Food Chemistry: X 5 (2020) 100072

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.journals.elsevier.com/food-chemistry-x

The impact of hybrid yeasts on the aroma profile of cool climate Riesling wines

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ARTICLE INFO

Chemical compounds studied in this article:
L-Malic acid (PubChem CID222656)
Sulphur dioxide (PubChem CID1119)
Isoamyl acetate (PubChem CID31276)
Hotrienol (PubChem CID5366264)
α – Terpineol (PubChem CID, 17100)
Citronellol (PubChem CID8842)
Linalool (PubChem CID6549)

Keywords:
Wine flavour
Aroma
Riesling
Hybrid yeasts
Saccharomyces cerevisiae var. *bayanus*
Saccharomyces kudriavzevii
Saccharomyces paradoxus
Monoterpenes

ABSTRACT

The current study highlights the effects of intra- and interspecific hybrid yeasts of the genus *Saccharomyces* (*S.*) on the alcoholic fermentation and formation of aroma compounds in cool climate Riesling wines. Three different hybrid yeasts: *S. cerevisiae* × *S. paradoxus* (SC × SP), *S. cerevisiae* × *S. kudriavzevii* (SC × SK) and *S. cerevisiae* var. *cerevisiae* × *S. cerevisiae* var. *bayanus* (SC × SB) were investigated. The species *S. cerevisiae* var. *bayanus* (SB) was chosen as control variant. It has been demonstrated that the hybrid yeasts have the ability to preserve positive properties while, suppressing undesired properties from the parental yeast species. The hybrid SC × SK showed an increase of desired acetate esters and monoterpenes. The concentrations of higher alcohols were higher in wines fermented by SC × SP, compared to the other variants. SC × SP fermentations resulted in decreased concentrations of L-malate and sulphites.

1. Introduction

Yeast and its metabolic products are important contributors to the sensory profile and consumers' preferences of wine. Increasingly, winemakers look for ways to optimise the sensory attributes of their wines in order to have a product differentiation and hence a competitive advantage.

During the second half of the 20th century, the use of selected yeasts of the species *Saccharomyces cerevisiae* var. *cerevisiae* as well as *var. bayanus* became widely accepted as a starter culture which reduces the risk of unwanted flavour compounds and ensures a high degree of fermentation (Benito et al., 2015). For some years, spontaneous fermentation and the use of unconventional yeasts has been increasingly used to achieve a more complex and unique wine style (Querol et al., 2018; Ruiz et al., 2019). The use of metabolic activities of certain yeast genera different from *Saccharomyces* may lead to new aromatic profiles with complex characteristics; nevertheless, among these genera, many are not able to properly perform an industrial fermentation process by themselves (Dittrich & Großmann, 2010). In order to counteract the problem of increased formation of sensory deficits through fermentation by non – *S. cerevisiae* yeasts and at the same time preserve individual positive aromas, genetic hybrids of *S. cerevisiae* and other *Saccharomyces* yeasts or isolated hybrids from the environment were bred (Querol et al., 2018).

The sexual reproduction of two yeast cells of the same genus but of different species results in a combination of the respective genomes. This type of hybrid is called interspecific. A representative is the bottom-fermenting brewer's yeast *Saccharomyces pastorianus* (syn. *S. carlsbergensis*), whose crossbreeding parental strains are believed to be *S. cerevisiae* and the cryotolerant *S. eubayanus* (Libkind et al., 2011;

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https://doi.org/10.1016/j.fochx.2019.100072
Received 1 May 2019; Received in revised form 22 November 2019; Accepted 24 November 2019
Available online 28 November 2019

Pérez-Través, Lopes, Querol, & Barrio, 2014; Vaughan-Martini & Martini, 2011). Hybrid yeasts that arise from the sexual reproduction of two *S. cerevisiae* yeasts are intraspecific hybrids (Dittrich & Großmann, 2010).

In the wine industry, the use of hybrid yeasts is less known. In the past, however, some natural hybrids have been isolated from vineyards, grapes, must and wine, which can now be purchased as assembled yeast preparations or serve as model organisms for the development of new hybrids (Borneman et al., 2012; González, Barrio, Gafner, & Querol, 2006). Hybridisation can either occur spontaneously or be constructed *in vitro* by methods like protoplast fusion and rare mating methods (Sipiczki, 2008).

The *in vitro* construction is a powerful tool that opens up the opportunity of creating genetic diversity without the use of – by definition – genetic engineering. Hereof the debates on genetically modified organisms (GMOs) in the food sector are to be mentioned. The use of GMOs experiences great resistance by consumers but also within the wine industry (Belda et al., 2017a), especially in traditional wine-growing countries such as France, Italy and Germany. Other than for instance in the United States of America within the European Union, the use of genetically modified yeasts is restricted. Wines containing GM yeasts need to be labeled accordingly (Benito, 2019; Regulation, 2008) and further increase the rejection by many consumers. Thus, it can be assumed that the application of GMO yeasts in the winemaking industry will not be accepted in the EU within the next few years (Gross, 2009; Pérez-Torrado, Barrio, & Querol, 2018). In this regard, hybrid yeasts could serve as an alternative.

Previous studies investigated the effect of different *Saccharomyces* yeasts, their hybrids and non – *Saccharomyces* yeasts on the fermentation and metabolic products of wine (Bellon et al., 2011; Benito et al., 2014, 2015; Ciani & Maccarelli, 1997; González et al., 2006; Majdak, Herjavec, Orlic, Redzepovic, & Mirosevic, 2002; Orlić, Redzepovic, Jeromel, Herjavec, & Iacumin, 2007; Romano, Suzzi, Comi, Zironi, & Maifreni, 1997; Stribny, Querol, & Pérez-Torrado, 2016; Swiegers et al., 2009). Strategies of inoculating *S. cerevisiae* together with non – *S. cerevisiae* yeasts resulted in mostly minor optimisation of the final wine flavour. This is due to the dominance of the *S. cerevisiae* which is characterised by a rapid cell propagation, tolerance to increased alcohol concentrations (up to ~15% vol) and osmotic stress tolerance (Bellon et al., 2011). On the other hand, sequential inoculation of non – *Saccharomyces* following *S. cerevisiae* can lead to significant differences in the aroma profile (Belda et al., 2017b; Benito et al., 2015).

Gamero, Manzanares, Querol, and Belloch (2011) performed small-scale fermentations of Muscat grape juice, indicating a dependency between the monoterpene concentration in the produced wine and the applied yeast. The resulting monoterpene concentrations would depend on the enzymatic activity of yeast to cleave the volatile, odour active compounds from their glycosidic bond by β-glucosidases. It was demonstrated that the yeast species *S. paradoxus* – which is the parent species of the hybrid SC × SP used in the present study – produces significantly higher concentrations of glycerol under conditions of increased concentrations of yeast available nitrogen source (YAN) and at the same time lower concentrations of volatile acidity than *S. cerevisiae* (Orlić et al., 2010). In several studies, *S. kudriavzevii* and its hybrids showed enhanced yields of the polyfunctional thiol 4-methyl-4-sulfanyl-pentan-2-ol (4 – MSP) in comparison to *S. cerevisiae* (Swiegers et al., 2009) which is described as tropical (passion fruit, guava). Polyfunctional thiols such as 4-MSP, 3-sulfanylhexanol (3-SH) and 3-sulfanylhexyl acetate (3-SHA) are of particular importance to the varietal character of grape varieties such as Sauvignon Blanc and Scheurebe. Since the main proportion of the mentioned compounds occur as cysteinylated, odourless precursors in the grape juice, enzymatic reactions are necessary to liberate the aroma active thiols. In order to enhance the concentrations of the polyfunctional thiols, non-*Saccharomyces* yeasts have been studied. *Torulaspora delbrueckii* has been proven to improve the thiol release due to its enhanced cysteine-S-conjugate cleaving β-lyase activity (Belda et al., 2017b). Therefore hybrid yeasts with similar properties may serve as a contributor to the typical varietal flavours of Sauvignon Blanc wine (Belda, Ruiz, Navascués, Marquina, & Santos, 2016).

The main objective was to examine the differential abilities of the hybrid yeasts to modulate the chemical composition of the resulting wine. Attention was paid to the quantification of volatile organic compounds (VOCs) that resulted from the fermentation trials and their comparison to the aroma profile of regular *S. cerevisiae* fermented Riesling wines. Furthermore, the fermentation kinetics and technological useful metabolic properties of hybrid yeasts were observed and discussed.

Hypothesis The expectation of enhanced desired VOCs for Riesling wine with simultaneous suppression of negative attributes resulting from the non – *S. cerevisiae* parent.

2. Materials and methods

2.1. Microorganisms

The strain EC 1118™ (Lallemand, Montréal, Canada) was selected as the control sample for the vinifications. It is a yeast of the species *S. cerevisiae* var. *bayanus* and is commonly used as a suitable comparative standard (Benito et al., 2015). It is characterised by a rapid and efficient fermentation performance, low formation of undesired by – products and increased tolerance towards low temperatures (~10 °C) and high alcohol concentrations. Table 1 shows the hybrid yeasts with different parents that were used for the trials. All yeasts were provided as commercial, granulated dry yeast, vacuum – packed in flexible aluminium composite film.

2.2. Vinification

For all fermentations, grape must of *Vitis vinifera* L. cultivar Riesling grapes grown at Hochschule Geisenheim University (Germany) (vintage 2017) was used. Constituent concentrations and conditions in the initial must were: total extract, 233.2 g L^{-1}; sugar, 208.3 g L^{-1}, pH, 3.36; yeast-assimilable nitrogen content (YAN), 211 mg L^{-1}; tartaric acid, 2.3 g L^{-1}; malic acid, 5.4 g L^{-1}; citric acid, 0.14 g L^{-1}; lactic acid < 0.1 g L^{-1}; acetic acid < 0.1 g L^{-1}; shikimic acid, 51 mg L^{-1}.

Using a microvinification method similar to that described by Benito et al. (2015), grape juice was sterilised by fine filtration (0.22 μm) and subsequent saturation with carbon dioxide gas and storage at a pressure of 600 kPa and a temperature of 0 °C. The grape juice

Table 1
The following selected, commercial yeast strains have been used to perform the fermentation. EC 1118™ serves as control; Exotics SPH™ is a diploid, interspecific hybrid yeast; VIN 7™ is an allotriploid, interspecific hybrid yeast; VIN 13™ is a diploid intraspecific hybrid yeast.

Product name	Brand (Company)	Yeast species	Nomenclature in figures/tables
EC 1118™	Lalvin (Lallemand)	*S. cerevisiae* var. *bayanus*	SB
Exotics SPH™	Anchor Yeast (Lallemand)	*S. cerevisiae* × *S. paradoxus*	SC × SP
VIN 7™	Anchor Yeast (Lallemand)	*S. cerevisiae* (diploid) × *S. kudriavzevii* (haploid)	SC × SK
VIN 13™	Anchor Yeast (Lallemand)	*S. cerevisiae* var. *cerevisiae* × *S. cerevisiae* var. *Bayanus*	SC × SB

was divided into 18 sterile glass vessels with a capacity of 2 L each. In order to ensure sufficient head space for the fermentation, the bottles were filled to a maximum of 1.8 L each. The inoculation of the rehydrated yeasts was carried out in triplicate (3 × 4 bottles) and was performed under strict aseptic conditions. To ensure a sufficient yeast population for an appropriate fermentation, the starter culture was set to a population of 1×10^6 cfu per mL of the commercial product. Due to the low YAN of ~ 210 mg L^{-1} in the grape must, the following yeast nutrient preparations were added: 0.4 g L^{-1} Fermaid E™ (Lallemand, Canada); 0.4 g L^{-1} LALVIN Go Ferm™ (Lallemand, Canada). Additionally, 0.3 g L^{-1} of the chitosan-based, antibiotic preparation Bactiless™ (Lallemand, Canada) was added to the grape must. All fermentation trials were carried out at 20 °C in a temperature-controlled room. Once the weight loss remained constant for 48 h, the wines were racked and stabilised for 7 days at 4 °C, concluding with the final product being bottled in 750 – mL bottles. Potassium metabisulfite ($K_2S_2O_5$) was added in order to achieve a concentration of 60 mg L^{-1} free sulphur dioxide. The bottles were sealed with aluminium screw caps and were placed in a climate chamber at 4 °C.

2.3. Analytical determinations

Fourier-Transform-Middle-Infrared-Spectroscopy (FT-MIR) was used to assess total extract, density, pH, glycerol and SO_2 in initial grape juice and wines. The method was used according to Baumgartner, Bill, and Roth (2001) and Patz, Kürbel, Dietrich, and Thente (1999) and the Standard Operating Procedure SOP-WG1-84 of the HGU's Department of Beverage Research.

Measurements of non-volatile organic acids, ethanol and residual sugars were performed by HPLC (High Performance Liquid Chromatography) according to Schneider, Gerbi, and Redoglia (1987) with modifications of Semmler, Sponholz, and Rauhut (2017). For this purpose, the '1100 Series' system by Agilent Technologies (Santa Clara, USA) equipped with an Allure Organic Acids column (Restek GmbH, Germany) (250 mm × 4.6 mm I.D. × 5 µm grain size × 60 Å pore size) preceded by a 4 mm × 3.0 mm I.D precolumn (Security Guard C18™, Phenomenex, Germany) was used. Detection was performed using a refractive index detector (RID) and a multi wavelength detector (MWD). The samples were analysed in scan mode.

The quantification of esters, higher alcohols and fatty acids were performed with a modified method of Rapp, Yavas, and Hastrich (1994) using a 'GC 5890 Series II' gas chromatograph (Hewlett-Packard, Palo Alto, USA). For the sample preparation, 2 g sodium chloride (Carl Roth, Karlsruhe, Germany) were weighed into a 15 mL sample vessel and 10 mL wine was added. 10 µL of the internal standard 2,6-dimethyl-5-hepten-2-ol (DMH) (stock concentration 1219 µg L^{-1}) (Carl Roth, Karlsruhe, Germany) was added for the quantification, 10 µL of the internal standard cumol (Honeywell, Morris Plains, USA) (stock concentration 170 µg L^{-1}) as control and 160 µL of 1,1,2-trichlorotrifluoroethane (Merck, Darmstadt, Germany) was added as extractant. The mixture was agitated for 20 min and centrifuged for 8 min (3000 rpm; 1700 *g*). The extract was removed with a glass pipette and transferred to a sample vessel for analysis. The cold feed system 'KAS 3′ (Gerstel, Mülheim an der Ruhr, Germany) was used for sample feeding. 2 µL of sample was injected in splitless mode (start temperature = 40 °C, heating rate: 3 °C min^{-1} to 125 °C, holding time: 4 min and 6 °C min^{-1} to 200 °C, holding time: 14.2 min). It was equipped with a Varian VF-5MS Agilent column (Santa Clara, USA) with the dimensions of 60 m × 320 µm × 1 µm. Helium (Linde Gas, Bingen, Germany) was used as carrier gas at a flow rate of 1 mL min^{-1}. The detection was performed by mass spectrometry ('5972 MSD', Hewlett-Packard) in scan mode covering a mass-to-charge ratio from *m/z* 35 to 250. Voltage of electron – impact was set at 70 mV. The analysis of the sulphur compounds was carried out using the method according to Rauhut, Beisert, Berres, Gawron-Scibek, and Kürbel (2005) with the modification according to Beisert and Rauhut (2017). Free monoterpenes and C13 – norisoprenoids were quantified using a HS-SPME-GC-MS according to Câmara, Alves, and Marques (2006) adapted by Brandt, Scheidweiler, Patz, Rauhut, Zorn, and Stoll (2018)

3. Statistical analysis

For the statistical evaluation of the data, the means and standard deviations of the wine sample triplicates were calculated. One way ANOVA and multiple range tests were performed using Statgraphics Centurion V17.2.05 software (Graphics Software Systems, Rockville, MD, USA). The significance level was set at $p < 0.05$. Multiple range test was used to compare and group the mean values of the variants according to the Fisher's Least Significant Difference (LSD) method. It is identified by letters a to f in the tables. A principal component analysis (PCA) was performed using Matlab (MathWorks Inc., Natick, MA, USA) version 9.5, PLS_Toolbox (MathWorks Inc.) version 8.6.2, and Statistics Toolbox (MathWorks inc.) Version 11.4.

4. Results and discussion

4.1. Fermentation kinetics

Fig. 1 shows the progress of the alcoholic fermentation of the different variants. It was measured by the method of weighing the fermentation vessels every 24 h. Since gaseous CO_2 escapes during the alcoholic fermentation, gravimetric measurements indicate the progress of sugar consumption and fermentation kinetics. The total weight loss varied between 98 g Lnorisoprenoids were quantified [1] (SC × SP) and 102 g L^{-1} (SC × SK). SC × SB and the reference strain SB started fermenting on day one after inoculation. The other yeasts showed the first weight loss 24 h later and hence had a delayed fermentation start. The main fermentation period of all samples went until day 7. From day 10, the daily weight losses for all variants were less than 2 g L^{-1} per day. SC × SK and SC × SB stopped fermenting on day 10. In contrast to SC × SK and SC × SB, the fermentation performed by SC × SP was completed on day 14. The kinetics of SB and SC × SB were similar, with the latter showing a slightly higher fermentation intensity. This could be explained by the fact that part of the genome of both yeasts originates from the species *S. cerevisiae* var. *bayanus*. It is known for its strong fermentation properties, thus its popularity for the production of sparkling wine.

4.2. Basic chemical parameters

Table 2 shows the basic chemical parameters of the studied wines. The final ethanol content is consistent with the observations mentioned concerning the fermentation kinetics. SC × SP had the lowest and SC × SK and SC × SB had the highest ethanol content, while SC × SP contained 8.3 g L^{-1} (± 1.0) residual sugars. SC × SK consumed 0.6 g L^{-1} more fructose than SB. This could indicate a higher fructophilic activity in the metabolism of *S. kudriavzevii* strains than *S. cerevisiae* var. *bayanus*.

All yeasts showed metabolic activity in consuming L-malic acid. The highest consumption of L-malic acid was recorded in SC × SP fermentations. The degradation of L-malic acid was likely due to a higher enzyme activity of the interspecific yeast hybrid SC × SP. Several studies demonstrated that fermentations with the species *S. paradoxus* lead to a degradation of malic acid (Majdak et al., 2002; Redzepovic et al., 2003). The enzyme malate dehydrogenase, catalysis an oxidative decarboxylation of L-malic acid to pyruvate and CO_2 in the presence of NAD^+ and Mg^{2+} or Mn^{2+} (Orlić et al., 2007).

The degradation of L-malic acid by SC × SP could be an alternative tool for the natural acid degradation without the need of lactic acid bacteria (LAB). The advantage over the heterofermentative LAB, i.e. *Oenococcus oeni*, which is popular for the degradation of acidity – is the reduced formation of undesirable fermentation by-products such as

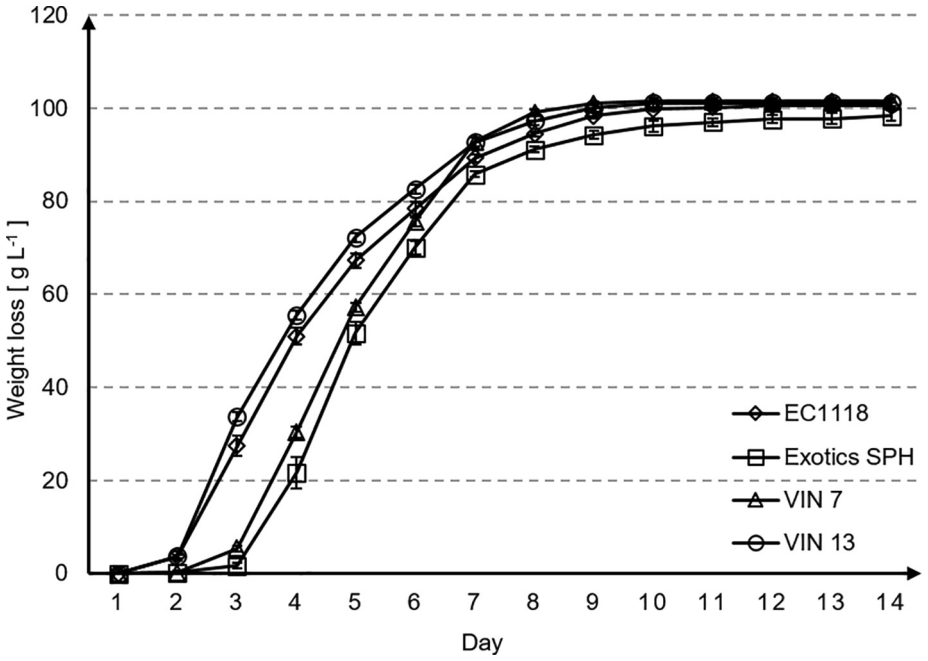

Fig. 1. Fermentation kinetics of the variants measured gravimetrically every 24 h by the total weight loss in the course of the fermentation. Mean values and standard deviations of the triplicate are shown.

acetic acid, acetoin, biogenic amines or diacetyl. In addition, the absence of LAB prevents contamination of other wines processed at the same production site.

Sulphur dioxide (SO_2) is commonly added to wine as preservative, but only the unbound, 'free' SO_2 is able to act toxic for microbiological cells and reductive in order to preserve the wine's aroma and colour. During fermentation, a high concentration of the metabolic intermediate compound acetaldehyde is present. It is a strong reaction partner of SO_2 and directly binds to the yeast's endogenous SO_2. Thus, it is undesirable that yeasts produce SO_2 since it cannot act as a preservative in the bound state (Osborne, Dubé Morneau, & Mira de Orduna, 2006). The analyses demonstrated distinct variations in the SO_2 formation among the yeasts. SB strain resulted in a wine with the highest concentration of total SO_2 with 34.7 mg L^{-1} (± 1.0). In contrast to SB, SC × SP had only 15 mg L^{-1} (± 1.0) of total SO_2. This could be due to the enzyme activity of the yeasts in reducing sulphate or to a lower production of combinable compounds such as acetaldehyde. For wines with a high content of endogenous total SO_2, it can be assumed that either a high activity of sulphate permease and ATP sulphurylase (sulphite formation) and/or low activity of sulphite reductase (sulphite degradation) existed (Heinzel, Dott, & Truper, 1979). Since SC × SP produced low concentrations of SO_2 during the alcoholic fermentation, it can be assumed that SC × SP hybrid yeasts possess the advantage of keeping the total SO_2 content low, prior to the addition of SO_2. This is of particular interest to wine producers aiming a 'clean label'. Wines that contain more than 10 mg L^{-1} of total SO_2 are required to bear the term 'contains sulphites' on the label, according to the EU legislation (Regulation (EC) No 1333/2008). The use of SC × SP for the alcoholic fermentation may be an advantage in achieving a concentration below the critical value.

Acetic acid was detected in concentrations equal or lower than 0.3 g L^{-1} which indicates the suppression of undesired production by the hybrid yeasts. Wines fermented with *S. kudriavzevii* showed enhanced concentrations of acetic acid in the final wine (González, Gallo, Climent, Barrio, & Querol, 2007).

Table 2
Final analysis of ethanol, sugar, organic acids, total SO_2 and pH – value from wines fermented by *S. cerevisiae* var. *bayanus* (SB), *S. cerevisiae* × *S. paradoxus* (SC × SP), *S. cerevisiae* × *S. kudriavzevii* (SC × SK) and *S. cerevisiae* var. *cerevisiae* × *S. cerevisiae* var. *bayanus* (SC × SB).

	SB	SC × SP	SC × SK	SC × SB
Ethanol [% *v/v*]	11.1 ± 0.1b	10.8 ± 0.1a	11.3 ± 0.1c	11.2 ± 0.1bc
Residual Sugars [g L^{-1}]	2.5 ± 0.2a	8.3 ± 1.0b	1.9 ± 0.0a	1.7 ± 0.1a
Glucose [g L^{-1}]	0.0 ± 0.0a	0.7 ± 0.6b	0.0 ± 0.0a	0.0 ± 0.0a
Fructose [g L^{-1}]	2.5 ± 0.2a	7.7 ± 1.5b	1.9 ± 0.0a	1.7 ± 0.1a
Glycerol [g L^{-1}]	8.6 ± 0.1c	8.7 ± 0.1c	7.7 ± 0.0a	8.2 ± 0.0b
Tartaric acid [g L^{-1}]	2.3 ± 0.1b	2.4 ± 0.0c	2.3 ± 0.0b	2.1 ± 0.0a
L-Malic acid [g L^{-1}]	4.4 ± 0.0d	3.6 ± 0.1a	4.2 ± 0.0b	4.3 ± 0.0c
Shikimic acid [mg L^{-1}]	45.6 ± 2.6a	47.4 ± 0.4a	46.7 ± 3.9a	47.1 ± 3.5a
L-Lactic acid [g L^{-1}]	0.2 ± 0.0a	0.3 ± 0.0b	0.2 ± 0.0a	0.2 ± 0.0a
Acetic acid [g L^{-1}]	0.1 ± 0.0a	0.2 ± 0.0b	0.3 ± 0.0c	0.1 ± 0.0a
Citric acid [g L^{-1}]	0.2 ± 0.0b	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0ab
Total SO_2 [mg L^{-1}]	34.7 ± 1.2c	15.3 ± 0.6a	21.7 ± 0.6b	21.7 ± 0.6b
pH	3.7 ± 0.0a	3.8 ± 0.0b	3.7 ± 0.0a	3.7 ± 0.0a

Shown are means and standard deviations of the fermentation triplicate; mean – values in the same row with the same letter are not significantly different from each other ($p < 0.05$).

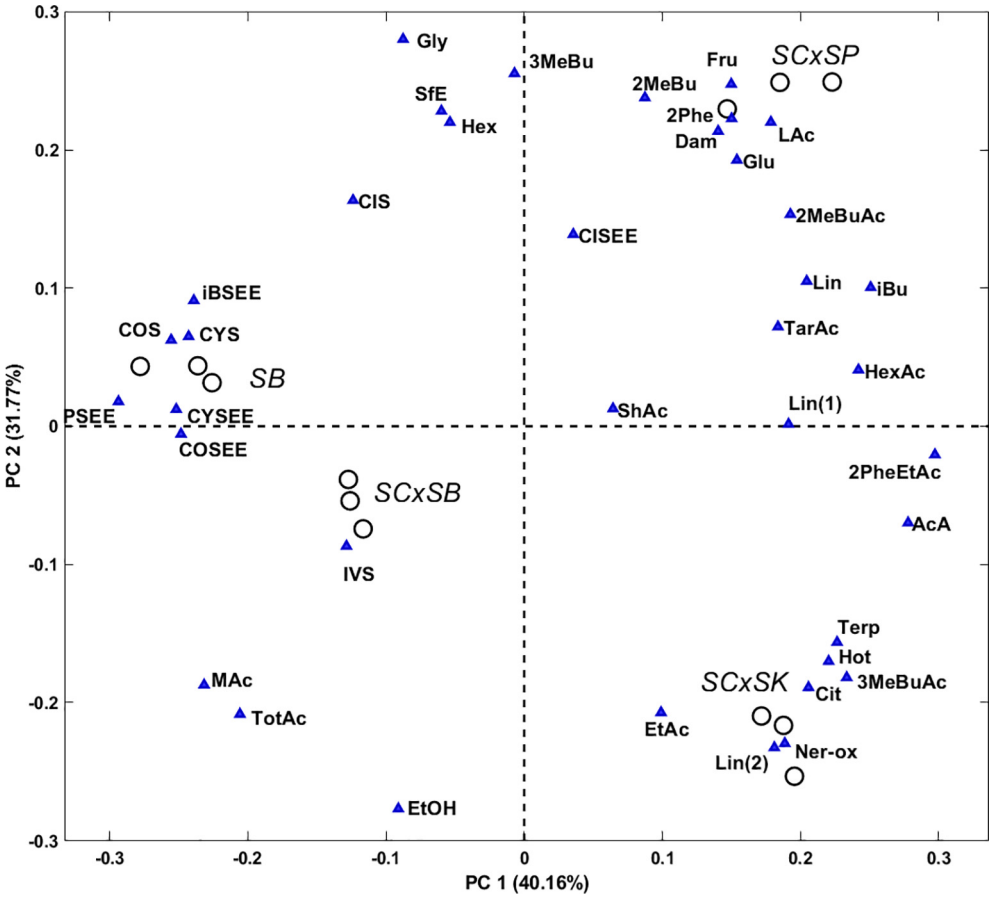

Fig. 2. PCA shows triplicates of yeast hybrids represented by solid circles (SB, SC × SP, SC × SK, SC × SB). Every yeast strain is located in a different quadrant indicating that there are significant differences among the fermentation products of the strains. PCA load shows differences between the variants (SB, SC × SP, SC × SK, SC × SB) regarding flavour formation. Substances in the same quadrant as respective yeast hybrid shows relatively high abundance in the wines fermented by the corresponding yeast hybrid. Abbreviations: Ethanol (EtOH), Sugar free extract (SfE), Glucose (Glu), Fructose (Fru), Glycerol (Gly), Total acidity (TotAc), Malic acid (MAc), Tartaric acid (TarAc), Lactic acid (LAc), Shikimic acid (ShAc), Acetic acid (AcA), Ethyl acetate (EtAc), Propionic acid ethyl ester (PSEE), 3-Methylbutanol (3MeBu), 2-Methylbutanol (2MeBu), isoButyric acid ethyl ester (iBSEE), Butyric acid ethyl ester (BSEE), Isovaleric acid (IVS), i-Butanol (i-Bu), 1-Hexanol (Hex), Isoamyl acetate (3MeBuAc), 2 – Methyl butyl acetate (2MeBuAc), Hexanoic acid (COS), Hexanoic acid ethyl ester (COSEE), Acetic acid hexyl ester (HexAc), 2-Phenylethanol (2Phe), Octanoic acid (CYS), Octanoic acid ethyl ester (CYSEE), 2 – Phenyl ethyl acetate (2PheEtAc), Decanoic acid ethyl ester (CISEE), Nerol oxide (Ner-ox), Linalool (Lin), Linalool oxide-1 (Lin (1)), Linalool oxide-2 (Lin (2)), Hotrienol (Hot), α – Terpineol (Terp), Citronellol (Cit), β – Damascenone (Dam).

4.3. Volatile compounds

The monitored yeast hybrids demonstrated the ability to modulate the wines' flavour profile during the alcoholic fermentation. A principal component analysis (PCA) (Fig. 2) identifies principal components based on the data of Table 3 and illustrates the major differences between the variants. The PCA plot (Fig. 2) of the first two principal components (PC1 and PC2) illustrating the association of measured variables of Riesling wine with treatments of the different hybrid yeasts explained 71.93% of the variation in the data. In general, the different hybrid yeasts treatments are separated and grouped reasonably well. Every treatment is located in a separate quadrant and variation between the triplicates is markedly low.

All analysed chemical components (Fig. 2) were pre-processed by auto scaling prior to the principal component analysis. The loadings (chemical variables) and scores (yeast treatments) were plotted together in a biplot. Fig. 2 gives a two-dimensional view of all analysed parameters together with the 4 × 3 fermentation trials (wines) of the Riesling must inoculated with different hybrid yeasts. The first principal component explained 40.16% and the second 31.77%, together 71.93% of the variation in the data set. The different wines are well separated in different quadrants of the biplot. This indicates that yeast fermentations with three replicates ended up with different chemical components in the wines and the variation between the triplicates is markedly low. The SB and SC × SB wines are close together, whereas the other wines are are oriented in the opposite direction to PC1. SC × SK and SC × SP wines show no correlation and are well separated in different quadrants, indicating a significant different chemical composition. The various composition of all wines fermented by different yeast can be seen by looking at each chemical component in the biplot.

SC × SK is an interspecific hybrid yeast of the species *S. cerevisiae* and *S. kudriavzevii*. The use of hybrids containing the genome of these species resulted in more aromatic wines (Bellon, Rose, Currie, & Bell, 2008; González et al., 2007).

In the current study SC × SK had the highest level of the sensory important acetate esters. Acetate esters contribute to the fruity olfactory impression. With a concentration of 80.6 mg L^{-1} (± 7.8), SC × SK had the highest content of ethyl acetate. Ethyl acetate is generally the most abundant ester present in wine. Its odour threshold is at ~12 mg L^{-1} in wine matrix (Waterhouse, Jeffery, & Sacks, 2016). At concentrations higher than 12 mg L^{-1}, it is perceived as pleasantly fruity. Between 90 mg L^{-1} and 150 mg L^{-1}, the odour is described as pungent and solvent-like (Plata, Millan, Mauricio, & Ortega, 2003). All variants (fermentations) had concentrations below the critical values. SC × SK produced the highest concentrations of 2 – phenyl ethyl acetate and isoamyl acetate (Table 3), which contribute to the fruity character in white wines (Francis & Newton, 2005). Previous studies on *S. kudriavzevii* and its hybrids substantiated similar results (Stribny et al., 2016). Ethyl esters were most abundant in the control variant SB. All variants had a content of higher alcohols below 350 mg L^{-1} (Table 3). In this concentration range, they are not yet perceived as unpleasant and contribute to the complexity of the sensory impression (Swiegers & Pretorius, 2005). SC × SK had low concentrations of higher alcohols and volatile fatty acids, compared to the other variants. The lower content of higher alcohols may be due to the enzymatic activity of alcohol acetyltransferase (Pérez-Torrado et al., 2018). The increased esterification by alcohol acetyltransferase reduced the concentration of the substrate, i.e. higher alcohols. SC × SP exhibited the highest content of higher alcohols, compared to the other variants. The most abundant aroma compounds produced by SC × SP are mainly derived from the amino acid metabolism of isoleucine, including i-butanol, 2-methylbutanol and its acetate ester 2 – methyl butyl acetate.

Notable concentration differences were evident among the monoterpenes (Fig. 3). The odorous compounds linalool, hotrienol, α-

Table 3
Volatile compounds measured after fermentation of SB, SC × SP, SC × SK and SC × SB; Abbreviations: not quantified (n.q.), not detected (n.d.). Values are.

	SB	SC × SP	SC × SK	SC × SB
Acetate esters				
Ethyl Acetate [mg L^{-1}]	65.7 ± 2.1a	59.6 ± 8.4a	80.6 ± 7.8b	60.2 ± 2.7a
Isoamyl acetate [μg L^{-1}]	4532.9 ± 108.9a	4991.8 ± 112.1b	5730.9 ± 52.2c	4924.2 ± 96.6b
2 – Methyl butyl acetate [μg L^{-1}]	204.2 ± 5.6a	342.8 ± 8.9d	255.6 ± 14.4b	288.0 ± 7.6c
Hexyl acetate [μg L^{-1}]	403.1 ± 3.5b	452.6 ± 22.5c	450.4 ± 1.2c	371.9 ± 20.0a
2 – Phenyl ethyl acetate [μg L^{-1}]	856.5 ± 26.5a	1276.3 ± 12.3c	1312.5 ± 30.7c	998.3 ± 28.6b
Σ Acetates [μg L^{-1}]	71713.3 ± 2222.1a	66633.5 ± 8556.8a	88389.4 ± 7861.0b	66764.0 ± 2835.9a
Ethyl esters				
Ethyl propanoate [μg L^{-1}]	89.6 ± 3.4c	50.3 ± 4.5a	49.0 ± 1.4a	73.0 ± 2.7b
Ethyl butanoate [μg L^{-1}]	497.6 ± 20.8c	429.1 ± 9.4b	339.5 ± 9.2a	551.5 ± 6.0d
Ethyl hexanoate [μg L^{-1}]	1394.6 ± 28.1c	873.8 ± 18.5a	951.3 ± 5.4b	984.7 ± 51.6b
Ethyl octanoate [μg L^{-1}]	1677.8 ± 47.5b	1298.5 ± 47.4a	1315.6 ± 53.1a	1429.8 ± 133.8a
Ethyl decanoate [μg L^{-1}]	645.9 ± 22.8a	663.8 ± 60.9a	613.2 ± 29.6a	595.7 ± 61.1a
Σ Ethyl esters [μg L^{-1}]	4305.4 ± 96.2c	3315.6 ± 119.5a	3268.5 ± 74.1a	3634.6 ± 252.1b
Higher alcohols				
i-Butanol [mg L^{-1}]	27.1 ± 0.9a	43.4 ± 2.5c	36.6 ± 0.9b	35.9 ± 1.0b
3-Methyl-butanol [mg L^{-1}]	145.5 ± 2.7b	149.2 ± 4.2b	134.7 ± 3.0a	136.1 ± 5.2a
2-Methyl-butanol [mg L^{-1}]	25.7 ± 1.1a	36.0 ± 2.1c	24.3 ± 0.2a	30.9 ± 1.4b
Hexanol [μg L^{-1}]	1940.2 ± 10.2b	1873.0 ± 24.9b	1425.3 ± 163.0a	1359.2 ± 158.5a
2-Phenyl-ethanol [mg L^{-1}]	49.2 ± 0.8a	80.6 ± 2.5c	52.2 ± 1.3a	62.2 ± 1.6b
Σ Higher alcohols [mg L^{-1}]	249.3 ± 3.7a	311.1 ± 7.7c	249.3 ± 4.0a	266.4 ± 8.6b
Fatty acids				
Isovaleric acid [μg L^{-1}]	1717.6 ± 8.0b	1664.2 ± 20.5a	1719.0 ± 31.3b	2104.5 ± 20.9c
Hexanoic acid [mg L^{-1}]	10.4 ± 0.4c	9.1 ± 0.3ab	8.8 ± 0.4a	9.7 ± 0.4b
Octanoic acid [mg L^{-1}]	14.7 ± 0.5b	11.2 ± 0.4a	10.7 ± 0.9a	11.6 ± 0.5a
Decanoic acid [mg L^{-1}]	7.1 ± 0.2c	6.1 ± 0.4b	5.1 ± 0.4a	5.0 ± 0.2a
Σ Fatty acids [mg L^{-1}]	34.0 ± 0.9c	28.1 ± 0.9ab	26.4 ± 1.5a	28.4 ± 0.3b
Monoterpenoids & C_{13} – norisoprenoids				
Linalool oxide-1 [μg L^{-1}]	2.5 ± 0.1a	2.6 ± 0.1a	2.7 ± 0.0a	2.5 ± 0.1a
Nerol oxide [μg L^{-1}]	1.3 ± 0.1a	1.5 ± 0.1ab	2.5 ± 0.1c	1.5 ± 0.1b
Linalool oxide-2 [μg L^{-1}]	7.3 ± 0.8a	8.3 ± 0.4ab	19.3 ± 0.6c	8.8 ± 0.5b
Linalool [μg L^{-1}]	13.0 ± 0.1a	18.1 ± 5.1c	15.6 ± 0.3ab	12.8 ± 0.1a
Hotrienol [μg L^{-1}]	21.9 ± 0.1a	84.6 ± 66.4a	259.9 ± 22.9b	22.2 ± 0.3a
α – Terpineol [μg L^{-1}]	6.9 ± 0.3a	30.6 ± 25.8a	84.0 ± 6.5b	6.8 ± 0.1a
Citronellol [μg L^{-1}]	2.7 ± 0.4ab	6.4 ± 4.5b	24.5 ± 1.5c	1.8 ± 0.1a
β – Damascenone [μg L^{-1}]	2.8 ± 0.3a	4.2 ± 1.1b	2.7 ± 0.0a	2.6 ± 0.1a
Σ **Monoterpenoids & C_{13} – norisoprenoids** [μg L^{-1}]	58.3 ± 2.3a	156.2 ± 97.8b	411.0 ± 31.5c	58.9 ± 0.4a
Sulphur Compounds				
Hydrogen sulphide [μg L^{-1}]	12.3 ± 1.3b	9.1 ± 1.0a	31.1 ± 3.4c	31.2 ± 1.2c
Methanethiol [μg L^{-1}]	1.9 ± 0.3a	n.q.	2.0 ± 0.1a	3.4 ± 0.1b

Shown are rounded means and standard deviations of the fermentation triplicate; means in the same row with the same letter are not significantly different from each other ($p < 0.05$).

terpineol and citronellol are major contributors to the floral and citrus-like odour of aromatic white wines (Strauss, Wilson, Gooley, & Williams, 1986). It is known that Riesling originates from the grape variety Weißer Heunisch (Gouais Blanc). The second parent is assumed to be Traminer crossed with the wild vine *Vitis sylvestris* (Regner, Stadlbauer, & Eisenheld, 2000). Traminer grapes generally contain high concentrations of several terpenes (Mateo & Jiménez, 2000). Hence, terpenes are present in Riesling grapes. The major terpenes in Riesling occur as odourless monoglucosides and disaccharide glycosides after fermentation, it is possible to achieve terpene – like notes in Riesling by application of certain yeasts to perform the alcoholic fermentation (Gamero et al., 2011). Depending on the enzymatic activities of the yeast strain, the potential to release terpenes from their odourless glycoconjugates varies. Hydrolysis can also be catalysed by acidic conditions and even induce molecular rearrangements of the monoterpenoid compounds (Williams, Strauss, Wilson, & Massy-Westropp, 1982). It has been demonstrated that terpenes not only derive from hydrolysis but can also be synthesised *de novo* by the yeast metabolism (Carrau et al., 2005; Gamero et al., 2011). The total content of terpenes in the wine fermented by SC × SK was nearly threefold in comparison to SC × SP and seven times higher than in SB and SC × SB (Fig. 3). The detected monoterpene hotrienol represented the main contributor and had a concentration of 259.9 μg L^{-1} (± 22.9) in variant SC × SK. The other wines had a low content of hotrienol and were below the odour threshold of about 110 μg L^{-1} (Simpson, 1979), while SC × SK had a value above. The PCA displays the results clearly. There is a grouping of the potent monoterpenes α – terpineol, hotrienol, citronellol, linalool oxide and nerol oxide in the same quadrant as SC × SK.

The current study gives rise to the suggestion that the monitored yeast hybrid SC × SK serves as a tool in order to achieve a statistically significant enhancement of the varietal character and a more complex aroma profile of Riesling wines. The results demonstrate high concentrations of terpenes in the wines fermented with SC × SK (Fig. 3 and Table 3). These results are in agreement with an earlier study in which genomic and transcriptomic analyses of VIN 7™ was performed (Gamero, Belloch, & Querol, 2015).

5. Conclusion

The current study demonstrated that hybrid yeasts had an impact on the aroma profile of Riesling wine. Three different hybrid yeasts were used for the alcoholic fermentation of German Riesling grape juice. The investigation proved major yeast – dependent modulations of the fermentative production of acetate esters, higher alcohols and monoterpenes – essential contributors to the overall olfactory impression of wine. Positive flavour attributes were enhanced in comparison to *S.*

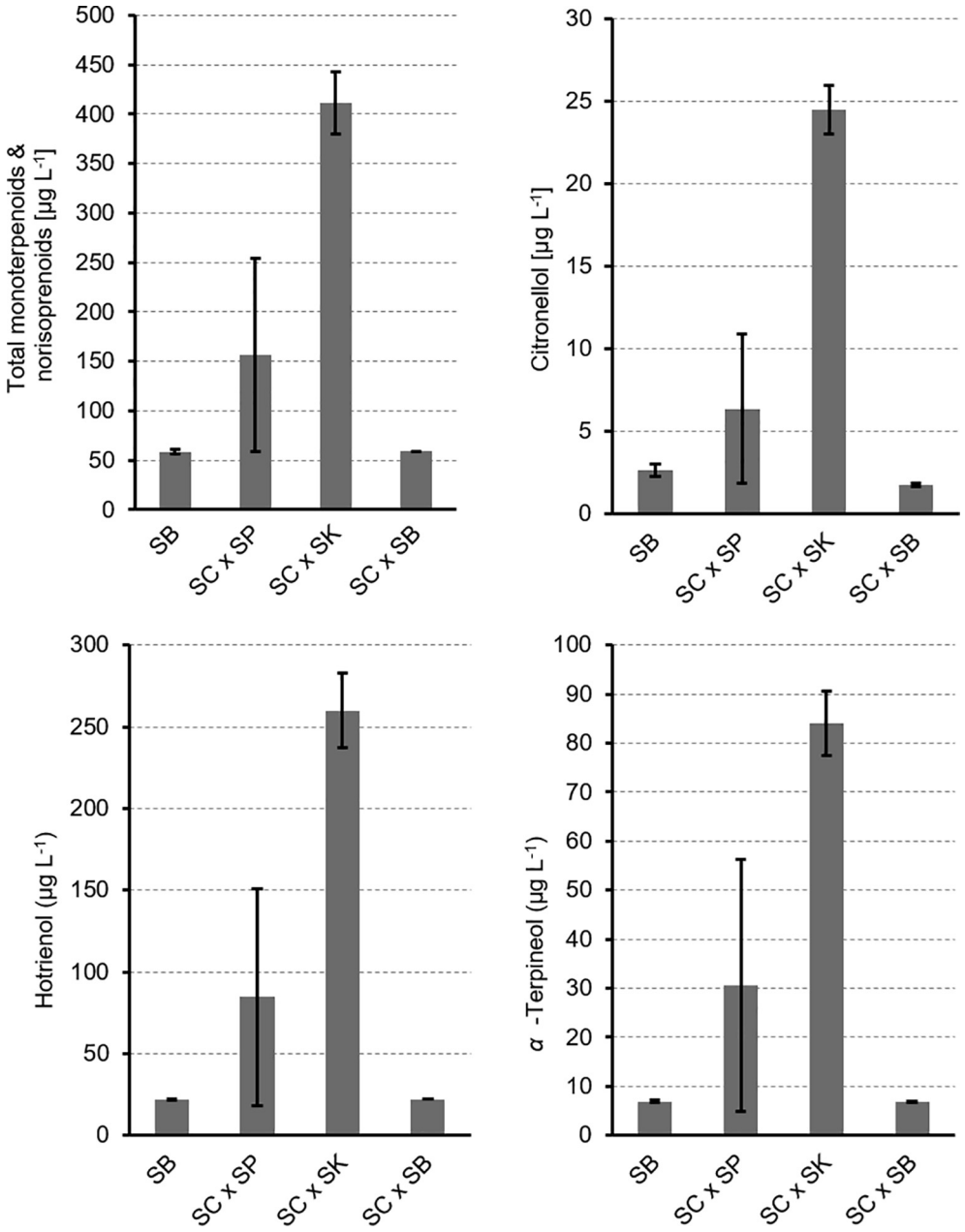

Fig. 3. Concentrations of total monoterpenoids and C13 – norisoprenoids, hotrienol, α-terpineol and citronellol detected in the wines fermented by SB, SC × SP, SC × SK and SC × SB.

cerevisiae var. *bayanus*, while specific negative properties – such as the production of acetic acid and sluggish fermentation – of the respective parent strain were suppressed. Additionally, the degradation of L-malic acid and reduced formation of sulphur dioxide by certain hybrid yeasts can serve as technological tools in the wine producing industry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Mrs Carmen Dost is gratefully acknowledged for her assistance to control the fermentation trial and Mrs Heike Semmler for her support in HPLC analysis (Hochschule Geisenheim University). Funding for Santiago Benito was provided by Ossian Vides y Vinos S. L under the framework of the project FPA1720300120 (Centre for the Development of Industrial Technology (CDTI), Spain).

Author Contributions

D.R., S.Be. and J-P.K. developed the experimental design; J-P.K. and S.Be. performed the vinifications; J-P.K. and S.Be. performed the formal data analysis and supervised the project; J-P.K., S.Be. and D.R. wrote the article; D.R., S.Br., B.B., and S.F. performed gas chromatographic analysis; C.-D.P. introduced to the FTIR analysis and assisted to data acquisition and statistical analysis. All authors discussed the results and contributed to the final manuscript.

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