The influence of yeasts on the aroma profile of Viennese Sauvignon blanc

Article /	cle <i>in</i> Mitteilungen Klosterneuburg · December 2015	
CITATIONS 0	IONS REAE 1,4	
1 autho	thor:	
(4)	Karin Mandl Höhere Bundeslehranstalt und das Bundesamt für Wein- und Obstbau in Klosterneuburg 45 PUBLICATIONS SEE PROFILE	3

THE INFLUENCE OF YEASTS ON THE AROMA PROFILE OF VIENNESE SAUVIGNON BLANC WINES

Dragos Pavelescu¹, Karin Mandl², Robert Steidl², Xandra Blesl² and Bernhard Spangl³

¹Winery Mayer am Pfarrplatz, VWG "Vienna 19" Weinmarketing GmbH A-1190 Wien, Kuchelauer Hafenstrasse 175 E-Mail: Dragos.Pavelescu@pfarrplatz.at

² Federal College and Federal Office of Viticulture and Pomology Klosterneuburg A-3400 Klosterneuburg, Wiener Straße 74

³ University of Natural Resources and Life Sciences Vienna, Institute of Applied Statistics and Computing (IASC) A-1190 Wien, Peter-Jordan-Straße 82

Five different yeasts (X5, VL3, Delta, Revelation Thiols and Alchemy II) and one yeasts mixture (X5 + Alchemy II) were tested for the ability to liberate volatile thiols during alcoholic fermentation. The chemical composition and the sensory attributes of the Sauvignon blanc wines were monitored. The yeasts applied provoked significant differences regarding the production of 4-mercapto-4-methylpentan-2-one (4-MMP) and 3-mercaptohexan-1-ol (3-MH). Fermentation aroma substances isoamyl acetate, phenylethyl acetate, 2-phenylethanol and ethyl hexanoate were as well significantly influenced by the yeasts used. Co-inoculated fermentation of different yeast strains can produce wines with different chemical composition and sensory profile, compared to wines produced from the same must with pure strains. In this experiment, the yeasts used for fermentation were a deciding factor not just for aroma production, but for a successful alcoholic fermentation. Not all the yeasts applied fermented the grape juice to dryness.

Der Einfluss von Hefen auf das Aromaprofil von Wiener Sauvignon blanc-Weinen. Fünf verschiedene Hefen (X5, VL3, Delta, Revelation Thiole und Alchemy II) und eine Hefemischung (X5 + Alchemy II) wurden auf ihre Fähigkeit getestet, während der alkoholischen Gärung flüchtige Thiole freizusetzen. Die chemische Zusammensetzung und die sensorischen Eigenschaften der Sauvignon blanc-Weine wurden beobachtet. Die verwendeten Hefen haben signifikante Unterschiede in Bezug auf die Herstellung von 4-Mercapto-4-methylpentan-2-on (4-MMP) und 3-Mercaptohexan-1-ol (3-MH) hervorgerufen. Die Gärungsaromen Isoamylacetat, Phenylethylacetat, 2-Phenylethanol und Ethylhexanoat wurden auch wesentlich von den verwendeten Hefen beeinflusst. Die co-geimpfte Gärung verschiedener Hefestämme, im Gegensatz zu Weinen von reinen Hefestämmen, kann Weine mit unterschiedlicher chemischer Zusammensetzung und mit unterschiedlichen sensorischen Profilen erzeugen. In diesem Experiment waren die verwendeten Hefen nicht nur für die Aromaproduktion ein entscheidender Faktor, sondern auch für eine erfolgreiche alkoholische Gärung. Nicht alle verwendeten Hefen konnten die Weine trocken vergären.

Schlagwörter: 'Sauvignon blanc', flüchtige Thiole, Gärungsaroma, Hefestämme, Hefemischung

Keywords: 'Sauvignon blanc', volatile thiols, fermentation aroma, yeast strains, yeasts mixture

'Sauvignon blanc' is one of the most important grape varieties all over the world. In Austria the surface planted with the variety 'Sauvignon blanc' is about 1000 ha. Between 1999 and 2009 the area planted with 'Sauvignon blanc' increased by 50 % and is still increasing (Statistik Austria, 2009). High quality Sauvignon blanc wines are produced in the Austrian region Styria, but smaller wine regions such as Vienna have also the potential to produce brilliant wines with typical varietal aroma.

The wines produced from this variety have intensive aroma due to volatile thiols, methoxypyrazine and fermentation aroma. The three most important volatile thiols are 4-mercapto-4-methylpentan-2-one (4-MMP), 3-mercaptohexan-l-ol (3-MH) and 3-mercaptohexyl acetate (3-MHA), the following concentrations were reported: 4 to 40 ng/l, 200 to 1800 ng/l and 0 to 2500 ng/l, respectively (Coetzee et al., 2013). Other grapevine varieties also contain volatile thiols above the perception thresholds. In wines produced from 'Gewürztraminer', 'Riesling', 'Colombard', 'Petit Manseng' and botrytized 'Semillon' the concentration of 4-MMP, 3-MH and 3-MHA was considerably higher than the perception thresholds (Tominaga et al., 2000).

The concentration of volatile thiols is influenced by many factors: grape origin, vineyard position, terroir (soil and microclimate), harvest date and grape processing (Tominaga, 1998). Additional other factors can influence the concentration of volatile thiols and different aroma substances produced during the fermentation: yeast strain, nutrients addition during fermentation and type of recipient. Fermentation temperature influences the production of volatile thiols irrespective of the yeast strain used. The levels of volatile thiols were higher when the fermentation was conducted at 20 °C than at 13 °C (MASNEUF-POMAREDE et al., 2006).

The cysteinylated and glutathionylated precursors of 4-MMP and 3-MH are present in grape juice and the volatile thiols are released during fermentation (Tominaga et al., 1998; Fedrizzi et al., 2009). The yeast possesses cysteine-lyase activity, which gives the ability to hydrolyze the non-volatile cysteinylated precursors (Tominaga, 1998).

The volatile thiol 3-MHA is the product of the esterification reaction of 3-MH and acetic acid. The reaction is catalyzed by the enzyme ester forming alcohol acetyltrans-

ferase (SWIEGERS et al., 2007).

The yeasts possess different abilities to liberate and convert the precursors of 4-MMP and 3-MH to aroma active thiols and 3-MH into 3-MHA, respectively (Swiegers et al., 2011; Murat et al., 2001; Dubour-DIEU et al., 2006; MASNEUF-POMAREDE et al., 2006). The effect of the yeast strain on Sauvignon blanc wine aroma was demonstrated even after three years of bottle aging (KING et al., 2011). The yeast strains can modulate the liberation of varietal thiols during fermentation, but the conversion yields are always less than 10 % for both classes of precursors demonstrating no explanation for total biogenesis of such molecules in wine (ROLAND et al., 2011). Other strategies were proposed to increase varietal aroma in Sauvignon blanc wines, like: producing yeasts with genetic modification technology which are able to liberate more volatile thiols or co-inoculation with different yeast strains.

In wines produced with mixed cultures, the yeast metabolism is affected by the interactions between different yeasts resulting in wines with different content of secondary compounds compared to fermentations performed by monocultures (Barrajon et al., 2011).

The aim of the study was to determine aroma levels in Viennese Sauvignon blanc wines and to investigate the influence of different yeast strains and one yeast mixture on the aroma profile of the experimental wines.

MATERIALS AND METHODS

MUST ORIGIN AND TREATMENTS

The Viennese Sauvignon blanc grapes (Mayer am Pfarrplatz Winery, Alsegg vineyard) were harvested on September, 12th, 2012. The grapes were destemmed and cold maceration was carried out at 5 °C for 12 hours. The grapes were pressed and juice was prepared for fermentation. The following products were added to the must: 1 g/hl Rapidase CB (DSM Food Specialties, Delft, The Netherlands), 10 g/hl Granucol GE (Erbslöh, Geisenheim, Germany), 150 ml/hl must Gelatine (Erbslöh, Geisenheim, Germany) and 1 g/l tartaric acid. After 48 hours the must was racked and the sediment was filtrated with lees filter.

YEAST STRAINS

Oenoferm Klosterneuburg (Erbslöh, Geisenheim, Germany); Zymaflore X5, VL 3, Delta (Laffort, Bordeaux, France); Alchemy II (Anchor, Cape Town, South Africa); IOC Revelation Thiols (Institut Oénologique de Champagne, Epernay, France). Yeast mixture X5 and Alchemy II, ratio 1:1. Dosage 25 g/hl. The yeasts were rehydrated according to manufacturer's instructions.

FERMENTATION PROCEDURE

The fermentation was carried out in 30 l glass carboys. Fermentation temperature 17.5 to 19.5 °C. 24 hours after fermentation start 20 g/hl Optimum-White (Lallemand, Madrid, Spain), a product rich in glutathione was added; 24 hours later also diammonium phosphate (Erbslöh, Geisenheim, Germany), 20 g/hl. When the sugar level was below 5 g/l, fermentation was considered completed. The wines were racked and prepared for bottling. All wines were replicated two times.

FERMENTATION KINETICS AND YEAST STRAIN IDENTIFICATION IN THE YEAST MIXTURE

The fermentation kinetics was monitored by means of portable density meter DMA 35 (Anton Paar, Graz, Austria).

DNA PREPARATION AND PCR AMPLIFICATION

The isolation of the yeast was done in main fermentation of each sample. 100 μ l of the wine were transferred to Wallerstein Nutrient Medium (Wallerstein Nutrient Agar, Oxoid, CM0309) petri dishes and 30 individual colonies per sample were analysed. The strains were grown on YM-medium (3 g/l malt extract, 5 g/l peptone, 10 g/l glucose, 20 g/l agar) for DNA isolation. DNA extraction was carried out with MasterPureTM Yeast Kit (Epicentre Technologies Corp., Madison, WI 53719, USA.) as described by the manufacturer. In order to identify the yeasts as the commercial yeast strains

that were used for the inoculation, a microsatellite multiplex PCR analysis with the primers C5, C11 and SCYOR267X was carried out according to Legras et al. (2005) and a PCR SC8132X according to GONZALEZ TECHERA et al. (2001) (Table 1). 2 μl (1:10) diluted DNA and 23 µl master mix, containing 20 pmol each of forward and reverse primer, dNTP (Fermentas), 2,5 units Taq DNA polymerase (Peqlab) were used for PCR. The reaction was performed in Mastercycler (Eppendorf, Hamburg, Germany). For the amplification of the loci SCYOR267C, C5, and C11 the following protocol was used: 94 °C/4 min – (94 °C/30 sec - 55 °C/30 sec -72 °C/1 min) x 35 -72 °C/7 min. The program for the locus SC8132X was 94 °C/4 min - (94 °C/30 sec - 64.4 $^{\circ}$ C/30 sec - 72 $^{\circ}$ C/1 min) x 35 - 72 $^{\circ}$ C/7 min. After the amplification the DNA samples were stored at 4 °C. An aliquot of each template was separated on an 2 % agarose gel (Biozym LE Agarose, Biozym, Wien, Austria) and compared with the fragments of the commercial yeasts and 100 Bp ladder (Biolab GmbH, Wien, Austria). The documentation was made with Ebox VX2 (VWR, Wien, Austria).

GENERAL COMPOSITION, VOLATILE THIOLS AND AROMA FERMENTATION

The general composition of wines, their content of volatile thiols and fermentation aroma were determined by Sarco Laboratories (Bordeaux, France).

SENSORY ANALYSIS

Sensory analysis was performed 6 months after the end of fermentation by a panel of 31 expert wine tasters. The panelists noted the intensity of each descriptor on a scale from 0 for low or no intensity to 10 for high intensity. The mean values for each descriptor were used to obtain a profile of the experimental wines. Eight aroma attributes were rated by the tasters (duplicate fermentation replicates): grassy, green bell pepper, citrus, elder, gooseberry, freshness, bitterness and hydrogen sulfide aroma.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS (15.0.1)

program. Univariate analysis of variance (ANOVA) was applied to detect significant differences provoked by different yeast strains. Significance level was p < 0.05. Student-Newman-Keuls test was used to evaluate the significance of the analysis between wines produced with different yeast strains. All the values were statistically analyzed. Principal component analyses (PCA) were applied on the mean attribute ratings obtained from the panel.

RESULTS AND DISCUSSION

FERMENTATION KINETICS AND GENERAL COMPOSITION OF THE EXPERIMENTAL WINES

The initial must density was $1.102~\rm g/dm^3$ which means 249 g/l sugar, pH 3.43, TA 5.7 g/l; the grapes maturity was optimal for the desired style of wine. Before the fermentation 1 g/l of tartaric acid was added. After tartaric acid addition the must presented the following values: pH 3.24, TA 6.9 g/l, free SO₂ 6.7 mg/l and total SO₂ 40 mg/l.

After 15 days the yeasts X5, Alchemy II, Revelation, VL3 and the yeast mixture X5 + Alchemy II completed the fermentation. This is a normal fermentation duration for a must containing high sugar concentration (Fig. 1). The fastest yeast strain was Revelation followed by the yeasts Alchemy II, X5 and VL3 and the yeasts mixture X5 + Alchemy II. The yeast Klosterneuburg was the slowest one. The fermentation curve reveals the fact that it stopped before the desired conversion of sugars. The yeast strain Delta slowed after one week of fermentation but completed the alcoholic fermentation after 17 days.

MOLECULAR YEAST STRAIN IDENTIFICATION IN THE YEAST MIXTURE

The molecular biological identification by multiplex PCR of co-inoculated yeast products is graphically presented in Figure 2. Based on the different band patterns clear assignment of isolated single yeast to the original yeast products was possible. This served as basis for the representation of the yeast strain percentage at three different moments during alcoholic fermentation.

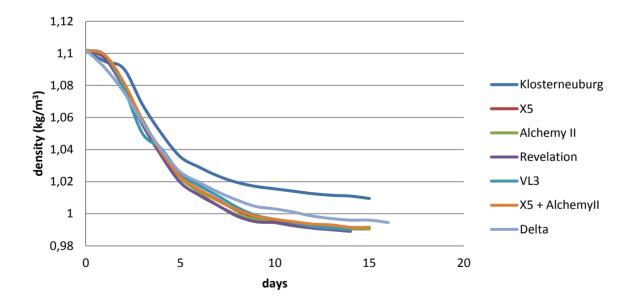


Fig. 1: Daily fermentation monitoring in all carboys (average of the two repetitions)

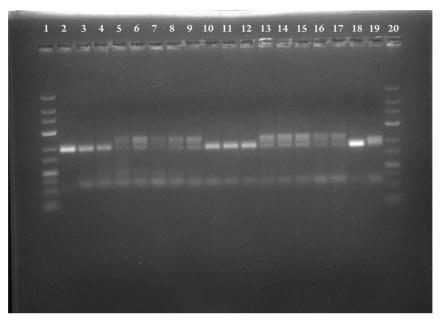


Fig. 2: Multiplex microsatellite PCR Lane 1 and 20 100 bp ladder, lane 2 to 17 wine samples, lane 17 yeast Zymaflore *X5 (Laffort), lane 18 yeast Alchemy (Anchor) C, lane 19 yeast Alchemy (Anchor) D

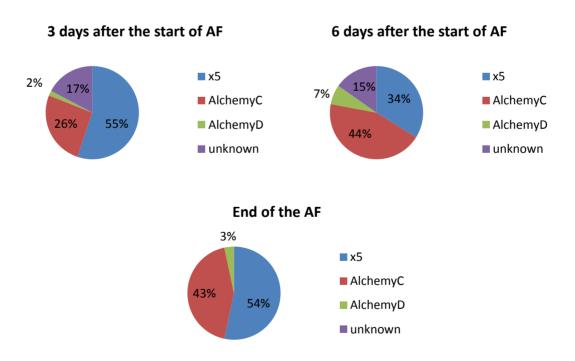


Fig. 3: Yeast strain percentages (averages of the two wines produced with each yeast) at three different moments during alcoholic fermentation (AF); Note: (Alchemy C+D is an intern code of the yeast mixture of Alchemy)

Molecular identification of individual colonies of the co-inoculated yeast strains used (ratio 1:1), showed that both yeasts were present during the entire AF process. 3 days after the start of AF, the relative proportion population of the yeast X5 was higher than the proportion population of yeast blend Alchemy II (Fig. 3). 17 % were unknown yeasts. Six days after the AF start, the population of the yeast blend was higher than the population of yeast X5, but the yeast X5 was still very present (34 %). The population of the unknown yeast slightly decreased to 15 %. At the end of the AF, both yeast products X5 and Alchemy II presented populations almost equal, 54 % and 46 %, respectively. The population of unknown yeasts dropped to 0. The yeast X5 might have a higher growth rate compared to the yeast Alchemy II, but during the AF the yeast Alchemy II was present in major quantities as well. The yeast X5 is killer positive and the yeast Alchemy II contains both killer positive and killer negative strains, but both yeasts carried out the AF. Probably the co-inoculation of the yeast X5 and the yeast blend Alchemy II had a distorting effect on the ratio of the Alchemy II strains. The yeast mixture X5 and Alchemy II ratio (1:1) was used during other vintages as well (2010 and 2011) and the results were in accordance with those: during the AF both yeasts presented viable populations (unpublished data).

GENERAL WINE COMPOSITION

Table 2 illustrates the general wine composition of the test wines. The yeast Revelation produced the wines with the highest content of alcohol (15 %vol.) and the lowest sugar content (1.6 g/l), therefore in this experiment Revelation was the strongest yeast. The alcohol level was high (about 14.8 %vol.) in all the wines except the wines produced with the yeast Klosterneuburg. The pH value presented no major differences. The sugar level

was below 5 g/l in all the wines except the wines produced with the yeast Klosterneuburg. Total acidity and malic acid were lower in the wines produced with the yeast Revelation, probably the yeast possesses the ability to metabolize malic acid during the fermentation. Volatile acidity showed small differences. The highest value for volatile acidity was found for the wines produced with the yeast Delta. All the parameters discussed in this section were significantly influenced by the factor yeast strain

VOLATILE THIOLS

The concentration of 4-MMP, 3-MH and 3-MHA was above the detection thresholds for each substance (0.8 ng/l, 60 ng/l and 4 ng/l, resp.).

The compound 4-MMP showed major differences between the experimental wines (Fig. 4). The yeast X5 released the highest amount of 4-MMP, followed by the yeast VL3. The yeast Alchemy II and the yeast mixture X5 + Alchemy II presented moderate quantity of this compound. The yeast mixture X5 + Alchemy II produced lower amounts of 4-MMP than the single strain X5 but higher amounts than the single strain Alchemy II. The wines produced with the yeasts Revelation, Klosterneuburg and Delta presented the lowest concentration of 4-MMP, close to the sensory detection threshold (Fig. 4). The factor yeast strain influenced the amount of 4-MMP in wines significantly. MURAT et al. (2001) also investigated the effect of Saccharomyces cerevisiae on the release of volatile thiols in Bordeaux Sauvignon blanc wine. The authors also concluded that the yeast strain plays a significant part in the release of 4-MMP. The ability of the yeast VL3 and other two yeasts to liberate 4-MMP was studied (Howell et al., 2004). The authors concluded that yeast strain and fermentation temperature strongly impacted 4-MMP production.

Table 1: PCR primer pairs used for amplification

Primer	Sequence	References
SCYOR267C fw	5'-tac taa cgt caa cac tgc tgc caa-3'	LEGRAS et al., 2005
SCYOR 267 rev	5'-gga tet act tgc agt ata egg g-3'	GONZALEZ TECHERA et al., 2001
C5fw	5'-tga caca at agc aat ggc ctt ca-3'	LEGRAS et al., 2005
C5 rev	5'-gca age gae tag aac aac aat cac a-3'	LEGRAS et al., 2005
C11 fw	5'-ttc cat cat aac cgt cgt gga tt-3'	LEGRAS et al., 2005
C11 rev	5'-tgc ctt ttt ctt aga tgg gct ttc-3'	LEGRAS et al., 2005
SC8132X fw	5'-ctg ctc aac ttg tga tgg gtt ttg g-3'	GONZALEZ TECHERA et al., 2001
SC8132 rev	5'-cet egt tae tat egt ett eat ett ge-3'	GONZALEZ TECHERA et al., 2001

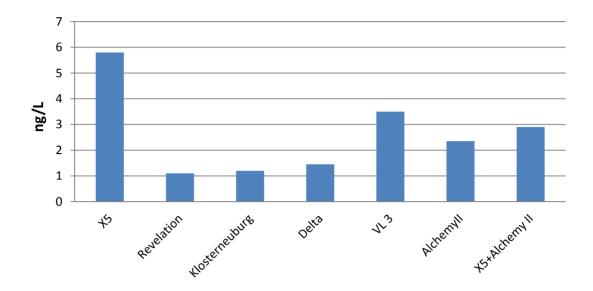


Fig. 4: The content of 4-MMP (4-mercapto-4-methylpentan-2-one) in Viennese Sauvignon blanc wines produced with different yeasts and one yeast mixture (averages of two repetitions)

The content of 3-MH was high in Viennese Sauvignon blanc wines, between 1200 and 700 ng/l (Fig. 5). The yeast strain VL3 produced the highest amount of 3-MH followed by the yeasts Klosterneuburg and Delta. Those two yeast strains released equal amounts of 3-MH. The yeast mixture X5 + Alchemy II produced slightly higher concentrations of 3-MH than the pure strains X5 and Alchemy II. The lowest concentration of 3-MH was produced by the yeast strain Revelation, but still much higher than the detection thresholds of this compound. The factor yeast strain provoked significant differences in the 3-MH content of the wines.

The yeast strains VL3 and Alchemy II produced during the fermentation the highest amount of 3-MHA, thus in our experiment those two yeast strains presented the highest ability to convert 3-MH to 3-MHA. All other yeast strains applied in this trial presented almost equal concentrations of 3-MHA. The wines produced with the yeast mixture X5 + Alchemy II showed the lowest amount of 3-MHA. Statistical analysis, however, revealed no significant differences between the yeast strains in regard to this compound (Fig. 6).

In the current study Sauvignon blanc wines with dif-

ferent levels of volatile thiols were produced from the same must using different yeast strains. This finding is in accordance with literature data. The differences in volatile sulfur aromas provoked by different S. cerevisiae strains also appeared in fermentation of model mediums added with S4-(4-methylpentan-2-one)-L-cysteine. VL3 was one of the yeasts with high ability to release 4-MMP (Dubourdieu et al., 2006).

Chemical composition of the wines produced with the yeast mixture X5 + Alchemy II differ from the wines produced with the pure yeast products. The mixture X5 + Alchemy II produced lower levels of 4-MMP than the pure yeast X5 but higher than the pure yeast Alchemy II. The levels of 3-MH were slightly higher in the wines fermented with the yeast mixture X5 + Alchemy II than in the wines fermented with pure strains X5 and Alchemy II

The levels of volatile thiols in Viennese Sauvignon blanc wines were lower as compared to the levels identified in Sauvignon blanc wines from Marlborough, New Zealand. Makhotkina et al. (2014) used samples from two different regions in New Zealand: southern Awatere Valley and northern side of the Wairau River. The wines produced from Awatere Valley presented higher concen-

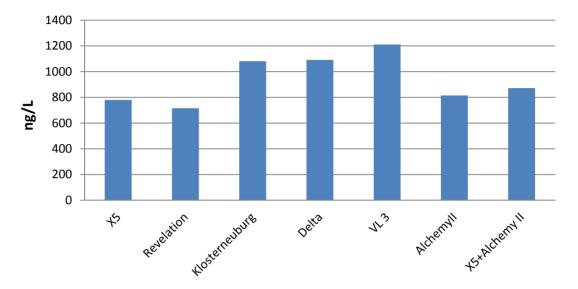
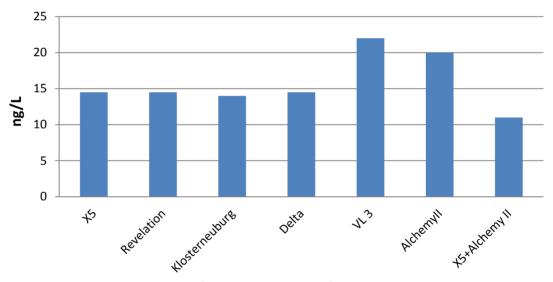


Fig. 5: The content of 3-MH (3-mercaptohexan-1-ol) in Viennese Sauvignon blanc wines produced with different yeasts and one yeast mixture (averages of two repetitions)



 $Fig. \ 6: The \ content \ of \ 3-MHA \ (3-mercap to hexyl \ acetate) \ in \ Viennese \ Sauvignon \ blanc \ wines \ produced \ with \ different \ yeasts \ and \ one \ yeast \ mixture \ (averages \ of \ two \ repetitions)$

Table 2: General wine composition of Sauvignon blanc wines produced with different yeast strains and one yeast mixture (averages of two repetitions)

Parameter	X5	Revelation	Klosterneuburg	Delta	VL3	Alchemy	X5+Alchemy II
pH value	3.35	3.39	3.40	3.35	3.31	3.33	3.33
Alcohol (% vol)	14.83	15.03	12.58	14.63	14.93	14.81	14.87
Sugar (g/l)	2.33	1.62	36.25	4.77	2.60	2.97	3.05
Acidity (g/l)	6.61	6.19	6.51	6.76	6.68	6.37	6.52
Malic acid (g/l)	2.27	1.97	2.20	2.21	2.21	2.13	2.20
Volatile acidity (g/l)	0.33	0.39	0.43	0.49	0.43	0.32	0.34

trations for the three thiols 4-MMP, 3-MHA and 3-MH (24, 580, 3000 ng/l) analyzed. The Wairau River wines presented different 4-MMP concentrations, one higher and one lower than the 4-MMP levels in Viennese Sauvignon blanc wines. The 3-MH concentration was almost equal in Viennese Sauvignon blanc wines and wines from the Wairau River and the levels of 3-MHA were higher in wines from Wairau River, Marlborough, New Zealand. Both studies were conducted on grapes from vintage 2012. Benkwitz et al. (2012) studied the aroma of Sauvignon blanc wines produced in different countries: New Zealand, United States, South Africa, Australia and France. The 3-MHA and 3-MH content of Marlborough wines (New Zealand) was significantly higher than in all other regions and countries.

FERMENTATION AROMA

In the current study the yeast Revelation produced the highest amount of isoamyl acetate, almost two times higher than all other yeast strains (10 mg/l). The other yeasts used in this study formed moderate quantities of isoamyl acetate during fermentation between 4.5 and 6 mg/l (Table 3). Isoamyl acetate is a fermentation ester, it is very important in young wines and is characterized by banana-like aromas. The amount of isoamyl acetate was as well higher in the wines produced with the mixture X5 + Alchemy II than in the same wines produced with the yeasts Revelation and Alchemy II the quantity of

phenylethyl acetate and 2-phenylethanol was higher than in the wines produced with the other yeast strains but below the sensory detection threshold. The lowest amount of those two substances was found in the wines produced with the yeast Klosterneuburg. Ethyl octanoate, ethyl decanoate, hexyl acetate and 2-methylbutanol presented no major differences between the experimental wines. The yeast Klosterneuburg produced the highest amount of ethyl hexanoate (5.1 mg/l) and the lowest amount was showed by the yeast mixture X5 + Alchemy II, 2.45 mg/l. The concentration of 2-phenylethanol, ethyl decanoate and hexyl acetate was lower than the aroma threshold of those substances in wine, but this does not mean that those substances did not contribute to the overall aroma impression. The presence of different esters can have a synergic effect on the individual flavours. Minor changes of esters concentration may provoke dramatic changes on wine flavours (MARULLO and Dubourdieu, 2010). Statistical analysis revealed that the factor yeast strain had significant influence on the production of isoamyl acetate, phenylethyl acetate, 2-phenylethanol and ethyl hexanoate.

Other aroma substances were present in lower quantities in the wines produced with the mixture X5 + Alchemy II compared to the ones produced with the pure yeast strains. Our results are in accordance with KING et al. (2008). The authors concluded that production of certain compounds can be promoted or inhibited by different interactive effects occurring within the co-inoculated fermentations.

Table 3: Fermentation aroma (mg/l) of Sauvignon blanc wines produced with different yeast strains and one yeast mixture (averages of two repetitions)

Compound	Aroma	Aroma threshold (mg/l)	X5	Reve- lation	Kloster- neuburg	Delta	VL3	Al- chemy II	X5 + Alchemy II
Isoamyl acetate	Banana	0.03	5.07	10.09	4.43	5.98	5.89	5.67	7.44
Phenylethyl acetate	Rose	0.25	0.13	0.22	0.1	0.13	0.15	0.18	0.13
2-phenylethanol	Rose	14	1.68	1.94	1.05	1.55	1.62	1.94	1.75
Ethyl octanoate	Floral	0.005	7.25	6.43	6.78	8.2	9.35	6.83	6.73
Ethyl decanoate	Floral	0.2	1.37	1.18	1.48	1.55	1.61	0.88	1.05
Hexyl acetate	Pear	0.7	0.25	0.3	0.25	0.25	0.28	0.3	0.28
2-methylbutanol	Ananas	1.2	7.25	4	7.25	4	3.75	6.5	4.75
Ethyl hexanoate	Green apple	0.014	4.4	4.05	5.1	4.45	4.8	4.2	2.45

SENSORY ANALYSIS

Sensory analysis of Viennese Sauvignon blanc wines revealed differences between wines produced with different yeast strains. The sensory data were analysed with the Univariate analysis of variance (ANOVA) using SPSS program.

Analysis of variance was applied and the results showed that 5 out of 8 rated attributes presented significant differences between the yeast strains ((p < 0.05): grassy, green bell pepper, citrus, freshness and bitter. The principal component analysis (PCA) biplot (Fig. 7) depicts the aroma attributes rated and the different yeast strains. The yeasts Alchemy II, X5 and the yeast mixture X5 +

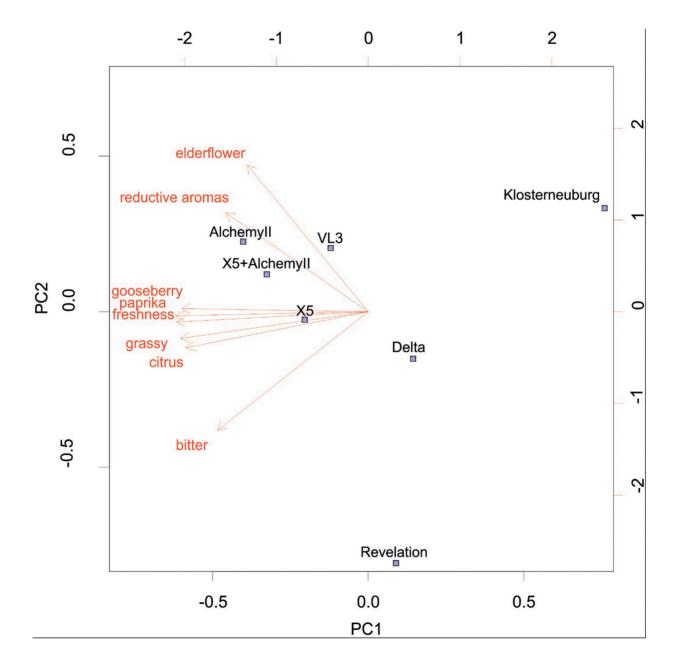


Fig. 7: Principal component analysis of Sauvignon blanc wines produced with different yeast strains and one yeasts mixture

paprika=green bell pepper

Alchemy II were rated with the highest intensity for the attributes grassy and green bell pepper, only minor differences were observed between the yeasts for those attributes. The yeast Klosterneuburg presented the lowest intensity for the attributes grassy and green bell pepper. The other two yeasts VL3 and Delta were rated with moderate intensity for the attributes grassy and green bell pepper. The attributes citrus and freshness were noted with the highest intensity for the yeast Alchemy II, and the yeast Klosterneuburg was rated with the lowest intensity. The yeasts Revelation, X5, Delta and the yeasts mixture X5 + Alchemy II were noted with moderate intensity for those attributes. The yeast Revelation presented the highest intensity for the attribute bitter and the yeast Klosterneuburg showed the lowest intensity for this attribute, this is correlated with the residual sugar. In the wines produced with Revelation the residual sugar was less than 2 g/l, and in the wines produced with Klosterneuburg the residual sugar was more than 30 g/l. The high residual sugar presented in the wines produced with the yeast Klosterneuburg probably influenced the ratings for other attributes as well. The position of the yeast Revelation revealed that the wines produced with this yeast presented totally different sensory profiles as it produces important amounts of fermentation aromas. The attributes gooseberry, green bell pepper and freshness were very highly correlated.

The wines were evaluated for overall sensory impression (Fig. 8). The wines produced with the yeast Alchemy II were rated as the best wines by the panel. The least favoured wines for the panel were the wines produced with the yeast Klosterneuburg. All those differences between the wines fermented with the yeast mixture and the pure strains were not high enough to produce wines with totally different sensory profiles, but the quality of the wines produced with the yeast mixture was rated very high by the panel. The wines produced with the yeast mixture X5 + Alchemy II, were rated as the second best wines for the overall sensory impression. The factor yeast strain presented significant influence on the overall sensory impression of the produced wines.

CONCLUSIONS

The yeasts used for alcoholic fermentation presented major influence on the chemical and sensory composition of Viennese Sauvignon blanc wines.

The yeasts X5, VL3, Alchemy II and the yeast mixture X5 + Alchemy II produced the most appreciated wines in terms of organoleptic characteristics. These yeasts produced during the fermentation the highest levels of 4-MMP and they were positively correlated with the desired sensory attributes for Sauvignon blanc wines. Differences were noticed also regarding other volatile

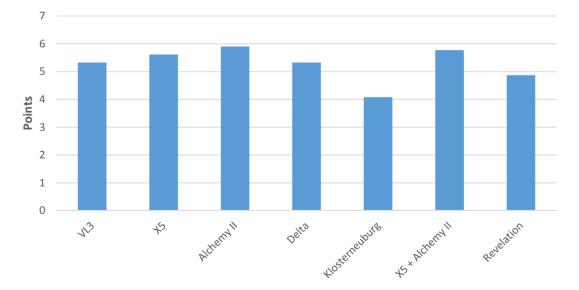


Fig. 8: Overall sensory impression of Sauvignon blanc wines produced with different yeast strains and one yeasts mixture (averages of two repetitions)

thiols (3-MH and 3-MHA), and their levels were much higher than the sensory thresholds of those substances. Volatile substances responsible for fermentation aroma were also influenced by the yeasts, high concentrations of the following aroma compounds were produced: isoamyl acetate (banana), 2-methylbutanol (pineapple), ethyl hexanoate (green apple) and ethyl octanoate (floral). These aroma substances lend aroma complexity. The differences between the pure strains were obvious. In our experiment, the yeasts used for fermentation were a decisive factor not just for the aroma production, but for a successful alcoholic fermentation as well. Not all the yeasts applied fermented the grape juice to dryness. It has to be mentioned that the results were obtained from a single vineyard, Alsegg, Dornbach, Vienna.

Co-inoculated fermentation of different yeast strains can produce wines with different chemical composition and sensory profiles, compared to wines produced from the same must with pure strains. Molecular quantification of the co-inoculated samples showed that both yeast products X5 and the yeast blend Alchemy II Molecular quantification of the co-inoculated samples showed that both yeast products X5 and the yeast blend Alchemy II presented viable populations during AF.

In Austria more than 400 different yeast products are available on the market. Therefore, choosing the best yeast for Sauvignon blanc production might be a difficult task. Only experience accumulated by trying different yeast products with crop from the same vineyard will allow the winemakers to use the best yeast for a certain type of wine. The yeast is an important tool in helping the winemakers to produce wines suitable for the continually changing demand of the consumers.

REFERENCES

Barrajon, N., Capece, A., Arevalo-Villena, M., Briones, A. and Romano, P. 2011: Co-inoculation of different Saccharomyces cerevisiae strains and influence on volatile composition of wines. Food Microbiol. 28: 1080-1086

- BENKWITZ, F., TOMINAGA, T., KILMARTIN, A.P., LUND, C., WOHLERS, M. AND NICOLAU, L. 2012: Identifying the chemical composition related to the distinct aroma characteristics of New Zealand Sauvignon blanc wines. Am. J. Enol. Vitic. 63(1): 62-72
- COETZEE, C., LISJAK, K., NICOLAU, L., KILMARTIN, P. AND JOHANNES DU TOIT, W. 2013: Oxygen and sulfur dioxide additions to Sauvignon blanc must: effect on must and wine composition. Flavour Fragrance J. 28: 155-167
- Dubourdieu, D., Tominaga, T., Masneuf, I., Peyrot des Gachons, C. and Murat, L.M. 2006: The role of yeasts in grape flavor development during fermentation: the example of Sauvignon blanc. Am. J. Enol. Vitic. 57(1): 81-88
- FEDRIZZI, B., PARDON, K.H., SEFTON, M.A., ELSEY, G.M. AND JEFFERY, D.W. 2009: First identification of 4-S-glutathionyl-4-methylpentan-2-one, a potential precursor of 4-mercapto-4-methylpentan-2-one, in Sauvignon blanc juice. J. Agric. Food Chem. 57(3): 991-995
- GONZALEZ TECHERA, A., JUBANY, S., CARRAU, F.M. AND GAGGERO, C. 2001: Differentiation of industrial wine yeast strains using microsatellite markers. Lett. Appl. Microbiol. 33(1): 71-5
- HOWELL, K.S., SWIEGERS, J.H., ELSEY, G.M., SIEBERT, T.E., BARTOWSKY, E., FLEET, G.H., PRETORIUS, I.S. AND DE BARROS LOPES, M. 2004: Variation in 4-mercapto-4-methyl-pentan-2-one release by Saccharomyces cerevisiae commercial wine strains. FEMS Microbiol. Letters 240: 125-129
- Legras, J., Ruh, O., Merdinoglu, D. and Karst, F. 2005: Selection of hypervariable microsatellite loci for the characterization of *Saccharomyces cerevisiae* strains. Int. J. Food Microbiol. 102(1): 73-83
- King, E.S., Francis, L., Swiegers, J.H. and Curtin, C. 2011: Yeast strain-derived sensory differences

- retained in Sauvignon blanc wines after extended bottle storage. Am. J. Enol. Vitic. 63(1): 62-72
- KING, E.S., SWIEGERS, J.H., TRAVIS, B., FRANCIS, L., BASTIAN, E.P. AND PRETORIUS, I.S. 2008: Coinoculated fermentations using Saccharomyces cerevisiae affect the volatile composition and sensory properties of the Vitis vinifera L. cv. Sauvignon blanc wines. J. Agr. Food Chem. 56(22): 10829-10837
- MAKHOTKINA, O., ARAUJO, D., OLEJAR, K., HERBST-JOHNSTONE, M., FEDRIZZI, B. AND KILMARTIN, P.A. 2014: Aroma impact of ascorbic acid and glutathione additions to Sauvignon blanc at harvest to supplement sulfur dioxide. Am. J. Enol. Vitic. 65(3): 388-393
- MARULLO, P. AND DUBOURDIEU, D. (2010): Yeast selection for wine flavor modulation. In: Reynolds, A.G. (ed.): Managing wine quality. Vol. 2: Oenology and wine quality, p. 293-345. Philadelphia: Woodhead Publ., 2010
- Masneuf-Pomarede, I., Mansour, C., Murat, M.L., Tominaga, T. and Dubourdieu, D. 2006: Influence of fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines. Int. J. Food Microbiol. 108: 385-390
- Murat, M.L., Masneuf, I., Darriet, P., Lavigne, V., Tominaga, T. and Dubourdieu, D. 2001: Effect of Sccharomyces cerevisiae yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. Am. J. Enol. Vitic. 52(2): 136-139
- ROLAND, A., SCHNEIDER, R., RAZUNGLES, A. AND CA-

- VELIER, F. 2011: Varietal thiols in wine: Discovery, analysis and applications. Chem. Rev. 111: 7355-7376
- STATISTIK AUSTRIA, 2009: Weingartengrunderhebung. (http://www.statistik.at/web_de/services/publikationen)
- SWIEGERS, J.H., CAPONE, D.L., PARDON, K.H., ELSEY, G.M., SEFTON, M.A., FRANCIS, I.L. AND PRETORIUS, I.S. 2007: Engineering volatile thiol release in Saccharomyces cerevisiae for improved WINE AROMA, YEAST 24: 561-574
- SWIEGERS, J.H., KIEVIT, R., SIEBERT, T., LATTEY, K., BRAMLEY, A., FRANCIS, L., KING, E.S. AND PRETORIUS, I.S. 2011: The influence of yeast on the aroma of Sauvignon Blanc wine. Food Microbiol. 26: 204-211
- TOMINAGA, T. (1998): Recherches sur l'arôme variétal des vins de Vitis vinifera L. cv. Sauvignon blanc et sa genèse à partir de précurseurs inodores du raisin. Thèse Doctorat ès Science, Université de Bordeaux II, 1998
- Tominaga, T., Baltenweck-Guyot, R., Peyrot des Gachons, C. and Dubourdieu, D. 2000: Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. Am. J. Enol. Vitic. 51(2): 178-181
- Tominaga, T., Peyrot des Gachons, C. and Du-Bourdieu, D. 1998: a new type of flavor precursors in Vitis vinifera L. cv. Sauvignon blanc: S-cysteine conjugates. J. Agric. Food Chem. 46: 5215-5219

Received April, 30th, 2015