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Indigenous Yeasts Perform Alcoholic Fermentation and Produce Aroma Compounds in Wine

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Abstract

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The spontaneous alcoholic fermentations of Moscato Bianco and Welschriesling must were carried out to retrieve indigenous yeasts. We confirmed that those fermentations, conducted with non-*Saccharomyces* and indigenous *Saccharomyces cerevisiae* yeasts, can generate high amounts of aroma compounds in wines. Consequently, two of the *S. cerevisiae* isolates were randomly chosen and further examined in Welschriesling and Sauvignon Blanc must for their ability and efficiency in performing alcoholic fermentation. Alcoholic fermentation with a commercial yeast strain was carried out for comparison. Indigenous isolates showed acceptable fermentation ability and efficiency. Moreover, Sauvignon Blanc produced with indigenous isolates contained significantly higher amounts of 3-mercaptohexyl acetate, linalool, geraniol and 2-phenylethanol and a significantly lower amount of 3-mercaptohexan-1-ol. Differences in Welschriesling wine were less striking but in this case indigenous isolates produced lower amounts of 3-mercaptohexan-1-ol and α-terpineol. Taken together, our results confirm that a suitable aromatic profile of wine can be produced with indigenous *S. cerevisiae* strains.

Keywords: *Saccharomyces*; non-*Saccharomyces*; thiols; monoterpene alcohols

The diversity of white wine types and winemaking methods has strongly decreased in the last 20–30 years because of strategies related to the world market, consumer tastes and preferences towards the use of a few universally appreciated techniques. Methods for inoculated alcoholic fermentation (AF) apply rather few commercially available yeast strains and the use of active dry yeasts (ADY) has replaced traditional practices that were based on indigenous yeast strains. A good-quality wild microflora, however, can produce spontaneous AF with excellent results and interesting wines characterised by a complexity of flavour, intense aroma persistency, overall distinction and vintage variability (Ribéreau *et al.* 2007). Recently, a new approach in managing inoculated AF consisting in the use of indigenous *Saccharomyces* or non-*Saccharomyces* yeasts has been implemented (Medina *et al.* 2013). The use of mixed starters of selected non-*Saccharomyces* yeasts and *S. cerevisiae* strains represents an alternative to both spontaneous and inoculated AF.

Although spontaneous AF on an industrial scale can be conducted either with residual ADY or indigenous strains (Scholl *et al.* 2016), the process is ecologically and biochemically complex involving yeasts of various genera but predominantly numerous strains of *S. cerevisiae* (Hall *et al.* 2011). These exhibit successive growth during the process and contribute to the final chemical and sensory quality of wine, which is specific for each combination of strains (Howell *et al.* 2006). Could this diversity be harnessed to gain satisfactory results, for in-

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stance, good fermentation kinetics involving complete processes, and balanced production of aroma compounds? The prevalence of different species or strains during spontaneous AF depends on various factors. The most important factors relate to the yeast killer phenotype, grape healthiness and ripeness, pesticide residues and residual population of ADY strains on winery equipment. Factors related to technological practices include time and amount of SO_2 addition, clarification degree, amount of dissolved oxygen in must and temperature during AF (Albertin *et al.* 2014).

The aim of our study was to perform a spontaneous AF with one aromatic (Moscato Bianco) and another more aromatically neutral variety (Welschriesling) of must, in order to isolate indigenous yeasts. We then conducted inoculated laboratory AF to examine the suitability of the naturally retrieved *S. cerevisiae* isolates, again with one aromatic variety (Sauvignon Blanc) and aromatically neutral variety (Welschriesling).

MATERIAL AND METHODS

Must handling for spontaneous AF. Entirely sound grapes of Moscato Bianco (synonym for the Muscat Blanc à Petits Grains variety) and Welschriesling were aseptically sampled in 2013 in the wine-producing region of Slovenian Styria (north-east of the country) and manually pressed in sterile plastic bags. Samples consisted of approx. 1.5 kg per replicate. Two replicates were taken for Moscato Bianco (MB1, MB2) and three for Welschriesling (WR1–WR3) from different parcels within the same vineyard. Before pressing, a mixture of L-ascorbic acid (35%) and potassium metabisulphite (demoisturised by 55%), and purified gallotannins (10%) were added into the bags in amounts of 0.1 g/kg. Must parameters for Moscato Bianco (Welschriesling) were 165.0 (185.0) g/l fermentable sugars, 8.1 (8.1) g/l total acids as tartaric acid, 101 (113) mg/l yeast assimilable nitrogen (YAN), and a pH of 3.40 (3.16).

Spontaneous AFs were conducted at room temperature (21–23°C). The weights of the fermentors and the amount of released CO_2 was monitored for 48 (Moscato Bianco must) or 39 days (Welschriesling must).

Microbiological analysis and isolation of indigenous isolates. During spontaneous AF, aliquots of must were aseptically sampled to allow determination of yeast species compositions and to count colony forming units (CFUs). The samples were diluted and cultured on Wallerstein Laboratory (WL) nutrient agar (Merck KGaA, Germany). Moscato Bianco must was sampled and microbiologically studied after day 0, 15 and 37, Welschriesling after day 0, 18, 22, and 35. Yeast colonies were classified into non-*Saccharomyces* and *Saccharomyces* groups according to overall macro- and microscopic characteristics described in the literature (Compendium of International 2016). Yeast strains of different morphological types were isolated from MB1 and WR2 fermentors, where the highest amount of CO_2 was released and isolation of *S. cerevisiae* strains was expected. A total of 17 representative isolates were retrieved from both fermentors and cryopreserved in glycerol (10%) at –80°C.

DNA barcode-based species identification. Ten isolates from both fermentors were grown as pure cultures on yeast-malt (YM) medium (yeast extract 3.0 g/l, malt extract 3.0 g/l, peptone 5.0 g/l, glucose 30.0 g/l, agar 20.0 g/l) for five days. Genomic DNA of cells harvested into 2-mm micro-centrifuge tubes was extracted using the NucleoSpin Plant II kit (Macherey-Nagel, Germany) including CTAB buffer according to the manufacturer's instructions. For sequencing the D1/D2 region of the 26S ribosomal gene and the internal transcribed spacers ITS1 and ITS2 including the 5.8S rDNA we followed Glushakova *et al.* (2010). Primers used for amplifications were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes & Bruns 1993) and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') (O'Donnell 1993). Reaction mixtures of 50 µl containing 3 µl $MgCl_2$ (25 mM), 1.0 µl dNTP mixture (10 mM; Promega), 0.25 µl primers ITS1F and NL4 (100 pM each), 5 µl buffer plus KCl, 0.2 µl Taq Polymerase (5 U/µl; Fermentas), and 1.5 µl genomic DNA were processed on a Veriti PCR machine (Applied Biosystems). The PCR programme consisted of an initial denaturation step of 2 min at 96°C, followed by 35 cycles of 30 s at 96°C (for denaturation), 50 s at 52°C (annealing), 90 s (elongation) at 72°C, and a final extension step of 7 min at 72°C. Agarose gel-checked PCR fragments were directly sequenced with the ITS1F, ITS4 (White *et al.* 1990), NL1 and NL4 primers (O'Donnell 1993) with the help of a commercial sequencing laboratory. Assembled contigs were compared with sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST searches (Altschul *et al.* 1990).

Microvinifications with isolated strains. As the results of spontaneous AF showed the great potential of indigenous strains to produce the volatile thiols (Table 2) that are typical aroma compounds

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of Sauvignon Blanc, we used musts of this variety and Welschriesling for these experiments. Two indigenous *S. cerevisiae* strains, randomly chosen from the isolates, were co-inoculated in both musts. For each variety, a separate AF with a commercial yeast strain was used for a comparison. Sauvignon Blanc must 2013 (fermentable sugars 180.0 g/l, total acids 7.8 g/l, YAN 76 mg/l, and pH = 3.12) obtained from 5 kg of grapes was self-settled after addition of 5–6% H_2SO_3 (0.6 ml/l). Two indigenous isolates of *S. cerevisiae* were inoculated for AF as described elsewhere (Jolly *et al.* 2003). The inoculum of both isolates was prepared on a shaker in liquid YM. After 18 h of cultivation, yeast cell concentration was measured with a haemocytometer and the yeast suspension was used for inoculation to reach a final concentration of 1×10^6 CFU/ml and 1 : 1 ratio. A control AF was carried out with the commercial yeast strain VL3 (Laffort, France). Both AFs were conducted in triplicates in 0.5 l fermentors in a thermostatic cabinet at 16–18°C. After 1/3 and 1/2 of the sugars were depleted, the yeast nutrient diammonium phosphate (each time 0.3 g/l) was added to the must.

The same experiment was performed with Welschriesling must 2013 (fermentable sugars 182.0 g/l, total acids 6.5 g/l, YAN 58 mg/l, and pH = 3.16); the commercial VIN7 strain (Lallemand, Canada) was used in the control AF.

Measurements of volatile compounds. Measurements were taken 2–3 months after completion of spontaneous AF (4 out of 5 samples) and inoculated AF. Measurements of free volatiles such as monoterpene alcohols, acetaldehyde, ethyl acetate and higher alcohols were performed using methods described in Bavcar *et al.* (2011). For α-terpineol, citronellol, geraniol, linalool, and nerol, wine samples were diluted with water (Milli-Q; Milipore, USA) (1 : 4) to achieve a 1 : 3 ratio between the liquid and the headspace of a 20-ml SPME vial. Samples were incubated at 40°C for 1 h and monoterpene alcohols were adsorbed to a PDMS/DVB fibre (Supelco, USA). These were identified and quantified with a gas chromatograph (Agilent 7890A; Agilent Technologies, USA) equipped with the MPS 2 automatic sampler (Gerstel, Germany) and coupled with a mass spectrometer (Agilent 5975C; Agilent Technologies) (GC-MS system). The concentrations of volatile thiols (4-mercapto-4-methyl-2-pentanone (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were measured in wine samples after completion of AF using the method described in Tominaga and Dubourdieu (2006), slightly modified by Jenko *et al.* (2013) on the same GC-MS system. Hydroxy mercury benzoate (5 ml of a 2-mM solution) and butylated hydroxylanisole (0.5 ml of a 0.02-mM solution) were added to 50 ml of wine sample. After mixing for 1 min, internal standards consisting of 4-methoxy-2-methyl-2-mercaptobutane (4M2M2MB), d3MH, and d3MHA were added and the procedure described in the references was followed.

Statistical analysis. The results were statistically analysed using ANOVA in Statgraphics® Centurion XVI software (StatPoint Technologies, USA).

RESULTS AND DISCUSSION

Spontaneous AF of Moscato Bianco and Welschriesling. The amounts of produced CO_2 per 100 ml of Moscato Bianco must were 9.2 g in MB1 and 8.8 g in MB2 (Figure 1). Both fermentations were characterised after 48 days when the concentrations of unfermented reducing sugars were 44 (MB1) or 35 g/l (MB2). The concentration of yeast cells increased in both fermentors during the experiment and the proportion of non-*Saccharomyces* and *S. cerevisiae* cells changed as expected (Table 1). Representatives of morphologically identified operational taxonomic groups isolated from MB1 at the second sampling time revealed molecular barcodes indistinguishable from deposited sequences of reference strains of the ascomycetous yeasts *Hanseniaspora uvarum* (retrieved ITS1F/NL4 amplification product, ca. 1400 bp), *Starmerella bacillaris* (ca. 1000 bp) and *Saccharomyces cerevisiae* (ca. 1450 bp).

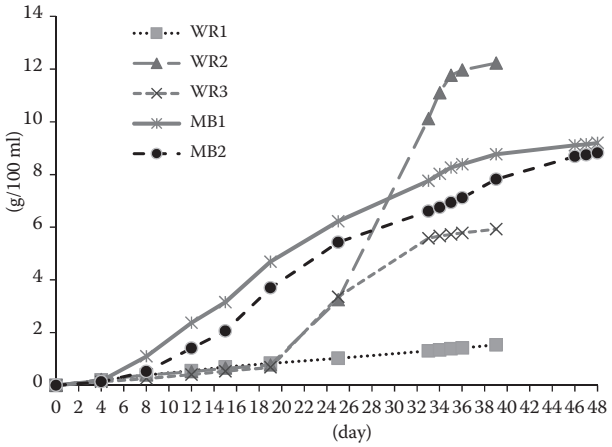

Figure 1. Amount of produced CO_2 (g/100 ml) in spontaneous alcoholic fermentation (days) of Welschriesling (WR) and Moscato Bianco (MB)

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Table 1. Concentration of viable yeast cells (log_{10} CFU/ml) and proportions of non-*Saccharomyces* and *S. cerevisiae* cells in spontaneous AF of Moscato Bianco (MB) and Welschriesling (WR)

Sampling	MB1		MB2	
	log_{10} CFU/ml	non-*Sacch.* : *S. cerevisiae*	log_{10} CFU/ml	non-*Sacch.* : *S. cerevisiae*
1st	4.5	100 : 0	5.2	100 : 0
2nd	6.4	50 : 50	6.1	90 : 10
3rd	5.8	0 : 100	6.3	0 : 100

	WR1		WR2		WR3	
	log_{10} CFU/ml	non-*Sacch.* : *S. cerevisiae*	log_{10} CFU/ml	non-*Sacch.* : *S. cerevisiae*	log_{10} CFU/ml	non-*Sacch.* : *S. cerevisiae*
1st	1.5	100 : 0	3.0	100 : 0	1.8	100 : 0
2nd	4.9	100 : 0	6.2	100 : 0	3.0	100 : 0
3rd	*	not relevant	7.3	100 : 0	6.5	100 : 0
4th	*	not relevant	5.8	5 : 95	*	not relevant

*must was not sampled due to weak AF

The amounts of released CO_2 per 100 ml of Welschriesling must were 1.5 g (WR1), 12.2 g (WR2), and 5.9 g (WR3) (Figure 1). The amounts of unfermented reducing sugars in wine were 178 g/l (WR1), 5 g/l (WR2), and 109 g/l (WR3) after 53 days. The CO_2 production correlated with the number of yeast cells in must. These increased in WR2 and WR3 but clearly reduced values were observed at the fourth sampling time in WR2. Because the AF was too weak in WR1 and WR3, no must samples were taken at that time. No *S. cerevisiae* was found in WR1 and WR3 (Table 1). According to morphological and microscopic examination, *Zygosaccharomyces bailii* (retrieved ITS1F/NL4 amplification product, ca. 1400 bp) prevailed in WR1 and *H. uvarum* and *Metschnikowia pulcherrima* (retrieved ITS1F/NL4 amplification product, ca. 950 bp) in WR3. In WR2, *Z. bailii* was isolated at second, third and fourth sampling times (day 18, 22, and 35) while *S. cerevisiae* only at the fourth (day 35).

It has been reported that spontaneous AF can proceed with sluggish kinetics or even cease because of the long duration of the process or the complete prevalence of non-*Saccharomyces* yeasts. The lack of nutrients and vitamins that are required for yeast growth, and the presence of inhibitors during the successive growth of different yeasts in must are the main reasons for such observations (Nguyen & Panon 1998; Mortimer 2000). Because the yeast composition and concentration is unique in must, spontaneous AF also shows unique kinetics in each fermentation process (Hall *et al.* 2011). The results of our study confirm these observations.

The concentrations of 4MMP and 3MHA were comparable in both Moscato Bianco assays (Table 2);

Table 2. Concentration of volatile thiols and monoterpene alcohols in wines of spontaneous AF (Moscato Bianco – MB, Welschriesling – WR)

	MB1	MB2	WR2	WR3	Olfactory perception threshold (ng/ll)
Thiols (ng/l)					
4MMP	78	67	11	12	0.8
3MHA	25	21	208	981	4
3MH	2 787	4 692	10 274	21 858	60
Monoterpene alcohols (µg/l)					
Linalool	2 848	2 451	*	*	50
Geraniol	1 177	1 272	*	*	130
α-Terpineol	3 041	3 200	*	*	400
Nerol	139	169	*	*	400
Citronellol	151	102	*	*	18

*concentrations of monoterpene alcohols were not measured in Welschriesling

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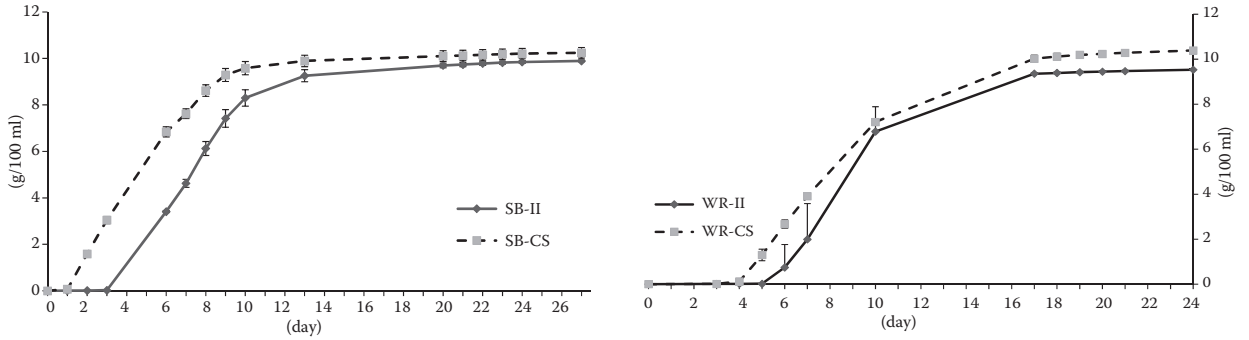

Figure 2. Kinetics of CO_2 release (g/100 ml) in inoculated alcoholic fermentation (days) of Sauvignon Blanc (SB) and Welschriesling (WR) with indigenous isolates (II) and commercial strains (CS) (mean ± SD)

the concentration of 3MH was almost 2000 ng higher in MB2. The concentrations of monoterpene alcohols also differed in the two wines: higher values were measured for geraniol, α-terpineol and nerol in MB2, while higher values for linalool and citronellol were registered in MB1.

As in the Moscato Bianco assay, the concentration of the 3MH compound also doubled in WR3.

It is clear that the persistence of non-*Saccharomyces* yeasts is responsible for increased concentrations of the above-mentioned volatiles because such species can release high amounts of monoterpene alcohols or thiols from their non-volatile precursors (Mendes Ferreira *et al.* 2001; Zott *et al.* 2011). The differing ratios between non-*Saccharomyces* and *S. cerevisiae* yeasts in each fermentor caused considerable differ-

Table 3. Concentrations of volatile thiols, monoterpene and higher alcohols in Sauvignon Blanc (SB) and Welschriesling (WR) (II-indigenous isolates, CS-commercial strains) (mean ± SD)

	SB-II	SB-CS	WR-II	WR-CS	Olfactory perception threshold
Volatile thiols (ng/l)					
4MMP	26 ± 3^a	44 ± 16^a	11 ± 2^a	16 ± 3^a	0.8
3MHA	213 ± 1^b	130 ± 22^a	122 ± 9^a	89 ± 21	4
3MH	2264 ± 18^a	2657 ± 35^b	1132 ± 97^a	1410 ± 75^b	60
Monoterpene alcohols (μg/l)					
Linalool	13 ± 2^b	7 ± 1^a	9 ± 4^a	16 ± 5^a	50
Geraniol	41 ± 12^b	17 ± 4^a	29 ± 20^a	37 ± 13^a	130
α-Terpineol	28 ± 1^a	28 ± 1^a	27 ± 3^a	36 ± 5^b	400
Nerol	nd	nd	nd	nd	400
Citronellol	12 ± 4^a	20 ± 3^a	13 ± 6^a	14 ± 2^a	18
Acetaldehyde, Ethyl acetate, and higher alcohols (mg/l)					
Acetaldehyde	23 ± 1^b	18 ± 1^a	14 ± 1^a	22 ± 7^a	100
Ethyl acetate	45 ± 5^a	42 ± 2^a	37 ± 1^a	40 ± 3^a	15
2-Bbutanol	nd	nd	nd	nd	30
1-Propanol	8 ± 0 ^{b}	6 ± 0^a	5 ± 0^a	5 ± 1^a	40
2-Methyl propanol	22 ± 8^a	39 ± 5^b	40 ± 6^a	35 ± 8^a	40
2-Propenyl alcohol	nd	nd	nd	nd	1
1-Butanol	1 ± 0	nd	nd	nd	30
2-Methyl butanol	32 ± 3^a	35 ± 1^a	39 ± 3^a	37 ± 2^a	15
3-Methyl butanol	171 ± 19^a	183 ± 7^a	201 ± 17^a	173 ± 9^a	30
2-Phenyl ethanol	83 ± 10^b	47 ± 2^a	69 ± 2^a	75 ± 8^a	10

^{a,b}significant differences among samples within the same grapevine variety at 95% confidence level; nd – not detected

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ences in 3MH, linalool and citronellol in Moscato Bianco and 3MHA and 3MH in Welschriesling.

Inoculated AF of Sauvignon Blanc and Welschriesling. The exponential growth phase of indigenous yeasts Sauvignon Blanc started only three days later, while that of the commercial strain began already on the first day. A similar delay was also observed for the start of the stationary growth phase. Both AFs lasted 27 days and the amount of released CO_2 was comparable. All AFs in Welschriesling were completed. Their kinetics hardly varied among replicates inoculated with the commercial strain. The AF with indigenous isolates took 24 days to reach a concentration of fermentable sugars below 2 g/l, the commercial strain, 31 days. The total amount of produced CO_2 was significantly lower in the AF with indigenous isolates (9.5 vs. 10.5 g/l).

Five of the six fermentations could be completed with indigenous yeasts, demonstrating that our isolates showed acceptable fermentation ability and efficiency. Only slight delays at the start of the exponential or stationary growth phases were observed. Indigenous isolates even allowed earlier terminations of the processes in the AF of Welschrielsing.

The concentrations of volatile thiols, citronellol (only in the wine produced with commercial strains), ethyl acetate, 2-methyl butanol, 3-methyl butanol, and 2-phenyl ethanol in Sauvignon Blanc wines exceeded the olfactory perception thresholds (Table 3). The concentrations of 3MHA, linalool, geraniol, 2-phenylethanol, 1-propanol, and acetaldehyde were significantly higher in the wine produced with indigenous isolates, but the concentrations of 3MH and 2-methyl propanol were significantly lower.

The concentrations of volatile thiols, ethyl acetate, 2-methyl propanol (only in the wine produced with indigenous isolates), 2-methyl butanol, 3-methyl butanol, and 2-phenyl ethanol in Welschriesling wines exceeded olfactory perception thresholds (Table 3). The concentrations of 3MH and α-terpineol were significantly lower in the wine produced with indigenous isolates. All other differences were not significant.

Thus, our results confirm that indigenous *S. cerevisiae* yeasts could produce a suitable aromatic profile in both wines.

CONCLUSIONS

Although the grape samples were small in size, we successfully isolated indigenous *S. cerevisiae* yeasts involved in the spontaneous AF of Moscato Bianco and Welschriesling must. In both processes, high concentrations of volatiles were released, particularly volatile thiols, by the indigenous non-*Saccharomyces* and *S. cerevisiae* yeasts. Therefore, two *S. cerevisiae* isolates were used in inoculated AF of Welschriesling and Sauvignon Blanc must, and they showed good fermentation abilities which were comparable to the commercial strains. Thus, we confirm that indigenous *S. cerevisiae* strains can be used to produce wines with suitable aromatic profiles. Our results underline the potential of indigenous yeasts in winemaking and may therefore encourage winemakers to continue with their use.

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References

Albertin W., Miot-Sertier C., Bely M., Marullo P., Coulon J., Moine V., Colonna-Ceccaldi B., Masneuf-Pomarede I. (2014): Oenological prefermentation practices strongly impact yeast population dynamics and alcoholic fermentation kinetics in Chardonnay grape must. International Journal of Food Microbiology, 178: 87–97.

Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. (1990): Basic local alignment search tool. Journal of Molecular Biology, 215: 403–410.

Bavcar D., Cesnik H.B., Cus F., Kosmerl T. (2011): The influence of skin contact during alcoholic fermentation on the aroma composition of Ribolla Gialla and Malvasia Istriana *Vitis vinifera* (L.) grape wines. International Journal of Food Science and Technology, 46: 1801–1808.

Compendium of International Methods of Wine and Musts Analysis (2016): Paris, Organisation International de la Vigne et du Vin: 415–449.

Gardes M., Bruns T.D. (1993): ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology, 2: 113–118.

Glushakova A.M., Maximova I.A., Kachalkin A.V., Yurkov A.M. (2010): *Ogataea cecidiorum* sp nov., a methanol-assimilating yeast isolated from galls on willow leaves. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 98: 93–101.

Hall B., Durall D.M., Stanley G. (2011): Population Dynamics of *Saccharomyces cerevisiae* during spontaneous fermentation at a British Columbia Winery. American Journal of Enology and Viticulture, 62: 66–72.

doi: 10.17221/398/2016-CJFS

Howell K.S., Cozzolino D., Bartowsky E.J., Fleet G.H., Henschke P.A. (2006): Metabolic profiling as a tool for revealing *Saccharomyces* interactions during wine fermentation. Fems Yeast Research, 6: 91–101.

Jenko M., Lisjak K., Kosmerl T., Cus F. (2013): The influence of yeast strain combinations on the quality of Sauvignon Blanc wine. Food Science and Technology Research, 19: 7–15.

Jolly N.P., Augustyn O.H.P., Pretorius I.S. (2003): The effect of non-*Saccharomyces* yeasts on fermentation and wine quality. South African Journal of Enology and Viticulture, 24: 55–62.

Medina K., Boido E., Fariña L., Gioia O., Gomez M.E., Barquet M., Gaggero C., Dellacassa E., Carrau F. (2013): Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. Food Chemistry, 141: 2513–2521.

Mendes Ferreira A., Clímaco M.C., Mendes Faia A. (2001): The role of non-*Saccharomyces* species in releasing glycosidic bound fraction of grape aroma components – a preliminary study. Journal of Applied Microbiology, 91: 67–71.

Mortimer R.K. (2000): *Kloeckera apiculata* controls the rates of natural fermentation. Rivista di Viticoltura e di Enologia, 53: 61–68.

Nguyen H.V., Panon G. (1998): The yeast *Metschnikowia pulcherrima* has an inhibitory effect against various yeast species. Sciences des Aliments, 18: 515–526.

O'Donnell K. (1993): *Fusarium* and its near relatives. In: Reynolds D.R., Taylor J.W. (eds): The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. Wallingford, CAB International: 225–233.

Ribéreau G.P., Dubourdieu D., Donéche B., Lonvaud A. (2007): Handbook of Enology. Vol. 1: The Microbiology of Wine and Vinifications. Chichester, John Willey & Sons: 209–211.

Scholl C.M., Morgan S.C., Stone M.L., Tantikachornkiat M., Neuner M., Durall D.M. (2016): Composition of *Saccharomyces cerevisiae* strains in spontaneous fermentations of Pinot Noir and Chardonnay. Australian Journal of Grape and Wine Research, 22: 384–390.

Tominaga T., Dubourdieu D. (2006): A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wines made from *Vitis vinifera* grape varieties. Journal of Agricultural and Food Chemistry, 54: 29–33.

White T.J., Bruns T., Lee S., Taylor W.J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Ninsky J.J., White T.J. (eds): PCR Protocols: A Guide to Methods and Applications. New York, Academic Press, Inc.: 315–322.

Zott K., Thibon C., Bely M., Lonvaud-Funel A., Dubourdieu D., Masneuf-Pomarede I. (2011): The grape must non-*Saccharomyces* microbial community: Impact on volatile thiol release. International Journal of Food Microbiology, 151: 210–215.

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