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Research Article

Effects of Commercial Yeast Strains and Nutrient Supplements on SO_2-binding and Aroma Compounds of Yellow Passion Fruit (*Passiflora edulis* Sims *f. flavicarpa* Degner) Wine

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Abstract

Information on the impact of yeast strain and nutrient supplement on SO_2-binding and aroma compounds of yellow passion fruit (YPF) wine is very limited. Therefore, the effects of two *Saccharomyces cerevisiae* var. *bayanus* strains (Lalvin-EC1118 and LittoLevure), one *Saccharomyces spp.* strain (Anchor-Alchemy I) and two *Saccharomyces cerevisiae* strains (Zymaflore VL3 and X5) in the combination with two nutrient sources (Diammonium hydrogen phosphate (DAP), and DAP plus thiamine, VCombi) at two levels (0.25 and 0.5 g L^{-1}) were examined to improve the formation of SO_2-binding and aroma compounds as well as varietal volatile thiols in the YPF wines. The results clearly demonstrated that supplementation of YPF juice with DAP plus thiamine at a high level (0.5 g L^{-1}) appeared to be the most effective way of producing YPF wine with more desirable aromas and a low level of reductive sulphur and SO_2-binding compounds for strains EC1118, VL3, X5 and LittoLevure. Interestingly, strain EC1118 was the greatest producer of varietal volatile thiols, 3-sulphanylhexanol (3SH) and acetic acid 3-sulphanylhexyl ester (3SHA), in the treatments of 0.25 g L^{-1} DAP and 0.5 g L^{-1} DAP plus thiamine. Strain LittoLevure with 0.5 g L^{-1} DAP plus thiamine addition also produced high amounts of 3SH.

Keywords: yellow passion fruit, *Passiflora edulis* Sims f. *flavicarpa* Degner, yeast strains, nutrient supplement, aroma compounds, Thailand.

Introduction

During alcoholic fermentation, *Saccharomyces* yeasts do not only convert sugars to ethanol and carbon dioxide [1], they also produce a wide range of metabolites, for example, acetic acid, acetaldehyde, pyruvate and lactic acid [2]. The keto acids, principally pyruvate and α-ketoglutarate, have implications for wine stability and quality due to their abilities to bind sulphur dioxide and to react with phenols [3, 4]. A vast number of volatile compounds are also formed and modulated by yeast during alcoholic fermentation that significantly impacts the flavour and overall quality of wines [5]. The use of different *Saccharomyces* strains for wine fermentation has been shown to result in wines with differing secondary metabolites, through varied relative concentrations of acetic acid esters, fatty acid ethyl esters and higher alcohols [2, 6], which are sensorially important volatile metabolites giving wines its vinous character. Some key varietal aromas are volatile thiols (thiol referring to the SH functional group) that derive from non-volatile precursors during the alcoholic process due to the bioconversion of yeasts [1, 7]. Nevertheless, it is well known that S-compounds can also be responsible for certain off-flavours (e.g. H_2S, methanethiol and thioacetic acid-S-methyl ester) in wine. Intensive research work demonstrated that one of the main causes for off-flavours occurring after fermentation is the chosen yeast strain (*Saccharomyces cerevisiae*) and its nutrient requirements and content in the grape musts [8, 9].

Yellow passion fruit (YPF) is known for its natural attractive colouring and the best tropical fruit having a floral, estery aroma with an exotic tropical sulphury note [10]. It is a typical example of a fruit, of which the flavour is established by volatile thiol compounds [11, 12, 13]. 3-sulphanylhexanol (3SH, which contributes to aromas of passion fruit and grapefruit) and acetic acid 3-sulphanylhexyl ester (3SHA, which contributes to aromas of boxwood, grapefruit zest and passion fruit) were identified in YPF and present in both free and conjugate form [11, 12, 14]. Whereas, Jordán *et al*. [15] reported that the most abundant compounds in yellow passion fruit aqueous essence are linalool, octanol, hexanoic acid ethyl ester and butanoic acid ethyl ester. The use of different *Saccharomyces* yeast strains for YPF wine fermentation has been shown to result in wines with different formation of secondary metabolites [16]. However, there is little research work on YPF wine production as well as improvement of its quality, especially the compounds responsible for the typical passion fruit wine aroma still need to be investigated. In addition, only little information is available on the improvement of passion fruit wine quality by optimal choice of yeasts as well as nutrient supplementation, additionally, nearly no information is available to optimize the release and preservation of volatile flavours in passion fruit wine during alcoholic fermentation.

Therefore, this work was aimed at studying the impact of commercial yeast strains and nutrient supplements on the formation of SO_2-binding (pyruvate and α-ketoglutarate), aroma and varietal thiol compounds of YPF wine.

Materials and Methods

The fermentation for this study was conducted at the Department of Microbiology and Biochemistry, Geisenheim Research Center, Germany.

Passion fruit juice

The frozen YPF puree employed for this study is the hybrid breeding variety of *Passiflora edulis* Sims *f. flavicarpa* Degner. It was purchased from Thai Nutri-Juice Co., Ltd., Thailand and kept at –

25°C until use. The properties of frozen YPF puree were TSS (total soluble solid) 9.0 ± 0.1 °Brix, 53.5 ± 3.5 g L^{-1} reducing sugar content, pH 2.9 ± 0.1, 40.5 ± 0.0 g L^{-1} citric acid and 2491.0 mg L^{-1} total amino acid (TAA) without proline (data not shown).

The frozen YPF puree was thawed at ambient temperature and diluted by addition of hot water until its pH was 3.1-3.2, therefore reducing sugar content of diluted YPF juice was 3.65 g L^{-1} and 123.1 mg L^{-1} TAA without proline) (data not shown). Then the sugar content was adjusted to provide sugar quantities of 200 g Kg^{-1} juice by addition of beet sugar (sucrose, Südzucker, Mannheim, Germany). The composition of prepared YPF juice was 170.5 ± 12.1 g L^{-1} inverted sugar content, pH 3.2 ± 0.1 and 3.1 ± 0.6 g L^{-1} citric acid (data not shown). Experiments were performed in triplicate in 1.50-litre bottles filled with 1.20 litres of YPF juice. Five commercial *Saccharomyces* yeast strains (Lalvin-EC1118, LittoLevure, Anchor-Alchemy I, Zymaflore VL3 and X5) in the combination with two nutrient sources (DAP, and DAP plus thiamine, VCombi) at two levels (0.25 and 0.5 g L^{-1}) were used, thus giving 20 different fermentation treatments. Addition of nutrients was performed in the YPF juice according to an experimental design prior to the alcoholic fermentation and 50 mg L^{-1} of sulphur dioxide as $K_2S_2O_5$ were added. Juice bottles were left to settle approximately12 hours to suppress undesirable microorganism growth as well as to function as an antioxidant.

Fermentation

Each yeast culture was rehydrated following the recommendations of the manufacturer prior to inoculation and was randomly assigned to each of three bottle replicates. The bottles were fitted with airlocks and the fermentations were carried out at 20°C in a controlled environment until finished. The YPF wines were cold stabilized at below 10°C for 7 days and racked into previously sterilized bottles. Then $K_2S_2O_5$ was added corresponding to 80 mg L^{-1} YPF wine and the bottles were sealed with sterilized crown caps. Bottled YPF wines were stored at below 15°C until analysed.

Analyses

SO_2-binding compounds (pyruvate and α-ketoglutarate) were determined enzymatically by an UV/VS spectrometer Lambda 2 (Perkin Elmer 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 according to instructions of Boehringer Mannheim [17].

Reductive sulphur compounds were 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 Rauhut *et al*. [18]. Esters, fatty acid and higher alcohols were detected by HP 5890 Series II gas chromatograph equipped with a cooled injection system CIS-3 (Gerstel GmbH, Mühlheim an der Ruhr, Germany) and HP 5972 mass selective detector (MSD) according to a modified procedure from Rapp *et al*. [19].

The varietal volatile thiols, 3SH and 3SHA, were specifically extracted by the reversible combination of the thiols with *p*-hydroxymercuribenzoate (*p*-HMB) according to a modified Ferreira *et al*. [20] method. They were then analyzed with a 6890N GC oven (Agilent Technologies, Böblingen, Germany) equipped with a cold Injection system 4 (CIS4), a Multi Purpose Sampler 2 (MPS2) both from Gerstel (Mülheim an der Ruhr) and a DB-WAX column 30 m x 0.32 mm x 0.25 µm (Agilent

Technologies, Böblingen, Germany). The detector used was a 5975C mass selective detector (MSD) from Agilent Technologies (Böblingen, Germany).

Statistical analysis

The one-way analysis of variance (ANOVA) and least significant difference (LSD) tests were performed using MSTATC statistical program (www.msu.edu/~freed/disks.htm) to interpret mean differences in mean values.

Results and Discussion

Excessive amounts of keto acids, α-ketoglutarate and pyruvate, in the YPF wines were produced by strains X5 and VL3 in the presence of DAP, especially at low levels, but significantly diminished with VCombi (DAP plus thiamine) addition (Figure 1). It has been suggested that when ammonium salts are added as sole major source, they will be converted to glutamate and mobilized in transamination reactions in yeasts resulting in the formation of other amino acids and keto acids, however depending on the yeast strain [21, 22]. In accordance with some studies, thiamine effectively reduces keto acid concentrations by the enzymatic decarboxylation [7, 23]. The result indicated that excessive levels of keto acids (more than 100 mg L^{-1}) detected in the YPF wines produced by strains X5 and VL3 in the DAP treatment seemed to influence the stability and quality.

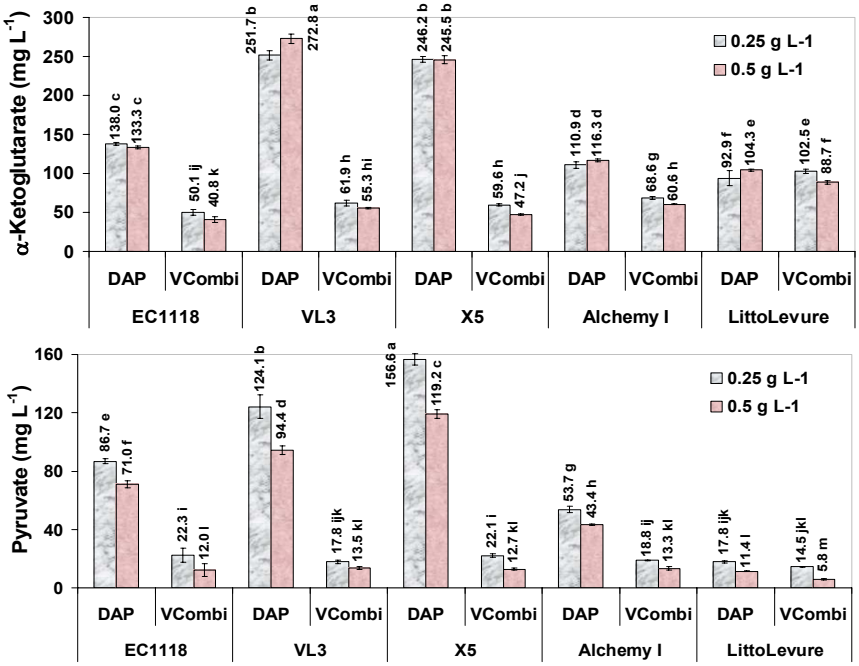

Figure 1. Concentration of SO_2-binding compounds found in finished YPF wines.

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound, whereas those followed by same letters on the top of the bar are not significantly different ($p>0.05$) according to the DMRT test.

Although DAP was added to compensate nitrogen deficiencies in the YPF juices, high formations of H_2S by most strains were observed (Figure 2). Its supplementation is not always effecting H_2S production because of other factors, e.g. methionine and other nitrogen that regulate amino acid transport into the yeast cell and sulphur metabolism, especially the SRS pathway, which generates

H_2S [24] and deficiencies in vitamins and micronutrients [25]. In addition, Rauhut [9] suggested that some yeast strains are constantly high or constantly low H_2S producers. On the other hand, its production significantly decreased when the VCombi was supplemented into the juice at 0.5 g L^{-1}. A high formation of H_2S by strains EC1118 and Alchemy I in the same nutrient condition might lead to a high production of methanethiol as well as a high production of thioacetic acid-S-methyl ester (MeSAc) by the Alchemy I strain. It has been reported that a high H_2S formation in the early phase of fermentation leads to high amounts of MeSAc [8, 9]. Although concentrations of H_2S and MeSAc detected in the YPF wines were below the aroma threshold (10-80 µg L^{-1} and 130 µg L^{-1}, respectively), levels of MeSH produced by strains EC1118 and Alchemy I under certain nutrient conditions may give a reductive off-flavour (0.02-0.3 µg L^{-1}) in YPF wines.

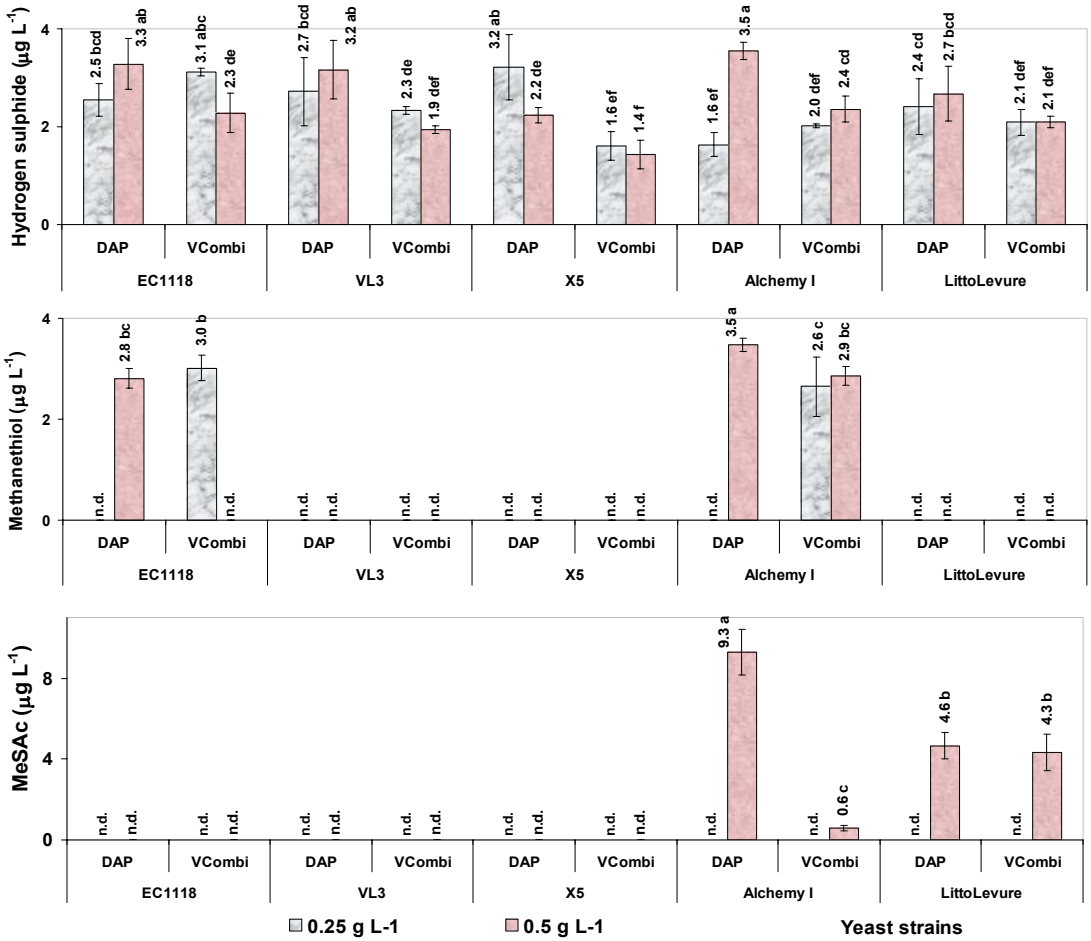

Figure 2. Concentration of hydrogen sulphide, methanethiol and MeSAc in finished YPF wines.
Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.
n.d. denotes not detected

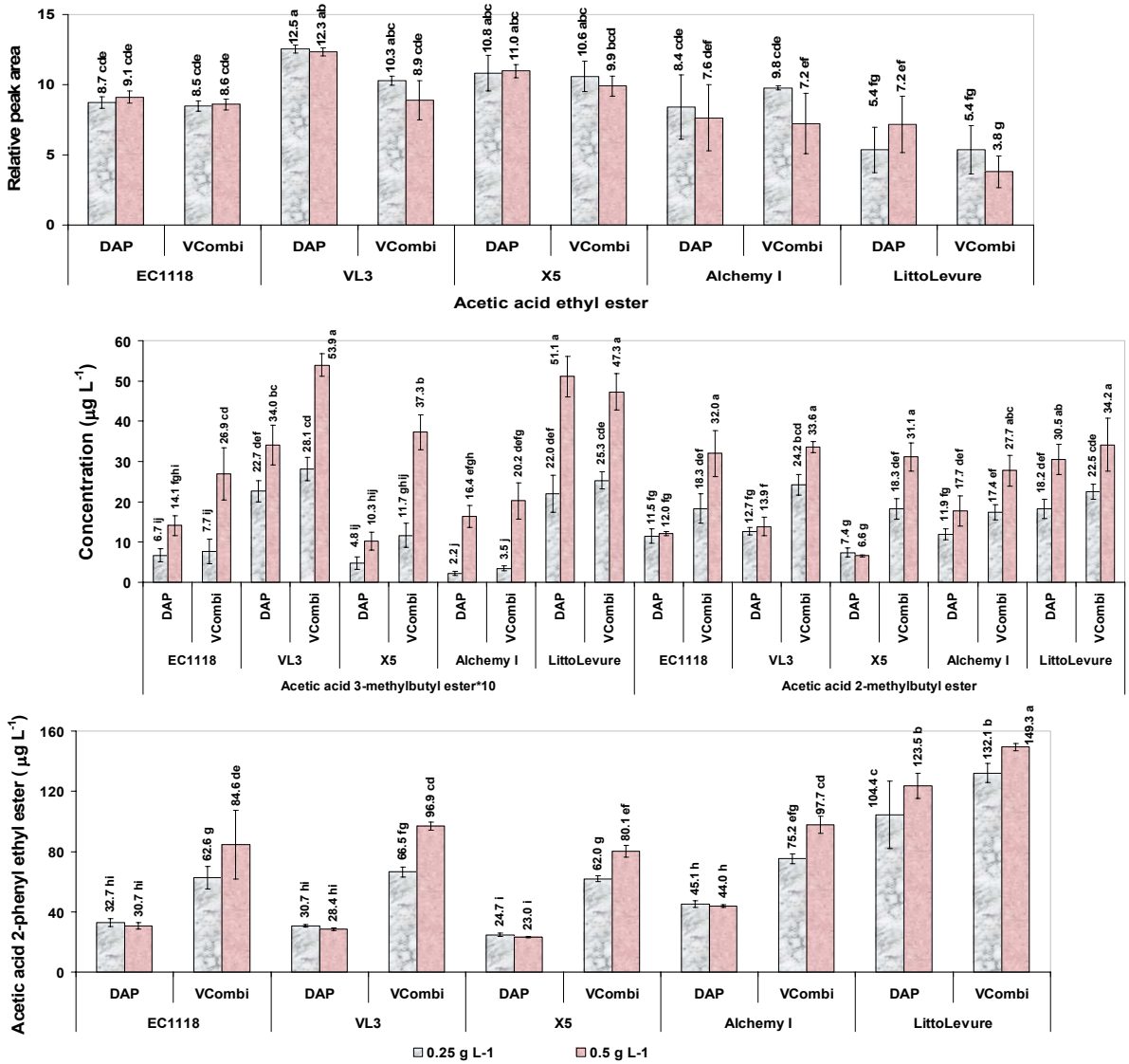

Figure 3. Concentration of acetic acid ester in finished YPF wines (*10 = concentration of compound times 10).

Acetic acid ethyl ester (pineapple-like aroma), acetic acid 3-methylbutyl ester (banana-like aroma), acetic acid phenylethyl ester (fruity, flowery favour with a honey note), hexanoic acid ethyl ester (apple-like aroma), octanoic acid ethyl ester (fruity aroma) and decanoic acid ethyl ester (pineapple-like aroma) [26]; www.flavornet.org).

Vertical bars represent standard deviations from three fermentation replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among treatment combinations of each compound according to the DMRT test.

Relative peak area = (peak area of sample)/(Peak area of internal standard)

Even though the YPF wines produced by strains VL3 and X5 in most nutrient supplements showed a tendency to develop high amounts of acetic acid ethyl ester, their concentrations were below the quantification limit as displayed in relative peak area (Figure 3). Strain LittoLevure, with the addition of either DAP or VCombi (DAP plus thiamine) at high level, produced prominent acetic acid esters. The addition of high level of VCombi resulted in the greatest formation of acetic acid 3-methylbutyl ester by strains VL3 and acetic acid 2-methylbutyl ester for all yeast strains. It is likely that a high

nutrient level promoted a great formation of acetic acid esters. Some studies have also reported that the increase in wine ester formation is directly related to a high nitrogen level [27, 28]. In addition, supplementation of DAP plus thiamine might enhance the metabolic activity of the yeast and also regulate the metabolic pathways of acetic ester production [22, 25, 28, 29]. Ribéreau-Gayon *et al*. [23] and Swiegers *et al.* [1] reported that acetic acid esters contribute positively to wine aroma at very low concentrations such as acetic acid ethyl ester 50-80 mg L^{-1}, acetic acid 3-methylbutyl ester 30 µg L^{-1} and acetic acid 2-phenylethyl ester 250 µg L^{-1}. The YPF wines might also impart an intensive banana aroma as the concentrations of acetic acid 3-methylbutyl ester were quite above the threshold.

Table 1. Concentration of fatty acid ethyl ester in finished YPF wines.

Strain	Nutrient	Content (g L^{-1})	Ethyl ester of fatty acid (µg L^{-1})		
			Hexanoic acid	Octanoic acid	Decanoic acid
EC1118	DAP	0.25	235.3 ± 38.1 bcd	267.4 ± 76.7 bcde	71.3 ± 18.2 def
		0.5	213.1 ± 17.7 bcde	306.6 ± 69.5 bcd	78.0 ± 14.0 cde
	DAP + Thiamine	0.25	369.4 ± 87.4 a	384.4 ± 45.8 ab	120.9 ± 29.3 ab
		0.5	412.7 ± 85.9 a	480.5 ± 80.0 a	139.4 ± 39.9 a
VL3	DAP	0.25	47.5 ± 8.4 h	65.9 ± 25.9 h	26.3 ± 5.6 gh
		0.5	73.3 ± 13.2 h	135.9 ± 31.2 efgh	39.2 ± 3.9 gh
	DAP + Thiamine	0.25	162.6 ± 34.9 def	248.8 ± 67.7 bcdef	71.9 ± 19.0 def
		0.5	243.3 ± 23.5 bc	371.9 ± 61.9 abc	102.9 ± 13.0 bc
X5	DAP	0.25	83.3 ± 21.6 gh	66.0 ± 28.7 h	31.1 ± 7.4 gh
		0.5	84.1 ± 11.4 gh	113.5 ± 35.1 fgh	28.9 ± 6.2 gh
	DAP + Thiamine	0.25	265.1 ± 35.4 b	313.3 ± 61.3 bcd	90.0 ± 9.4 cd
		0.5	344.8 ± 25.5 a	496.5 ± 82.0 a	139.0 ± 7.3 a
Alchemy I	DAP	0.25	113.6 ± 13.3 fgh	102.2 ± 30.0 gh	24.1 ± 1.9 h
		0.5	166.7 ± 26.0 def	206.7 ± 35.9 defg	50.5 ± 4.1 efgh
	DAP + Thiamine	0.25	150.3 ± 9.0 efg	163.7 ± 38.7 efgh	42.7 ± 5.9 fgh
		0.5	252.0 ± 39.8 bc	305.3 ± 75.1 bcd	87.1 ± 10.6 cd
LittoLevure	DAP	0.25	120.8 ± 11.4 fgh	200.7 ± 47.7 defgh	53.8 ± 10.6 efgh
		0.5	235.3 ± 38.1 bcd	267.4 ± 76.7 bcde	71.3 ± 18.2 def
	DAP + Thiamine	0.25	213.1 ± 17.7 bcde	306.6 ± 69.5 bcd	78.0 ± 14.0 cde
		0.5	369.4 ± 87.4 a	384.4 ± 45.8 ab	120.9 ± 29.3 ab

Each value shows the mean ± standard deviation from three fermentation replicates.
Values displaying different letters are significantly different ($p<0.05$) according to the DMRT test.

As shown in Table 1, the addition of DAP plus thiamine at high level stimulated strains EC1118 and X5 to produce a high amount of ethyl esters of medium-chain fatty acids (ethyl esters of hexanoic, octanoic and decanoic acids). As mentioned above, the addition of DAP plus thiamine seemed to enhance the metabolic activity of the certain yeast strain and then regulated the metabolic pathways of ethyl ester production. Nevertheless, concentrations of only ethyl ester of hexanoic and octanoic acid were beyond the odour threshold (5-14 and 2-5 µg L^{-1}, respectively), which elicit pleasant floral and fruity aromas in wine [1, 26]. Thus, it can be suggested that depending on the yeast strain utilized,

the formation of acetic acid ester and ethyl esters showed different responses when nutrient/nitrogen was added to the medium as demonstrated in some studies [29, 30].

As shown in Figure 4, the results confirmed that high amounts of 3SH can be found as the naturally occurring volatile thiols in YPF juice [11, 12, 13, 14]. Although the prepared YPF juice in this study was diluted by water addition, these volatile thiols were detected. The results clearly showed that commercial *Saccharomyces* yeasts released approximately 7.1-fold to 11.0-fold of 3SH in YPF wines in comparison to detected 3SH in the YPF juice, however this depended on the yeast strain. 3SH can be generated by the action of yeast C-S β-lyase from its S-cysteine conjugate (Cys-3SH) in YPF juice [14]. In the study, strain EC1118 was the most effective producer of 3SH and 3SHA in the YPF wines. The LittoLevure strain seemed to follow a similar pattern of 3SH liberation, but less 3SHA production. Strain VL3 appeared to be the least efficient in releasing the 3SH aroma. This result confirmed previous studies in grape wines [1, 5, 6] that the release and the modulation mechanisms of volatile thiol compounds in YPF wines were also yeast strain dependent.

It has been shown that during fermentation 3SHA is generally formed when acetic acid esterifies the 3SH that has been released. Swiegers *et al*. [1, 31] found out that 3SHA is formed from 3SH by the action of the yeast ester-forming alcohol acetyltransferase, encoded by the *ATF1* gene. Lilly *et al*. [32] suggested that the overexpression of *ATF1* in a VIN13 yeast strain resulted in increased 3SHA concentrations. The present study also revealed that a higher concentration of 3SH induced an increased production of 3SHA in YPF wines for most yeast strains (6.5 to 9.0 % released 3SHA), except for strain LittoLevure (5.0 %). Some specific gene, which encodes an ester-degrading enzyme, might be involved [1, 31].

To examine the effect of the nutrient source (DAP and DAP plus thiamine; VCombi) at different levels (0.25 and 0.5 g L^{-1}) on the production of volatile thiols, the two yeast strains of EC1118 and X5 were used. As demonstrated in Figure 5, the result clearly indicated that the low level of DAP addition modulated higher 3SH and 3SHA productions by strain EC1118 than the high level. This result confirmed the investigation of Subileau *et al*. [33] that a complementation with DAP induces a decrease of 3SH production. They also concluded that in synthetic medium, Cys-3SH enters the cell through at least one identified transporter, GAP1p, whose activity is limiting the release of volatile thiols. The uptake of the precursor through GAP1p is not confirmed, but the effect of the addition of DAP, eventually prolonging nitrogen catabolite repression, is shown to decrease thiol production. On the other hand, the addition of VCombi (DAP plus thiamine) at high level tended to enhance higher expression of these thiols in strain EC1118. In addition, the nutrient source and concentration had no significant influence on the 3SH release in strain X5.

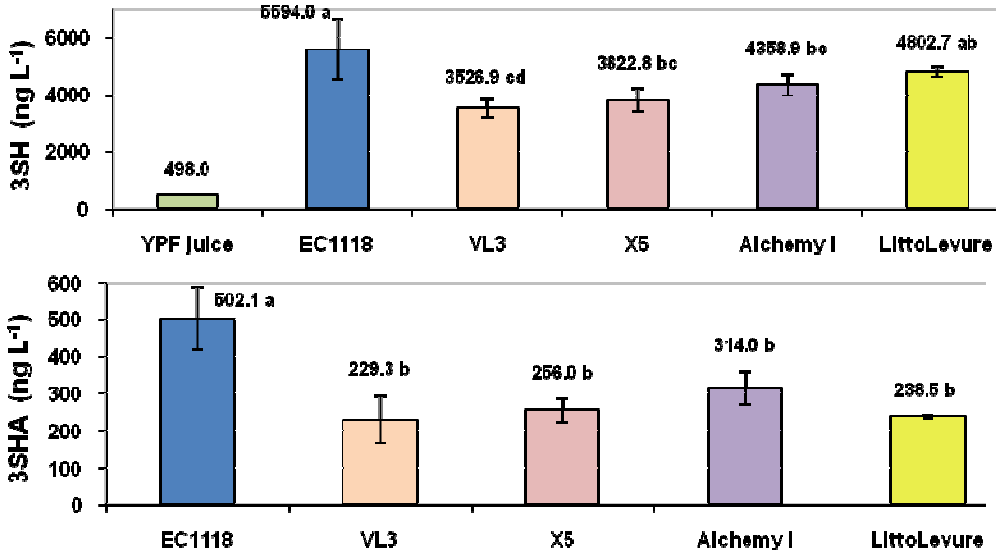

Figure 4. Concentration of 3SH and 3SHA detected in prepared YPF juice with the addition of VCombi at the high level and finished YPF wines.

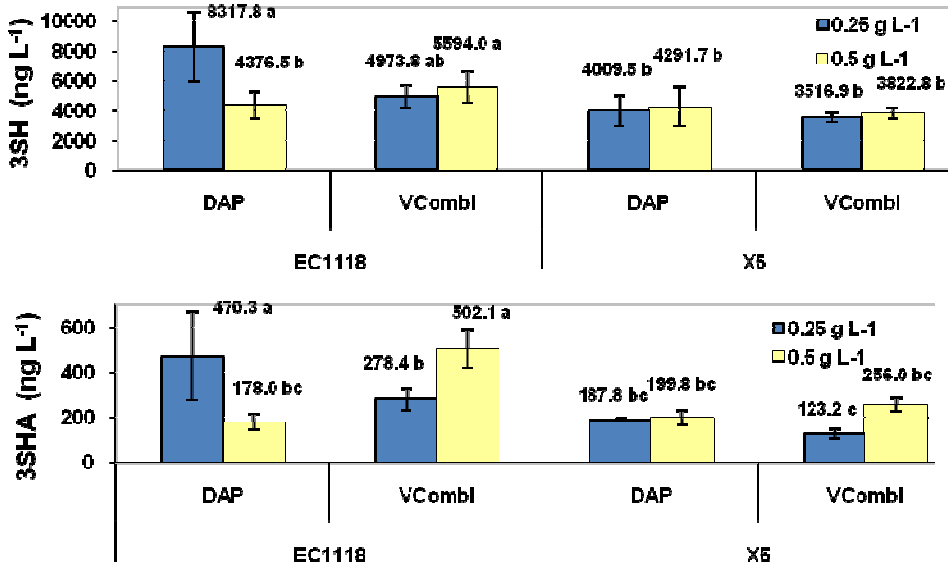

Figure 5. Concentration of 3SH and 3SHA detected in YPF wines produced by two yeast strains with the addition of two nutrient sources at two levels.

Vertical bars represent standard deviations from three fermentation replicates, except YPF juice obtained from two sample replicates. Means followed by different letters on the top of the bar are significantly different ($p<0.05$) among yeast strains according to the DMRT test.

Conclusion

It can be concluded that certain yeast strains and optimal nutrient supplementations had a great impact on minimizing the formation of SO_2-binding and undesirable sulphur compounds and improving desirable aroma compounds in final YPF wine products. Nevertheless, the choice of nutrient supplements and their concentrations is yeast strain-dependent. Nutrient supplements like

DAP plus thiamine at the levels normally recommended for grape wine production (0.5 g L^{-1}) were the best nutrient condition to improve the formation of SO_2-binding and aroma compounds in the YPF wines for most yeast strains studied. The EC1118 strain with the addition of 0.25 g L^{-1} DAP and 0.5 g L^{-1} DAP plus thiamine appeared to be a high producer of varietal volatile thiols, 3SH and 3SHA. Strain LittoLevure also formed the highest amounts of 3SHA in the 0.5 g L^{-1} DAP plus thiamine treatment.

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