher content of titratable acids were generally produced using the commercial *S. cerevisiae* yeast strain Zymaflore VL3. This trend has always manifested itself in all wines in the lactic acid content. A similar trend was also observed in relation to the higher alcohol content of wines produced by commercial yeasts. The results of measurements and analyses showed that wines produced with selected *Saccharomyces* starter cultures are more complex and may be more acceptable to consumers, despite the fact that the concentration level of all determined monoterpenes did not reach the detection threshold in any of the evaluated samples. This fact is also supported by the results of sensory evaluation. The samples of wines made with *Saccharomyces* starter cultures were better evaluated in combination with lower temperatures, at which the varietal profile of Sauvignon Blanc wines with fragrant tones of lime, nutmeg, and roses was better evaluated.

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