Effect of different *Saccharomyces cerevisiae* strains and nutrients on the formation of SO_2-binding and aromatic compounds of Sauvignon blanc wines

Pattharaporn Srisamatthakarn[1*] and Doris Rauhut[2]

Abstract

This research aimed to compare the formation of SO_2-binding compounds (α-ketoglutarate, pyruvate, acetaldehyde), and various aroma compounds in Sauvignon blanc wines produced by two co-inoculations of three *Saccharomyces cerevisiae* strains (Alchemy I and Alchemy II) and an inoculation of a single *S. cerevisiae* strain (X5) in combination with addition of the complex nutrient product Fermaid E (diammonium hydrogen phosphate plus thiamine, yeast cell walls and ammonium sulfate) at 0.30 and 0.40 gL^{-1} or the inactivated yeast product OptiWhite at 0.30 and 0.50 gL^{-1}. The results showed that the lowest amount of acetaldehyde was detected in the samples fermented with Alchemy II and addition of Fermaid E ($P<0.05$). All fermentation treatments with addition of 0.40 gL^{-1} Fermaid E had the lowest concentrations of α-ketoglutarate and pyruvate. In none of the wines, hydrogen sulfide (H_2S) could be detected above the odor threshold value. The addition of 0.40 gL^{-1} Fermaid E and 0.50 gL^{-1} OptiWhite only led to a slight increase of 2-phenyl ethanol (floral and rose-like aromas) and α-terpineol (lilac-like aroma) in the wines fermented with Alchemy II. In general, Alchemy II yeasts were the highest producer of acetic acid 2-phenyl ethyl ester (flowery and honey note aroma). Alchemy I and II fermented wines had higher amounts of acetic acid 3-methylbutyl ester (banana-like aroma) in the variants with Fermaid E treatments than the wines fermented with X5, however they contained lower ethyl esters of medium-chain fatty acids (fruity and floral aroma). Although, yeast strains and nutrient additions had various effects on the formation of some of the investigated compounds, they had no significant effect on the formation of ethyl decanoate and ethyl hexanoate in the wines ($P>0.05$). In conclusion, the optimal choice of yeast strain and nutrient addition for fermentation of the Sauvignon blanc wines in this trial was the Alchemy II and Fermaid E at 0.40 gL^{-1}.

Keywords: Sauvignon blanc wine, yeast strain, yeast nutrient, hydrogen sulfide, wine aromatic compounds

[1] Agricultural Technology Research Institute, University of Technology Lanna, Lampang 52000

[2] Department of Microbiology and Biochemistry, Hochschule Geisenheim University, 65366 Geisenheim, Germany

[*] Corresponding author. E-mail: patr99@hotmail.com

Received: April 16, 2020; Revised: May 5, 2020; Accepted: June 8, 2020

Introduction

It is known that *Saccharomyces* yeasts do not only convert sugars to ethanol and carbon dioxide during wine fermentation, they also produce a wide range of secondary metabolites, for example, acetaldehyde, pyruvate, glycerol, ketoglutarate and organic acids (Pretorius, 2016; Goold et al., 2017). Acetaldehyde is the major carbonyl compound found in wine, which contributes to flavor with aroma descriptors such as “bruise apple” and nutty. However, it can also be a sign of wine oxidation, especially in white wine (Varela et al., 2012; Goold et al., 2017). A great number of volatile compounds are also formed and modulated by yeast during wine fermentation which significantly affects final wine flavors and overall characteristics (Bellon et al., 2011; Pretorius, Curtin, & Chambers, 2012; Bellon, Schmidt, Capone, Dunn, & Chambers, 2013). It is well-known that some sulfur containing compounds can be responsible for certain off-flavors (e.g. hydrogen sulfide (H_2S) methanethiol (MeSH) and thioacetic acid-S-methyl ester (MeSAc)) in wine. It is reported that one of the main causes for off-flavors occurring after wine fermentation is the chosen yeast strains and its nutrient requirement in the grape musts (Carrau, Gaggero, & Aguilar, 2015; Rauhut, 2017). The use of different *Saccharomyces* strains for wine fermentation has been shown to result in final wines with different secondary metabolites, through varied relative concentrations of higher alcohols, acetic acid esters and fatty acid ethyl esters. They are sensorial important aromatic compounds giving wines vinous characters. The volatile esters represent the largest and most important group of flavoring compounds produced by wine yeast during fermentation (Pretorius, Curtin, & Chambers, 2012; Pretorius, 2016; Dutraive et al., 2019). The characteristic fruity aromas of wines and other grape-derived alcoholic beverages are primarily due to a mixture of 2-phenyl ethanol, acetic acid 2-phenyl ethyl ester, acetic acid hexyl ester, ethyl hexanoate and ethyl octanoate (apple-like aroma) and acetic acid 3-methylbutyl ester (Lewin, 2010; Pretorius, 2016).

It has been revealed that mixtures of amino acids and vitamins give higher growth rates during wine fermentation than the most preferred single nitrogen sources (i.e. ammonium, glutamate and asparagines) in juice (Crépin, Nidelet, Sanchez, Dequin, & Camarasa, 2012; Crépin et al., 2017), while thiamine addition effectively reduces SO_2-binding compounds (acetaldehyde, pyruvate and ketoglutarate) levels by enzymatic decarboxylation (Wells, & Osborne, 2011; Comuzzo, & Zironi, 2013). Among other factors, nitrogen content affects the pattern of both higher alcohols and esters formed during

fermentation, via regulation of the Erhlich pathway, fatty acid, and ester synthesis pathways (Mendes-Ferreira, Barbosa, Falco, Leão, & Mendes-Faia, 2009; Styger, Prior, & Bauer, 2011; Pretorius, Curtin, & Chambers, 2012). Srisamatthakarn (2011) reported that yeast strains of Alchemy I and II, X5, EC1118 and VL3 in combination of nutrient sources of 0.4 gL^{-1} Fermaid E blanc and 0.5 gL^{-1} DAP and 0.3 gL^{-1} Superstart (inactivated yeast and yeast cell walls) seemed to be the most effective for the fermentation of Sauvignon blanc wine. The yeasts X5, VL3, Alchemy II and the yeast mixture of X5 and Alchemy II also have been reported to produce the most desirable wines in terms of organoleptic attributes for Viennese Sauvignon blanc wines (Pavelescu, Mandl, Steidl, Blesl, & Spangl, 2015). Therefore, this research aimed to examine the effect of different amount of nutrient addition on SO_2-binding compounds, undesirable sulfur containing compounds and volatile aroma compounds produced in the Sauvignon blanc wines by different *Saccharomyces* strains (co- and single strain fermentations).

Methodology

Sauvignon blanc grape juice. The fermentation was carried out in fresh Sauvignon blanc grape juice. The composition of initial grape juice was total soluble solid 17.10 ^{o}Brix, reducing sugar content 168 gL^{-1}, pH 3.10, ammonium content 0.09 gL^{-1}, free amino nitrogen 92.51 mgL^{-1}, total acidity 10.3 gL^{-1}, tartaric acid 6.48 gL^{-1}, malic acid 6.26 gL^{-1} and glycerol 0.10 gL^{-1}. Triplicate experimental fermentations were carried out in 0.75-liter bottles containing 620 mL grape juice. Addition of two nutrient sources of Fermaid E (diammonium hydrogen phosphate (DAP) plus thiamine, yeast cell walls and ammonium sulfate) and OptiWhite (inactivated yeast) was performed in the grape juice according to an experimental design consisting of two different concentrations (0.3 and 0.4 gL^{-1} of Fermaid E and 0.3 and 0.5 gL^{-1} of OptiWhite; Lallemand Inc.) prior to the alcoholic fermentation. Then 25 mgL^{-1} of sulfur dioxide was added in grape juice as $K_2S_2O_5$ to suppress undesirable microorganism growth as well as to function as an antioxidant.

Fermentation. The following three commercial yeast products were applied for fermentation: Zymaflore X5 (single *Saccharomyces cerevisiae* strain; Laffort) and Alchemy I and Alchemy II (blends of three *Saccharomyces cerevisiae* strains; Anchor Yeast; Cordente, Tran, & Curtin, 2014). The yeast cultures were rehydrated following the recommendations of the manufacturer prior to inoculation of each

strain. The bottles were fitted with airlocks and the fermentations were carried out at 20 °C in a controlled environment. The progress of fermentation was followed by monitoring CO_2 production, which was determined by weight loss during fermentation. After the weight losses of the samples were constant, wines were cold stabilized at below 10 °C for 7 days and racked into previously cleaned bottles. Then $K_2S_2O_5$ was added corresponding to 80 mgL^{-1} free SO_2 in finished wines, and bottled wines were then stored at below 15 °C until analytical investigations.

Analysis.

1. SO_2-binding compounds analysis. The SO_2-binding compounds (acetaldehyde, pyruvate and α-ketoglutarate) were determined enzymatically by an UV/VS spectrometer Lambda 2 (PerkinElmer GmbH, Überlingen, Germany) and wavelength at 340 nm equipped with a refrigerated/heating circulator, Model F25-ME (JULABO Labortechnik GmbH, Seelbach, Germany) and controlled at 25 °C isothermic condition.

2. The hydrogen sulfide and aromatic compound analysis. The hydrogen sulfide was analysed by an HP 6890 gas chromatograph equipped with automatic headspace sampling (Multipurpose Sampler MPS 2) and a cooled injection system CIS-4 (Gerstel GmbH, Mülheim an der Ruhr, Germany) then detected by an OI 5380 pulse flame photometric detector (PFPD) (OI Analytical, USA) according to some publications (Rauhut, Beisert, Berres, Gawron-Scibek, & Kürbel, 2005; Rauhut, & Beisert, 2017). Wine samples were extracted according to the 'Kaltron' method by liquid-liquid extraction with 1,1,2-trifluorotrichloroethane according to Rapp, Yavas, & Hastrich (1994), and a modified procedure from Fritsch, Brezina, Ebert, & Rauhut (2017) and wine aromatic compounds (esters, higher alcohols and fatty acid esters) were analysed by gas chromatograph (Hewlett-Packard, HP 5890 Series II) equipped with a cooled injection system CIS-3 (Gerstel GmbH, Mülheim an der Ruhr, Germany) and detected by HP 5972 MSD operating in electron impact mode. Terpenes were determined by the application of solid phase extraction (SPE), gas chromatography, and mass spectrometry following the method of Schüttler, Friedel, Jung, Rauhut, & Darriet (2015).

Statistical analysis. The one-way analysis of variance (ANOVA) was performed by using the statistical package MSTAT-C program, where mean comparisons were tested by Duncan's new multiple range test (DNMRT) at $P<0.05$.

Results and discussion

1. The Formation of SO_2-binding compounds and hydrogen sulfide of the Sauvignon blanc wines

For the fermentation behavior, the fermentation of all wine samples was finished in

a range from 14-24 days. The Alchemy II led to the faster fermentation in both Fermaid E levels in 14 days (data not shown). Acetaldehyde, pyruvate and α-ketoglutarate were some of the most active SO_2-binding compounds that can bind bisulfite ion, reducing the concentration of free SO_2, which is the most active component in controlling wine oxidations and microbial spoilage. The production of these compounds in wine depends on the choice of the yeast strain and on the composition of the grape must (Wells, & Osborne, 2011; Comuzzo, & Zironi, 2013). The results in (Figure 1) show that the production of SO_2-binding compounds by the three different yeast fermentations varied depending on nutrient sources and concentrations. Within the yeast products, Alchemy II seemed to lead to a higher production of pyruvate and α-ketoglutarate, but low acetaldehyde. This is in accordance with Srisamatthakarn (2011), who reported that the Alchemy I and X5 strains were low producers of pyruvate and α-ketoglutarate in white wine. The addition of OptiWhite at both levels significantly stimulated the Alchemy II strains to produce higher amounts of pyruvate (11.20±1.40 and 12.00±0.60 mgL^{-1}, respectively), while higher amounts of α-ketoglutarate were produced at 0.5 gL^{-1} addition of OptiWhite (31.50±1.30 mgL^{-1}). Addition of inactive dry yeasts may provide low amounts of nitrogen availability, then formation of these compounds was increased, which was in agreement with previous studies (Wells, & Osborne, 2011; Comuzzo, & Zironi, 2013). Nevertheless, the concentrations of these metabolites in all wines were in usual concentration ranges (1-50 and 1-128 mgL^{-1}, respectively). These SO_2-binding compounds typically accumulate in wine at concentrations less than 50-100 mgL^{-1} when nitrogen is adequate (Wells, & Osborne, 2011; Crépin et al., 2017).

Overall, the higher the Fermaid E addition, the lower the amounts of SO_2-binding compounds produced. Fermaid E is a nutrient mixture of DAP, ammonium sulfate, yeast cell wall and thiamine, which has been shown to effectively decrease α-ketoglutarate and pyruvate concentrations by decarboxylation (Crépin, Nidelet, Sanchez, Dequin, & Camarasa, 2012; Comuzzo, & Zironi, 2013; Crépin et al., 2017). OptiWhite is an inactive dry yeast product, which has been revealed to contain amino acids, particularly, ornithine, α-alanine, γ-aminobutyric and glutamic acids (Andújar-Ortiz, Chaya, Martín-Álvarez, Moreno-Arribas, & Pozo-Bayón, 2014). The glutamic acid has been reported to stimulate the higher production of α-ketoglutarate by yeast than other nitrogen sources (Mas et al., 2014) and they suggested that glutamic acid is the donor of nitrogen in many of the biosynthetic pathways of amino acids, consequently, an excess of

α-ketoglutarate is to be formed in glutamic acid-grown cultures as a de-amination product (Crépin, Nidelet, Sanchez, Dequin, & Camarasa, 2012; Crépin et al., 2017).

Although acetaldehyde production was higher in wines fermented with strain X5 and strains of Alchemy I in the presence of low OptiWhite level (18.60±0.40 and 18.80±1.00 mgL^{-1}, respectively), its formation decreased with higher supplementation. Concentrations of acetaldehyde found in all wine treatments were quite below the threshold value (100 mgL^{-1}), which contributes to "bruised apple" and "nutty" flavors, but did not cause wine oxidation (Varela et al., 2012; Goold et al., 2017).

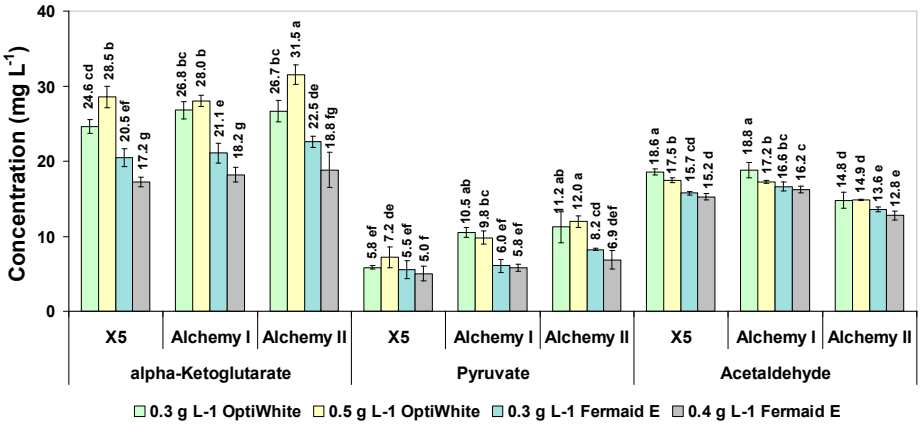

Figure 1 Concentration of SO_2-binding compounds found in Sauvignon blanc grape wines fermented by three commercial yeast products with two nutrient sources at two different concentrations. Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($P<0.05$) according to the DNMRT test.

2. The formation of hydrogen sulfide and aromatic compounds during fermentation of Sauvignon blanc wines

This research finding indicated that the three yeast strains did not revealed hydrogen sulfide in amounts above the odor threshold value, thus it can be suggested that addition of either OptiWhite or Fermaid E at both levels provided sufficient and suitable nutrients for yeast metabolism (data not shown). Alchemy II produced the highest amounts of 2-phenyl ethanol in the presence of the higher OptiWhite (17.50±0.90 mgL^{-1}) and Fermaid E level (16.30±0.70 mgL^{-1}). It is likely that supplementation of Optiwhite and

Fermaid E at higher level tended to increase the concentrations of 2-phenyl ethanol in final wines (Table 1). Srisamatthakarn (2011) also found that the Alchemy I and II strains formed increased amounts of 2-phenyl ethanol in Scheurebe wines. Mas et al. (2014) reported that the addition of organic nitrogen and yeast extract prior to yeast inoculation stimulated the yeast cell to produce higher amounts of higher alcohols like 2-phenyl ethanol. Higher production of this compound by strain Alchemy II might be reflected a less efficient usage of nitrogen, resulting in an increase of carbon flux related to branched-chain amino acid metabolism, which was consistent with the results of Carrau, Boido, & Dellacassa (2017). Concentrations of 2-phenyl ethanol, particularly in the Alchemy II wine, were above the aroma threshold of 10 mgL^{-1} (in wine-like model solution), which impart floral and rose aroma (Swiegers, Bartowsky, Henschke, & Pretorius, 2005; Darriet, & Pons, 2017). In summary, depending on the yeast strain utilized, the formation of higher alcohols presented different responses of nitrogen addition.

In addition, the results showed that different yeast strains significantly produced different concentrations of medium-chained fatty acids (MCFAs) and their corresponding ethyl esters and their formation strongly depended on nutrient sources (Table 1). For instance, strain X5 seemed to be a higher producer of ethyl esters of MCFAs, but the wines differed in concentrations of the individual compounds in response to nutrient sources. It produced the highest amount of ethyl hexanoate in the presence of Fermaid E at both levels (676.90$\pm$32.30 and 708.50$\pm$21.80 μgL^{-1}, respectively), whereas higher concentrations of ethyl octanoate were found in wines supplemented with both nutrient sources and concentrations (1,060.40$\pm$50.80 to 1,221.40$\pm$131.10 μgL^{-1}). Regards to the ethyl decanoate, all white wines had similar concentrations ranging from 285.90$\pm$54.70 to 416.60$\pm$82.00 μgL^{-1}.

Alchemy II strains appeared to be the most acetic acid esters producer, particularly acetic acid 2-phenyl ethyl ester, in all nutrient treatments (65.30$\pm$2.6 to 70.50$\pm$4.30 μgL^{-1}). It has been demonstrated that this ester was directly derived from the corresponding higher alcohol through condensation with acetyl-CoA (Swiegers, Bartowsky, Henschke, & Pretorius, 2005; Eder et al., 2018). Fermentation with Alchemy II also led to the greatest amount of acetic acid 2-methylbutyl ester at low OptiWhite level (28.50$\pm$3.30 μgL^{-1}). The Alchemy I strain followed similar pattern in the formation of acetic acid esters, however their concentrations varied

depending on nutrient sources and concentrations (Figure 2). Regarding to the acetic acid 3-methylbutyl ester, the Alchemy I and II strains formed the highest concentration in both Fermaid E additions (446.30±41.20, 449.30±39.60, 455.20±33.30 and 458.50±53.60 μgL^{-1}, respectively). In agreement with Pavelescu, Mandl, Steidl, Blesl, & Spangl (2015), they revealed that the yeasts of X5, VL3 and Alchemy II produced the most desirable aromatic compounds in Viennese Sauvignon blanc wines. In addition, the higher supplementation of Fermaid E seemed to stimulate the production of acetic acid 3-methylbutyl ester for all yeast strains. This effect is in agreement with Torrea et al. (2011), who found that amino acid plus ammonium nitrogen more strongly affected the production of this compound of Chardonnay wine, and presumed that the type of nitrogen source influences expression of the ester synthetic/hydrolytic genes. The addition of a mixture of amino acid and ammonium nitrogen exhibited the highest rating of pleasant fruity aromas. It might be due to a large pool of ammonium nitrogen in the DAP treatment, the amino acids were not used for yeast cellular structure and growth, but were available to produce high amounts of secondary metabolites such as volatile esters. On the other hand, Alchemy II strain produced the highest amounts of acetic acid 2-methylbutyl ester at low level of OptiWhite (inactive dry yeast), which was in good agreement with Andújar-Ortiz, Chaya, Martín-Álvarez, Moreno-Arribas & Pozo-Bayón (2014). It has been reported that the final formation of acetic acid esters of branched-chain alcohols is the result of the balance between of alcohol acyl transferase enzymes promoting acetic acid ester biosynthesis and esterase enzymes promoting their hydrolysis (Torrea et al., 2011). The behaviour of Alchemy II strain might reflect either reduced alcohol acyl transferase or increased esterase activity promoting acetic acid ester hydrolysis under this condition. In addition, all yeast strains exhibited similar concentration of ethyl hexanoate (acetic acid hexyl ester, $P>0.05$).

The result in (Figure 3) showed that the fermentations with the three yeast products led to slightly different concentrations of monoterpenes as α-terpineol varying depending on nutrient treatment. Other monoterpenes, like *trans*-linalool oxide, *cis*-linalool oxide and linalool were detected only in trace amounts (data not shown). Monoterpenes exist in grape juice principally as mono- and disaccharide terpenes and are released by acidic hydrolysis or by the enzymatic hydrolysis. Yeasts can exhibit a specific enzymatic activity to release certain monoterpenes from involatile

precursors (Fischer, Meyer, Claudel, Bergdoll, & Karst, 2011; Hjelmeland, & Ebeler, 2015; Carrau, Boido, & Dellacassa, 2017). This finding demonstrated that the three *Saccharomyces* yeast fermentations possessed only a little difference in the release of α-terpineol. It has been shown that terpene biosynthesis by industrial yeasts in relation to nitrogen metabolism and high assimilable nitrogen content of the medium (400 $mgNL^{-1}$) stimulates monoterpene formation (Carrau, Boido, & Dellacassa, 2017). Nonetheless, concentrations of α-terpineol (lilac-like aroma) were quite below the aroma threshold at 400 μgL^{-1} in all wines from the different variants (Hjelmeland, & Ebeler, 2015; Carrau, Boido, & Dellacassa, 2017; Jeromel, Korenika, & Tomaz, 2019).

Table 1 Concentration of 2-phenyl ethanol and ethyl esters of medium-chain fatty acids found in finished Sauvignon blanc wines fermented by three commercial yeast products with two nutrient sources at two different concentrations.

yeast	nutrient	2-phenyl ethanol (mgL^{-1})	ethyl hexanoate (μgL^{-1})	ethyl octanoate (μgL^{-1})	ethyl decanoate (μgL^{-1})
X5	0.3 gL^{-1} OptiWhite	$12.10^{h}\pm0.70$	$588.10^{b}\pm21.50$	$1,060.40^{ab}\pm50.80$	$394.50^{a}\pm21.90$
	0.5 gL^{-1} OptiWhite	$13.00^{gh}\pm0.90$	590.60^{b} 16.80	$1,072.50^{ab}\pm41.40$	$394.00^{a}\pm6.70$
	0.3 gL^{-1} Fermaid E	$13.50^{efg}\pm0.60$	$676.90^{a}\pm32.30$	$1,206.90^{a}\pm169.30$	$400.90^{a}\pm93.70$
	0.4 gL^{-1} Fermaid E	$13.40^{fg}\pm0.30$	$708.50^{a}\pm21.80$	$1,221.40^{a}\pm131.10$	$416.60^{a}\pm82.00$
Alchemy I	0.3 gL^{-1} OptiWhite	$14.90^{cde}\pm0.50$	$490.70^{c}\pm31.30$	$881.90^{c}\pm59.20$	$364.50^{a}\pm17.50$
	0.5 gL^{-1} OptiWhite	$16.30^{abc}\pm0.30$	$483.30^{c}\pm5.30$	$856.00^{c}\pm28.90$	$343.70^{a}\pm4.30$
	0.3 gL^{-1} Fermaid E	$14.50^{def}\pm0.50$	$506.10^{c}\pm12.60$	$921.10^{bc}\pm65.40$	$356.10^{a}\pm9.10$
	0.4 gL^{-1} Fermaid E	$14.80^{def}\pm0.40$	$506.70^{c}\pm49.50$	$855.70^{c}\pm136.30$	$285.90^{a}\pm54.70$
Alchemy II	0.3 gL^{-1} OptiWhite	$15.80^{abcd}\pm1.30$	$510.20^{c}\pm20.50$	$924.70^{bc}\pm107.10$	$358.10^{a}\pm31.20$
	0.5 gL^{-1} OptiWhite	$17.50^{a}\pm0.90$	$492.70^{c}\pm29.10$	$841.50^{c}\pm46.40$	$315.50^{a}\pm53.80$
	0.3 gL^{-1} Fermaid E	$15.40^{bcd}\pm0.10$	$534.50^{c}\pm23.50$	$962.30^{bc}\pm70.30$	$367.10^{a}\pm28.60$
	0.4 gL^{-1} Fermaid E	$16.30^{ab}\pm0.70$	$512.50^{c}\pm33.90$	$940.30^{bc}\pm83.50$	$373.00^{a}\pm40.50$

Values are means ± standard deviation. Values displaying the same letter (only 'a') within the same column indicate no significant difference ($P>0.05$), whereas those displaying different letters are significantly different ($P<0.05$) according to the DNMRT test.

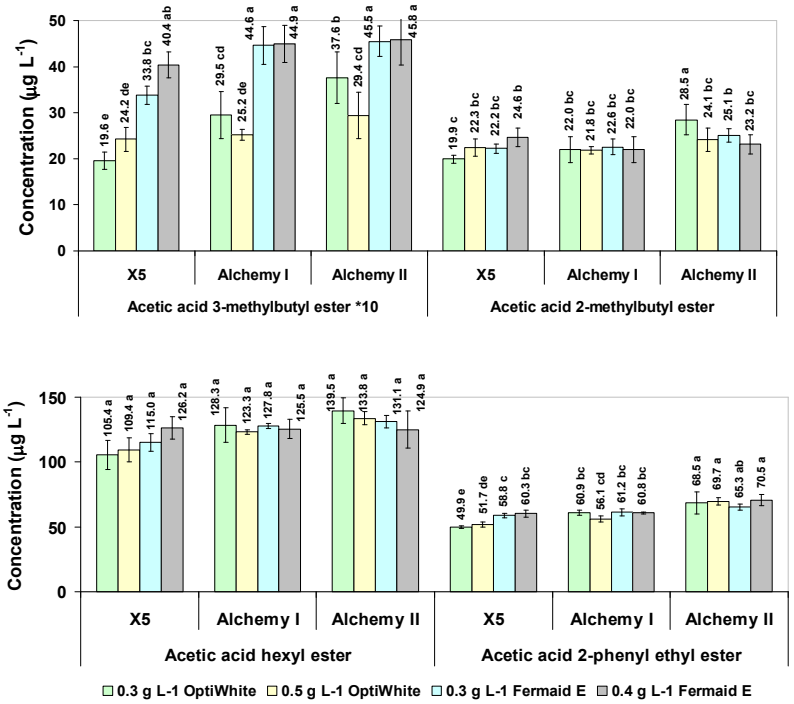

Figure 2 Concentration of acetic acid ester analysed in Sauvignon blanc wines produced by three different commercial yeast strains with two nutritive sources at two different levels. Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($P<0.05$) according to the DNMRT test.

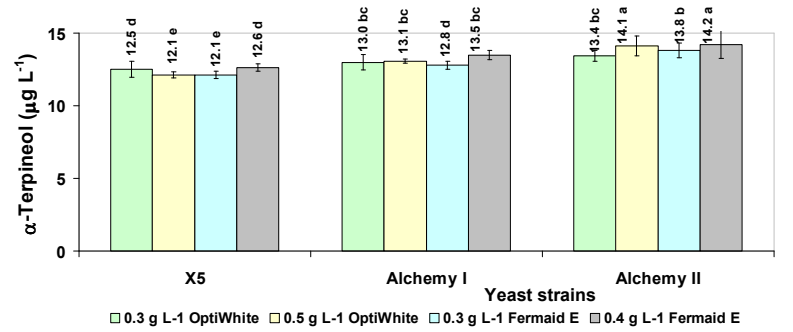

Figure 3 Concentration of α-terpineol analysed in finished Sauvignon blanc wines produced by three commercial yeast strains with two nutritive sources at two different concentrations. Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($P<0.05$) according to the DMRT test.

Conclusion

The fermentation trial with three commercial yeast products (X5, Alchemy I and Alchemy II) showed that some significant differences with regard to secondary metabolites and volatile aromatic compounds in the Sauvignon blanc wines were depended on the nutrient sources and their concentrations. The complex nutrient product, Fermaid E, seemed to be the most effective nutrient composition to lower SO_2-binding compounds and to enhance the formation of desirable metabolic compounds in the wines fermented with Alchemy II yeasts. Both Alchemy I and II strains increased the formation of acetic acid 3-methylbutyl ester in the wines fermented with Fermaid E treatment. Whereas the X5 strain contributed to the highest ethyl esters of medium-chain fatty acids in the presence of Fermaid E. It can be concluded that the most suitable choice for an adequate formation of SO_2-binding and aromatic compounds in the Sauvignon blanc wines was the yeast blend of Alchemy II and Fermaid E at 0.4 gL^{-1}.

Acknowledgement

Authors are sincerely grateful to staff members of the Department of Microbiology and Biochemistry, Hochschule Geisenheim University, Germany, for their technical and analytical support and assistance. The Agricultural Technology Research Institute, Rajamangala University of Technology Lanna are also gratefully acknowledged for the research financial support.

References

Andújar-Ortiz, I., Chaya, C., Martín-Álvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayón, M. A. (2014). Impact of using new commercial glutathione enriched inactive dry yeast oenological preparations on the aroma and sensory properties of wines. *International Journal of Food Properties*, *17*(5), 987-1001.

Bellon, J. R., Eglinton, J. M., Siebert, T. E., Pollnitz, A. P., Rose, L., de Barros Lopes, M., & Chambers, P. J. (2011). Newly generated interspecific wine yeast hybrids introduce flavour and aroma diversity to wines. *Applied and Environmental Microbiology*, *91*, 603-612.

Bellon, J. R., Schmidt, F., Capone, D. L., Dunn, B. L., & Chambers, P. J. (2013). Introducing a new breed of wine yeast: interspecific hybridisation between a commercial *Saccharomyces cerevisiae* wine yeast and *Saccharomyces mikatae*. *PLOS One*, *8*, 1-14.

Carrau, F., Boido, E., & Dellacassa, E. (2017). Yeast diversity and flavor compounds. In J. M. Mérillon, & K. Ramawat (Eds.), *Fungal Metabolites*. *Reference Series in Phytochemistry* (pp. 1-29). Cham: Springer.

Carrau, F., Gaggero, C., & Aguilar, P. S. (2015). Yeast diversity and native vigor for flavor phenotypes. *Trends in Biotechnology*, *33*, 148-154.

Comuzzo, P., & Zironi, R. (2013). Biotechnological strategies for controlling wine oxidation. *Food Engineering Reviews*, *5*(4), 217-229.

Cordente, T., Tran, T., & Curtin, C. (2014). Enhancing red wine complexity using novel yeast blends. *AWRI, Technical Review*, 212, 9-13.

Crépin, L., Truong, N., Bloem, A., Sanchez, I., Dequin, S., & Camarasa, C. (2017). Management of multiple nitrogen sources during wine fermentation by *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, *83*(5), e02617-16.

Crépin, L., Nidelet, T., Sanchez, I., Dequin, S., & Camarasa, C. (2012). Sequential use of nitrogen compounds by *Saccharomyces cerevisiae* during wine fermentation: a model based on kinetic and regulation characteristics of nitrogen permeases. *Applied and Environmental Microbiology*, *78*, 8102-8111.

Darriet, P., & Pons, A. (2017). Wine. In A. Buettner (Ed.), *Springer Handbook of Odor* (pp. 143-170). Cham: Springer.

Dutraive, O. Benito, S., Fritsch, S., Beisert, B., Patz, C.D., & Rauhut, D. (2019). Effect of sequential inoculation with non-*Saccharomyces* and *Saccharomyces* yeasts on Riesling wine chemical composition. *Fermentation, 5*(3), 79.

Eder, M., Sanchez, I., Brice, C., Camarasa, C., Legras, J.L., & Dequin, S. (2018). QTL mapping of volatile compound production in *Saccharomyces cerevisiae* during alcoholic fermentation. *BMC Genomics*, *19*, 166.

Fischer, M. J., Meyer, S., Claudel, P., Bergdoll, M., & Karst, F. (2011). Metabolic engineering of monoterpene synthesis in yeast. *Biotechnology and Bioengineering*, *108*, 1883-1892.

Fritsch, S., Brezina, S., Ebert, K., & Rauhut, D. (2017). *Standard Operating Procedure (SOP) for the Analysis of the Fermentation Bouquet in Wines*. Geisenheim, Germany: Hochschule Geisenheim University.

Goold, H. D., Kroukamp, H., Williams, T. C., Paulsen, I. T., Varela, C., & Pretorius, I. S. (2017). Yeast's balancing act between ethanol and glycerol production in low-alcohol wines. *Microbial Biotechnology*, *10*(2), 264-278.

Hjelmeland, A. K., & Ebeler, S. E. (2015). Glycosidically bound volatile aroma compounds in grapes and wine: a review. *American Journal of Enology and Viticulture*, *66*, 1-11.

Jeromel, A., Korenika, A. J., & Tomaz, I. (2019). An influence of different yeast species on wine aroma composition. In A. Grumezescu, & A. M. Holban (Eds.), *Fermented Beverages: Volume 5* (pp. 171-285). Duxford, UK: Woodhead .

Lewin, B. (2010). *Wine Myths and Reality*. Dover, UK: Vendange Press.

Mas, A., Guillamón, J. M., Torija, M. J., Beltran, G., Cerezo, A. B., Troncoso, A. M., & Garcia-Parrilla, M. C. (2014). Bioactive compounds derived from the yeast metabolism of aromatic amino acids during alcoholic fermentation. *BioMed Research International,* 2014, 898045.

Mendes-Ferreira, A., Barbosa, C., Falco, V., Leão, C., & Mendes-Faia, A. (2009). The production of hydrogen sulphide and other aroma compounds by wine strains of *Saccharomyces cerevisiae* in synthetic media with different nitrogen concentrations. *Journal of Industrial Microbiology and Biotechnology*, *36*(4), 571-583.

Pavelescu, D., Mandl, K., Steidl, R. Blesl, X., & Spangl, B. (2015). The influence of yeasts on the aroma profile of Viennese Sauvignon blanc wines. *Mitteilungen Klosterneuberg*, *65*, 157-169.

Pretorius, I. S. (2016). Conducting wine symphonics with the aid of yeast genomics. *Beverages*, *2*(4), 36.

Pretorius, I. S., Curtin, C. D., & Chambers, P. J. (2012). The winemaker's bug: From ancient wisdom to opening new vistas with frontier yeast science. *Bioengineered Bugs*, *3*(3), 147-156.

Rapp, A., Yavas, I., & Hastrich, H. (1994). Einfache und schnelle Anreicherung ("Kaltronmethode") von Aromastoffen des Weines und deren quantitative Bestimmung mittels Kapillargaschromatographie (Easy and fast enrichment (Kaltron method) and quantitative determination of wine volatiles with capillary gas chromatography). *Deutsche Lebensmittel-Rundschau*, *90*, 171-174.

Rauhut, D. (2017). Usage and formation of sulfur compounds. In H. König, G. Unden, & J. Fröhlich (Eds.), *Biology of Microorganisms on Grapes, in Must and in Wine* (pp. 255-291). Cham, Switzerland: Springer International Publishing AG.

Rauhut, D., Beisert, B., Berres, M., Gawron-Scibek, M., & Kürbel, H. (2005). Pulse flame phytometric detection: an innovative technique to analyse volatile sulphur compounds in wine and other beverages. In T. Hofmann, M. Rothe, & P. Schieberle (Eds.), *State-of-the-Art in Flavour Chemistry and Biology Garching* (pp. 363-367). Geisenheim, Germany: Deutsche Forschungsanstalt für Lebensmittelchemie.

Rauhut, D., & Beisert, B. (2017). *Standard operating procedure (SOP) for the analysis of low volatile sulfur compounds using headspace and GC-PFPD*. Geisenheim, Germany: Hochschule Geisenheim University.

Schüttler, A., Friedel, M., Jung, R., Rauhut, D., & Darriet, P. (2015). Characterizing aromatic typicality of Riesling wines: Merging volatile compositional and sensory aspects. *Food Research International*, *69*, 26-37.

Srisamatthakarn, P. (2011). *Improvement of varietal aroma in grape and tropical fruit wines by optimal choice of yeasts* (Doctoral dissertation). Justus-Liebig-University Giessen, Germany.

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology & Biotechnology*, *38*(9), 1145-1159.

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavor. In R. J. Blair, M. E. Francis, & I. S. Pretorius (Eds.), *Advances in Wine Science-commemorating 50 Years of The Australian Wine Research Institute* (pp. 159-187). Glen Osmond: The Australian Wine Research Institute.

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Francis, I. L., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice-effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. *Food Chemistry*, *127*(3), 1072-1083.

Varela, C., Kutyna, D. R., Solomon, M. R., Black, C. A., Borneman, A., Henschke, P. A., Pretorius, I. S., & Chambers, P. J. (2012). Evaluation of gene modification strategies for the development of low-alcohol wine yeasts. *Applied and Environmental Microbiology*, *78*, 6068-6077.

Wells, A., & Osborne, J. P. (2011). Production of SO_2 binding compounds and SO_2 by *Saccharomyces* during alcoholic fermentation and the impact on malolactic fermentation. *South African Journal of Enology and Viticulture*, *32*(2), 267-279.