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SPECIAL ISSUE

# Influence of *Starmerella lactis-condensi* inoculation on the aromatic behaviour of Austrian Grüner Veltliner wines

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## ABSTRACT

Over the past 20 years, a number of non-Saccharomyces strains have entered the market, promising various benefits for winemaking. Some strains have been reported to increase the aromatic diversity in wine. In the present study, the non-commercially available strain *Starmerella lactis-condensi* was tested for this purpose in the production of dry Grüner Veltliner wines. Two experiments were conducted. In the large-scale experiment, 200 L batches of pasteurised Grüner Veltliner must were fermented with *St. lactis-condensi*, *Saccharomyces cerevisiae* or both *St. lactis-condensi* and *S. cerevisiae* co-inoculation, with three replicates per vintage and variants over two vintages. The wines were characterised analytically, focusing on fermentation and primary aromas, and submitted to sensory analysis. In the small-scale experiment, 34 L of unpasteurised Grüner Veltliner must was compared with unpasteurised Pinot blanc must, using the same fermentation strategy as in the large-scale experiment. These wines were characterised with a focus on fermentation aromas. The results showed that *St. lactis-condensi* was able to ferment the musts dry, but the finished wines hat a lower concentration in thiols, rotundone, some esters and monoterpens and in terms of organoleptic properties, received fewer mentions for aroma descriptors. In comparison, the unpasteurised must fermented with *St. lactis-condensi* also showed lower concentrations of some free monoterpenes and minor esters but in general, fewer compounds were significantly affected. In contrast to the other aroma groups, it is noteworthy that the mixed varieties showed the highest concentrations of major ethyl esters in both pasteurised and unpasteurised fermentations. Since thiols, rotundone and minor esters are crucial for the aroma profile of Grüner Veltliner wines, the hypothesis that the non-commercial yeast *St. lactis-condensi* has an advantage over *S. cerevisiae* in producing particularly aromatic dry white wines can be rejected. Further research is recommended, especially regarding the influence of yeast selection on the rotundone content of Grüner Veltliner, which has been documented for the first time in this study.

**KEYWORDS:** non-*Saccharomyces*, rotundone, thiols, monoterpenes, fermentation aromas, IVAS 2024

## INTRODUCTION

Grüner Veltliner (GV) is Austria's most important grape variety; it is cultivated on 14,409 ha, which represents 32.4 % of the total area under vines in Austria (Österreich Wein, 2024). It is known for its spicy, fruity and sometimes tropical aromas. The sesquiterpene rotundone, fruity thiols and ester compounds are important aroma compounds for the typical taste of this grape variety (Nauer *et al.*, 2018; Philipp *et al.*, 2024b). Winemakers strive to intensify these aromas in wines and to produce even more typical wines.

Yeasts play a crucial role in aroma formation and the conversion of aroma precursors during alcoholic fermentation. Since the successful testing of pure culture yeasts and the favourable production of *Saccharomyces cerevisiae*, alcoholic fermentation on an industrial scale is typically performed with commercial active dry yeast (Ribéreau-Gayon *et al.*, 2000) strains. Over the years, *S. cerevisiae* strains have been extensively tested and characterised, resulting in the selection of strains with desirable oenological properties. In Austria, unlike in other countries, wine treatment agents are subject to additional general registration. Currently, there are about 600 different yeasts on the market (Federal College and Research Institute, 2024). While for decades only *S. cerevisiae* strains were available, there have been efforts to bring so-called non-Saccharomyces strains to the market for approximately 20 years.

The growing list of commercially available non-*Saccharomyces* yeasts includes *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Metschnikowia fructicola*, *Schizosaccharomyces pombe*, *Wickeranomyces anomalus*, *Kluyveromyces wickerhamii*, *Starmerella bacillaris*, *Zygosaccharomyces bailii*, *Zygosaccharomyces parabaili* and *Pichia kluyveri* (Vejarano *et al.*, 2021). These yeasts are used in winemaking for a variety of reasons, including bio-protection (*e.g.*, preventing the growth of Brettanomyces or other wild unwanted yeasts and bacterial strains), protecting against oxidation, increasing or reducing acidity, improving fermentation characteristics (*e.g.*, remedying stuck fermentations), reducing alcohol and/or improving flavour (Morata *et al.*, 2019; Petruzzi *et al.*, 2017; Roudil *et al.*, 2020; Vejarano *et al.*, 2021). At the same time, new strains are constantly being tested. *Starmerella lactis-condensi* (previously known as *Candida lactis-condensi*) is currently not on the market. It was isolated during the 2006 vintage from a refermentation to a Late Harvest wine from the winery owned by the monastery in Klosterneuburg, Austria. Since 2007, there has been a collaboration with Erbslöh GmbH to produce active dry yeast from St. lactis-condensi, but this endeavour has not yet been successful (Walter Kaltzin, 2009). The yeast has also been independently characterised by other institutes, mainly in the Tokaj region of Hungary. The yeast is considered to be osmotolerant as well as highly fructophilic and fermentative (Csoma *et al.*, 2020; Csoma *et al.*, 2023). There has been some research into the flavour-enhancing properties of this yeast. The use of *St. lactis-condensi* can have a positive effect on the fruitiness and floral aroma of sweet wines without causing any wine defects (Francesca *et al.*, 2024). However, this study has generally only looked at a few secondary metabolites such as higher alcohols, carboxylic acids and esters. At present, there are no available studies on the complete characterisation of aroma properties. In addition, the studies refer only to the production of sweet wines and not to dry white wines.

The present study tested the hypothesis that fermentation with *St. lactis-condensi* has a positive effect on the aroma of dry white GV wine, making the products more typical and the aroma more intense. Specifically, dry GV wine was produced on a practical scale by alcoholic fermentation with *St. lactis-condensi* and its aromatic characteristics were compared analytically with a focus on volatile substances and sensorially with wines fermented with *S. cerevisiae* or both *St. lactis-condensi* and *S. cerevisiae* (co-inoculation). The influence of two vintages and grape varieties (GV and Pinot blanc [PB]) were evaluated in two separate experiments.

## MATERIALS AND METHODS

### 1. Grape material

All grapes originated from the Agneshof experimental vineyard of the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, Austria (48° 17' 44" N, 16° 19' 31" E). The experiments were carried out at two different scales: 200 L (the large-scale experiment [LE]) and 34 L (the small-scale experiment [SE]). For the LE, GV grapes from the 2020 (harvest date: 30 September 2020) and 2021 (harvest date: 1 October 2021) vintages were used. For the SE, GV grapes harvested on 13 October 2020 and PB grapes harvested on 7 October 2020 were used. All grapes were free of visible mould and harvested by hand in 400 kg boxes. The basic parameters of the grapes are shown in Table 1. The parameters were measured by Fourier-transform infrared (FTIR) spectroscopy (Castellucci, 2010).

**TABLE 1.** Basic parameters of the grapes used in the experiments.

| Description | Sugar content (°Babo) | Titratable acid c.a. tartaric acid | pH | Total nitrogen (mg/l) |
|---|---|---|---|---|
| LE GV 2020 | 17.2 | 6.0 | 3.08 | 235 |
| LE GV 2021 | 19.4 | 6.8 | 3.23 | 320 |
| SE GV 2020 | 18.0 | 5.8 | 3.25 | 340 |
| SE PB 2020 | 18.2 | 7.3 | 3.13 | 318 |

*LE, large-scale experiment; GV, Grüner Veltliner; PB, Pinot blanc; SE, small-scale experiment.

## 2. Experimental design

### 2.1. The large-scale experiment

Grapes were pressed as whole bunches in a Willmes-Merlin press (Willmes GmbH, Lorsch, Germany) with a capacity of 3200 L. Before pressing, 50 mg/kg $SO_2$ (potassium pyrosulfite, Institut Oenologique de Champagne [IOC], Mardeuil, France) and 0.04 g/kg of pectinase (Vinozym FCE-G, Novazymes, Bagsværd, Denmark) were added. The must was transferred to a stainless-steel tank to settle overnight; the next day, the racked clear must was pasteurised at 75 °C for 1 min. After heating, everything in contact with the must (pipes, glass bulbs, steel tanks, pumps, etc.) was cleaned with hydrogen peroxide (TM Bisteril (concentration 2.0 % in water), Thonhauser GmbH, Giesshübl, Austria) and sodium hydroxide (TM Tartarex (concentration 2.0 % in water), Thonhauser GmbH). The heated must was distributed into nine 200 L steel tanks and three 54 L glass balloons. Table 2 lists the different variants and their replicates. The first variant (1A–1D) was inoculated with *St. lactis-condensi*, the second variant (2A–2D) was inoculated with *St. lactis-condensi* (a liquid supplied by Erbslöh GmbH, Geisenheim, Germany; it already contained a biological activator [Vitadrive F3, Erbslöh GmbH]) plus 1 g/100 L of *S. cerevisiae* (Önoferm Klosterneuburg, Erbslöh GmbH) and the third variant (3A–3D) was inoculated with *S. cerevisiae* (Önoferm Klosterneuburg, Erbslöh GmbH). The test was carried out in triplicate, and the fourth replicate (D) was carried out to top up the other three replicates after fermentation was complete. All results refer to the three replicates (A–C) in the 200-l tanks. The quantity of yeast used for the third variant was 20 g/100 L and 20 g/100 L of the biological activator (Vitadrive F3, Erbslöh GmbH). Both yeasts were slowly introduced into the must for 36 h by using two Yeastbutlers (Sitt GmbH, Lebring, Austria). In variant 2, *S. cerevisiae* was added 2 days after inoculation with *St. lactis-condensi*. The tanks were fermented in a temperature-controlled room (18 ± 1 °C) with sufficient air circulation. The progress of fermentation was monitored using FTIR spectroscopy (Castellucci, 2010). The fermentation curves are presented in Figures S1 and S2.

To check that the correct yeasts were carrying out fermentation, the three variants were subjected to microbiological testing on day 7 after yeast addition. Using the Resolution OIV-MA-AS4-01: R2010, the number of cells was estimated and the yeasts (International Organisation of Vine and Wine, 2010) were morphologically identified. The dilution method used Wallerstein nutrient agar (CM309, OxoidTM, Thermo Scientific, Waltham, USA), and the yeasts were observed under the microscope. At this time, the cell count was > $10^6$ in all three variants for both vintages, with a cell count of > $10^7$ for the 2020 and 2021 vintages of variant 3 and the 2020 vintage of variants 1 and 2. *St. lactis-condensi* and *S. cerevisiae* could be correctly assigned to the variants, with only *St. lactis-condensi* being found in variant 2 (Table S1).

At the end of fermentation, the wine from the glass container was used to top up the steel tanks, and 75 mg/L $SO_2$ was added. As the fermentation of the 2021 vintages of variants 1 and 2 took longer, the tanks were topped up after 21 days, and 75 mg/L $SO_2$ was added at the end of fermentation (day 96) (Figures S1 and S2). When fermentation had been completed for all variants from one year, 200 g/100 L bentonite (NaCalit, Erbslöh GmbH) was added. One week later, the wines were centrifuged, filtered and bottled in 34 L glass balloons. For tartaric stabilisation, the glass balloons were stored at –4 °C for 2 weeks. Then, the $SO_2$ levels were analysed; the free $SO_2$ level was set at 45 mg/l. The variants were filtered (0.3–0.5 µm; PILOT37046, Seitz, Pall/Filtra, Guntramsdorf, Austria) and bottled in 0.5-l bottles with screw caps. The 2020 wines were bottled in February 2021 and the 2021 wines were bottled in April 2022, and then stored at 4 °C until chemical and sensory analyses between June and September of the same year.

**TABLE 2.** The variants and yeast used for the large-scale experiment.

| Variants | Variant/replicate | Yeast | Volume (L) |
|---|---|---|---|
| Var 1 | LE GV/1A–1C | *Starmerella lactis-condensi* | 200 |
| | LE GV/1D | *Starmerella lactis-condensi* | 54 |
| Var 2 | LE GV/2A–2C | *Starmerella lactis-condensi* + 1 g/100 L *Saccharomyces cerevisiae* | 200 |
| | LE GV/2D | *Starmerella lactis-condensi* + 1 g/100 L *Saccharomyces cerevisiae* | 54 |
| Var 3 | LE GV/3A–3C | *Saccharomyces cerevisiae* | 200 |
| | LE GV/3D | *Saccharomyces cerevisiae* | 54 |

*LE, large-scale experiment; GV, Grüner Veltliner.

### 2.2. The small-scale experiment

The processing of grapes for SE was carried out in the same way as LE. The same variants were run, but the must was not heated. The fermentation process was not monitored by daily FTIR spectroscopic analyses, but only by a hand-held bending oscillator. When the bending oscillator measurements indicated the end of fermentation, FTIR spectroscopy was performed. If the residual sugar content was < 1 g/L, then the next steps were taken. The young wine was removed from the tank, centrifuged (SA1-01-175, Siebtechnik Zentrifugen West, Mühlheim an der Ruhr, Germany), pre-filtered with a sheet filter (PILOT37046, Seitz, Pall/Filtra) with 700 µm layers (Pall/Filtra) and added to 20 L glass balloons at the same time as sulphurisation (75 mg/L). Bentonite fining was not performed. After tartaric stabilisation, the wines were bottled in February 2021. These wines were only chemically characterised, focusing on the fermentation aromas and monoterpenes.

## 3. Analyses of the volatile profile by gas chromatography

A total of 89 volatile compounds—esters, higher alcohols, carboxylic acids, free monoterpenes, rotundone, thiols, C13-norisoprenoids, lactones, volatile phenols and carbonyl compounds –were analysed in the finished wines from the LE using eight different methods. The finished wines from the SE were analysed for a total of 64 volatile compounds—esters, higher alcohols, carboxylic acids, free monoterpenes and C13-norisoprenoids—using four different methods. A total of three different gas chromatographs (Agilent Technologies, Santa Clara, CA, USA) were used to analyse the different volatile compounds. The first system, consisting of a 6890 N GC instrument with a 5975 inert mass-selective detector (MSD) and an autosampler from CTC Analytics (Zwingen, Switzerland), was equipped with a ZB-Wax plus (length: 60 m, internal diameter 0.25 mm, df = 0.25 µm) from Phenomenex (Torrance, CA, USA) and was used to analyse the most important quantitative aroma compounds (higher alcohols, carboxylic acids, carbonyl compounds, some important ethyl esters [in the milligram per litre range] and C13-norisoprenoids). The second system, consisting of a 7890A GC instrument with a 5975C inert MSD with a triple-axis detector and a CTC Analytics autosampler, was used to analyse the minor ester compounds (in the microgram per litre range), free monoterpenes, lactones, volatile phenols and carbonyl compounds. This system was equipped with a ZB-5MS column (length: 60 m, internal diameter 0.25 mm, df = 0.25 µm) from Phenomenex. The third system comprised a 7890 B A instrument with an injector, controller and autosampler from CTC Analytics and a triple quad mass spectrometer (TQMS) detector (7010 B GC/MSMS Triple Quad) equipped with (a) a ZB-5MS column (length: 60 min, internal diameter 0.25 mm, df = 0.25 µm) from Phenomenex to analyse rotundone, and (b) a Zebron FFAP capillary column (length: 30 m, internal diameter 0.25 mm, df = 0.25 µm) from Phenomenex to analyse thiols.

The compounds were identified and quantified by calibration against analytical standards. Calibration was performed against an internal standard (in some cases labelled with deuterium), and the methods were validated. Information on calibration and validation can be found in Tables S2 and S3. All analyses were performed in duplicate except for the very complex thiol analyses, where every fifth sample was repeated. At least one calibration standard and one blank sample per day were included in each series of samples as a backup. Briefly, 14 relevant monoterpenes were quantified by headspace solid-phase microextraction-gas chromatography-selective ion monitoring-mass spectrometry (HS-SPME-GC-SIM-MS) according to a published method (Philipp *et al.*, 2020). Thirty-two ester compounds were determined by a partial stable isotope dilution assay (SIDA)-HS-SPME-GC-SIM-MS (Philipp *et al.*, 2019a). The major aroma compounds—relevant higher alcohols, relevant short and medium-chain carboxylic acids, carbonyl compounds and some major ester compounds—were quantified by a partial SIDA-HS-SPME-GC-MS method (Philipp *et al.*, 2019b). Lactones, volatile phenols and carbonyl compounds were quantified by solid phase extraction (SPE)-GC-MS according to a published method (Philipp *et al.*, 2020). Rotundone was analysed based on SPE followed by SPME determined by stable isotope dilution with multiple reaction monitoring-triple quadruple mass spectrometry (SPE-SPME-SIDA-GC-MRM-TQMS) developed by Nauer *et al.* (2018) and refined by Philipp *et al.* (2023). The thiols 3-sulfanylhexane-1-ol (3-SH), 3-sulfanylhexyl acetate (3SHA) and 4-methyl-4-sulfanylpentan-2-one (4-MSP) were quantified by GC-MRM-TQMS based on a published protocol (Coetzee *et al.*, 2018).

## 4. Sensory analysis

A sensory evaluation of the samples was performed to support the interpretation of the analytical results. The data were generated by a panel of experts and not by a panel trained in descriptive analysis. Only samples of the LE variants were analysed. All test samples were analysed using the check-all-that-apply (CATA) method. In this method, products are described using a pre-defined vocabulary. The tasters taste the samples and mark all the characteristics that apply to the product from a list of sensory terms. After tasting, the number of tasters who marked each characteristic is counted. The advantage of this method is that it is quick and the tasters do not need to be trained as in a descriptive panel (Derndorfer, 2020). The aroma descriptors used are fresh grass, tropical fruit, flowery, green apple, yellow fruit, black pepper, nut, herbal, citrus and honey. They are based on the selection by Nauer *et al.* (2018).

Prior to testing, a pooled sample was created from three replicates. The samples were given three-digit codes and tasted blind. Each wine was tasted twice by the tasters. The three wines per vintage were tasted together. Therefore, there were two rounds of three wines per vintage. The test was conducted with 26 tasters (19 males, aged 18–46 years (male), aged 18–50 years (female)). This resulted in 52 individual ratings per wine. The tasters were master's students in the Viticulture, Oenology and Wine Business programme at the University of Natural Resources and Applied Life Sciences in Vienna, employees of the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, students at the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, or winemakers. All tasters were official, certified wine tasters, were members of the sensory panel for Districtus Austriae Controllatus (DAC) Weinviertel (for GV wines), declared that they had been part of the panel at various wine tastings (including the Austrian Wine Challenge) and stated that they regularly consumed Austrian GV wines and were familiar with their typicality. The sensory analysis was carried out in an ISO 17025–accredited sensory laboratory and was conducted in accordance with the Declaration of Helsinki (1964). The environment in the tasting room was odourless and temperature-controlled and tasting cabins ensured anonymous tasting. The experiments were carried out with the knowledge and consent of the volunteers and were approved by the Ethics Committee of the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg.

## 5. Statistics

The statistical analyses were carried out using XLSTAT (Lumivera, Denver, CO, USA), SPSS Statistics 26.0 (IBM, Armonk, NY, USA) and Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Initially, the two vintages of the LE and the two varieties of the SE were analysed separately. Whether the variant had a significant influence on the volatile compounds was assessed. The analysis was carried out using analysis of variance (*F*-test), whereby, due to the large number of dependent variables tested, the results were corrected according to the Benjamin–Hochberg method. In the event of significance after correction, the Tukey-B *post hoc* test was used for multiple comparisons (Thissen *et al.*, 2002). Subsequently, the two experiments were evaluated using multivariate analysis (LE: vintage and variant as the factors, as well as their interaction; SE: cultivar and variant as the factors, as well as their interaction). Again, the Benjamin–Hochberg method was used for correction. To simplify the presentation of the results of the numerous substances, a principal component analysis (PCA) was carried out using XLSTAT; the results are presented with loading and scatter diagrams. The sensory results were also evaluated using a PCA, and the results are presented as a biplot.

# RESULTS

## 1. The large-scale experiment

Figure 1 shows the PCA of the analysed concentrations of volatile compounds of the different experimental wines in the LE. The replicates of the variants agree very well. Together, F1 (x-axis) and F2 (y-axis) explain 75.47 % of the variance (a good value). Of note, all variables have been used, including those that are not significant (Figure 1B). There is a clear separation by vintage. This separation occurs along F1, with the 2020 vintage wines having positive values on the x-axis and the 2021 vintage wines having negative values on the x-axis. The separation of the variants along F2 is also clearly visible. LE Var3 is in the positive area of the y-axis, LE Var1 is in the negative area of the y-axis and LE Var2 is exactly in the middle. This is true for both vintages, and the 2021 vintage allows an even clearer distinction.

The sum parameters of the aroma compounds are presented in Table 3, and the corresponding individual results are shown in Table S4. Both tables have a similar structure. First, the significant differences between the 2020 vintage variants are shown, followed by the differences between the 2021 vintage variants and then the multivariate analysis, where significant differences in yeast selection, vintage and the yeast selection × vintage interaction are indicated by asterisks.

For the 2020 vintage, there were significant differences in rotundone, the sum of C6 compounds, the sum of thiols, higher alcohols, aromatic esters, major ethyl esters and isoamyl esters with medium-chain carboxylic acids. Among these major aroma groups, except for the C6 compounds, the Var3 wines *(S. cerevisiae* control*)* always had the highest concentration, while the Var1 wines *(St. lactis-condensi* alone) had the lowest. The Var2 (*St. lactis-condensi* + *S. cerevisiae)* wines (mixed inoculation) were intermediate in terms of the concentrations. There were significant differences between the variants for 21 individual compounds. The 3-SH, 3-SHA, isobutanol, isovaleric acid, ethyl phenylacetate, ethyl butanoate, ethyl myristate, ethyl palmitate, isobutyl hexanoate, isobutyl octanoate, isoamyl isovalerate, isoamyl butyrate, isoamyl hexanoate and 5-acetoxymethyl-2-furaldehyde concentrations were significantly higher in LE Var3 2020 compared with LE Var1 2020. In contrast, the hexanol, ethyl acetate, delta-decalactone and o-cresol concentrations were significantly higher in LE Var1 2020 compared with LE Var3 2020. In general, LE Var2 2020 showed intermediate concentrations, except for guaiacol and butyric acid, for which LE Var1 2020 and LE Var3 2020 had significantly higher concentrations compared with LE Var2 2020.

For the 2021 vintage, there were significant differences in the sum of monoterpenes, the sum of thiols, the sum of carboxylic acids, the sum of aromatic esters, the sum of major ethyl esters, the sum of carbonyls and the sum of C13-norisoprenoids. Among these important aroma groups, the LE Var3 2021 wines always had the highest concentrations, except for the sum of the major ethyl esters. LE Var2 2021 presented intermediate concentrations, except for the sum of carbonyl compounds. There were significant differences between the variants for 23 individual compounds. The trans-linalool oxide, cis-linalool oxide, linalool, hotrienol, nerol oxide, alpha-terpineol, citronellol, geraniol, 4-MSP, 3-SH, 3SHA, decanoic acid, ethyl phenylacetate, diethyl succinate, furfural, guaiacol, 2,6-dimethoxyphenol, vitispiran and 1,1,4-trimethyl-1,2-dihydronaphthalene (TDN) concentrations were significantly higher in LE Var3 2021 compared with LE Var1 2021. In contrast, the syringaldehyde and 5-methylfurfural concentrations were significantly higher in LE Var1 2021 compared with LE Var3 2021. LE Var2 2021 generally showed intermediate or equal concentrations, except for 2,6-dimethoxyphenol, for which LE Var1 2021 and LE Var3 2021 showed significantly higher concentrations compared with LE Var2 2021, and nerol, for which LE Var1 2021 and LE Var3 2021 showed significantly lower concentrations compared with LE Var2 2021.

In the multivariate analysis, the effect of yeast selection was significant for 46 compounds, the effect of vintage was significant for 66 compounds, and the yeast selection × vintage interaction was significant for 12 compounds. Considering the sum parameters, there was a significant effect of yeast selection on the sum of monoterpenes, rotundone, the sum of C6 compounds, the sum of thiols, the sum of higher alcohols, the sum of aromatic esters, major ethyl esters, acetate esters, esters of higher alcohols and medium-chain carboxylic acids and isoamyl esters of medium chain carboxylic acids. For all sum parameters, except for the sum of monoterpenes, vintage had a significant influence. Finally, the yeast selection × vintage interaction was only significant for the sum of C6 compounds and the sum of carbonyl compounds.

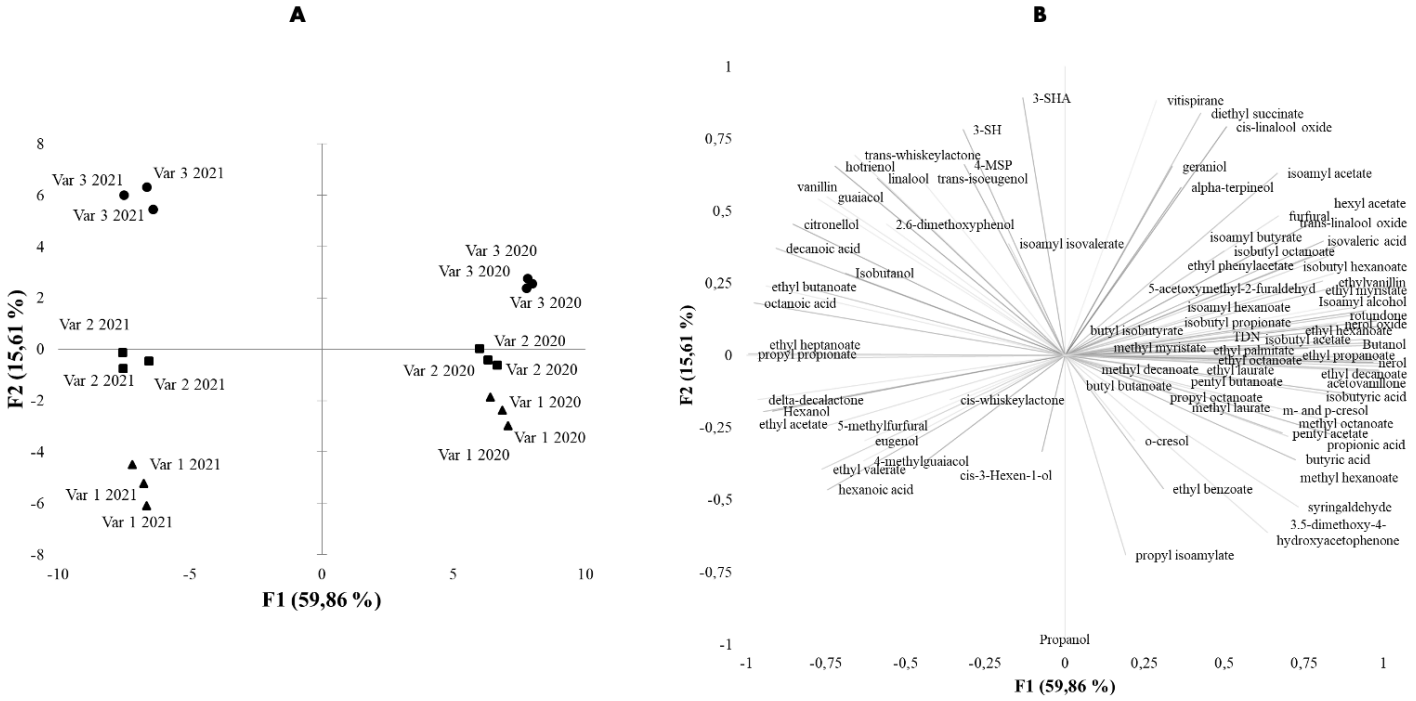



**FIGURE 1.** Principal component analysis of the large-scale experiment, with the (A) scatter plot and (B) loading plot. The concentrations of all significant and non-significant volatile compounds in the experimental wines of the vintages 2020 and 2021 were analysed for the scatter plot and the loading plot. Abbreviations: Var 1 (*St. lactis-condensi* alone), Var 2 (*St. lactis-condensi* + *S. cerevisiae)*, Var 3 (*S. cerevisiae control*).

## 2. The small-scale experiment

Figure 2 shows the PCA of the analysed concentrations of volatile compounds of the different experimental wines in the SE. Together, F1 (x-axis) and F2 (y-axis) explain 67.95 % of the variance (an acceptable value). It is worth noting that all variables have been used, including those that are not significant (Figure 2B). There is a clear separation based on the grape variety. This separation occurs along F1, with the GV wines taking positive values on the x-axis and the PB wines taking negative values on the x-axis. The variants are separated along F2: SE Var3 are in the positive area of the y-axis, while SE Var1 is in the negative area of the y-axis. The distinction between SE Var1 and SE Var2 is not possible for the PB wines and is only shown for the GV wines.

The sum parameters of the aroma compounds are shown in Table 4, and the corresponding individual results are presented in Table S5. Both tables have a similar structure. First, the significant differences between the variants of the GV 2020 wines are shown, followed by the differences between the variants of the PB 2020 wines and then the multivariate analysis, where significant differences in yeast selection, grape variety and the yeast selection × grape variety interaction are indicated by asterisks.

For the GV wines, there were significant differences in the sum of monoterpenes, the sum of higher alcohols and the major ethyl esters. Among these major aroma groups, the concentration of higher alcohols was significantly higher in SE Var3 GV compared with SE Var1 GV and SE Var2 GV. The concentration of monoterpenes was significantly higher for SE Var3 GV and SE Var2 GV compared with SE Var1 GV, and the concentration of major ethyl esters was significantly higher in SE Var1 GV and SE Var2 GV compared with SE Var3 GV. There were significant differences between the variants for only three individual compounds. The isoamyl alcohol and isobutanol concentrations were significantly higher in SE Var3 GV compared with SE Var1 GV. In contrast, the ethyl acetate concentration was significantly higher in SE Var1 GV compared with SE Var3 GV. SE Var2 GV showed significantly higher mean isobutanol and isoamyl alcohol concentrations. There was no significant difference between SE Var2 GV and SE Var1 GV and SE Var3 GV for ethyl acetate and no significant difference between SE Var2 GV and SE Var1 GV for isoamyl alcohol.

For the PB wines, there were significant differences in the sum of monoterpenes, the sum of major ethyl esters, the sum of esters of higher alcohols and medium-chain carboxylic acids, the sum of isoamyl esters of medium-chain carboxylic acids and the sum of methyl esters. Among these important aroma groups, except for the sum of esters of higher alcohols and medium-chain carboxylic acids and the sum of methyl esters, the SE Var3 PB wines always showed significantly higher concentrations compared with SE Var1 PB. In the case of methyl esters, SE Var1 PB showed the highest concentration, whereas SE Var2 PB showed the highest concentration of the sum esters of higher alcohols and medium-chain carboxylic acids. There were significant differences between the variants for 16 individual compounds. The cis-linalool oxide, nerol oxide, alpha-terpineol, geraniol, isobutanol, butyric acid, diethyl succinate, isobutyl acetate, hexyl acetate, ethyl

**TABLE 3.** The sum parameters for the large-scale experiment.

| | LE GV Var1 2020 | LE GV Var2 2020 | LE GV Var3 2020 | LE GV Var1 2021 | LE GV Var2 2021 | LE GV Var3 2021 | Yeast | Vintage | Yeast × vintage |
|---|---|---|---|---|---|---|---|---|---|
| Sum of free monoterpenes (µg/L) | 24.5a ± 1.9 | 23.0a ± 0.7 | 23.7a ± 0.8 | 17.3a ± 1.1 | 18.8a ± 0.1 | 30.8b ± 1.6 | * | | |
| Rotundone (ng/L) | 28a ± 1 | 33b ± 1 | 39c ± 1 | 5.2a ± 0.2 | 6.6a ± 0.7 | 7.5a ± 0.4 | * | * | |
| Sum of C6 compounds (mg/L) | 0.62c ± 0.0 | 0.59b ± 0.02 | 0.48a ± 0.01 | 0.77a ± 0.01 | 0.74a ± 0.02 | 0.74a ± 0.02 | * | * | |
| Sum of thiols (ng/L) | 223a ± 7 | 345b ± 2 | 448c ± 6 | 277a ± 14 | 398b ± 10 | 472c ± 18 | * | * | |
| Sum of higher alcohols (mg/L) | 140.21a ± 8.42 | 143.04a ± 5.37 | 224.33b ± 25.40 | 66.88a ± 2.14 | 73.04a ± 8.98 | 76.51a ± 5.38 | * | * | |
| Sum of carbon acids (mg/L) | 23.26a ± 1.65 | 22.50a ± 0.57 | 22.82a ± 0.84 | 41.87a ± 1.78 | 46.92b ± 1.92 | 57.80c ± 1.21 | | * | * |
| Sum of aromatic esters (µg/L) | 4.0a ± 0.2 | 4.6a ± 0.3 | 6.6b ± 0.4 | 1.7a ± 0.1 | 1.8a ± 0.3 | 3.0b ± 0.2 | * | * | |
| Sum of major ethyl esters (mg/L) | 41.83b ± 1.78 | 44.55b ± 0.24 | 31.75a ± 1.86 | 63.75b ± 1.79 | 65.32b ± 5.64 | 59.58a ± 1.34 | * | * | |
| Sum of acetate esters of higher alcohols (µg/L) | 1.034.7a ± 36.3 | 1.040.9a ± 116.0 | 1.275.7a ± 31.6 | 645.0a ± 31.7 | 716.7a ± 38.2 | 1.035.7a ± 122.5 | * | * | |
| Sum of ethyl esters of carboxylic acids with an even number of carbon atoms (µg/L) | 3.762.1a ± 75.5 | 3.777.6a ± 139.5 | 3.835.0a ± 205.1 | 1.467.6a ± 58.1 | 1.479.1a ± 29.2 | 1.486.2a ± 112.6 | | * | |
| Sum of esters of higher alcohols and medium-length chain carboxylic acids (µg/L) | 29.4a ± 0.2 | 29.5a ± 0.4 | 30.2a ± 0.2 | 26.0a ± 0.2 | 25.4a ± 0.2 | 26.3a ± 0.4 | * | * | |
| Sum of ethyl esters of carboxylic acids with an odd number of carbon atoms (µg/L) | 2.7a ± 0.2 | 2.6a ± 0.0 | 2.7a ± 0.0 | 3.8a ± 0.3 | 3.4a ± 0.2 | 3.2a ± 0.3 | | * | |
| Sum of ester of branched carboxylic acids (µg/L) | 8.8a ± 0.7 | 8.6a ± 0.6 | 8.2a ± 0.4 | 2.4a ± 0.1 | 2.0a ± 0.2 | 2.2a ± 0.3 | | * | |
| Sum of isoamyl ester of medium-long chain carboxylic acids (µg/L) | 1.8a ± 0.1 | 1.9a ± 0.1 | 3.1b ± 0.2 | 0.5a ± 0.0 | 0.5a ± 0.0 | 0.6a ± 0.1 | * | * | |
| Sum of methyl esters (µg/L) | 8.2a ± 0.3 | 7.9a ± 0.2 | 8.0a ± 0.2 | 3.5a ± 0.3 | 3.3a ± 0.4 | 3.1a ± 0.3 | | * | |
| Sum of minor esters (µg/L) | 4.851.7a ± 106.6 | 4.873.7a ± 242.2 | 5.169.4a ± 232.0 | 2.150.4a ± 63.4 | 2.232.2a ± 58.6 | 2.560.5a ± 235.1 | | * | |
| Sum of carbonyl compounds (µg/L) | 26.7a ± 1.7 | 27.0a ± 1.4 | 27.1a ± 1.2 | 17.0b ± 1.3 | 11.6a ± 1.1 | 24.8c ± 1.8 | | * | * |
| Sum of lactones (µg/L) | 2.9a ± 0.2 | 2.8a ± 0.2 | 3.1a ± 0.1 | 12.0a ± 1.4 | 11.5a ± 1.8 | 11.0a ± 0.9 | | * | |
| Sum of volatile phenols (µg/L) | 32.7a ± 2.4 | 28.5a ± 2.2 | 34.9a ± 3.0 | 19.3a ± 1.7 | 19.8a ± 0.9 | 23.6a ± 0.4 | | * | |
| Sum of C13-norisoprenoids (µg/L) | 3.4a ± 0.1 | 3.5a ± 0.1 | 3.7a ± 0.1 | 2.4a ± 0.1 | 2.6a ± 0.1 | 3.6b ± 0.1 | | * | |

*Significant differences are indicated by different letters in the same line (based on Tukey's *B*-test with the Benjamin–Hochberg correction, $\alpha = 0.05$ for three degrees of freedom), with the results for the 2020 and 2021 vintages marked separately. Significant differences in the multivariate analysis are marked with an asterisk (Benjamin–Hochberg correction, $\alpha = 0.05$).

butanoate, isobutyl octanoate, isoamyl butyrate and isoamyl hexanoate concentrations were significantly higher in SE Var3 PB compared with SE Var3 PB. In contrast, the butanol, propyl propionate and methyl myristate concentrations were significantly higher in SE Var1 PB compared with SE Var3 PB. In general, SE Var2 PB showed intermediate or equal concentrations.

In terms of multivariate analysis, the effect of yeast selection was significant for 24 compounds, the effect of grape variety was significant for 44 compounds, and the yeast selection × grape variety interaction was significant for 18 compounds. Considering the sum parameters, yeast selection had a significant influence on the sum of monoterpenes, the sum of C6 compounds, the sum of higher alcohols, the sum of major ethyl esters, the sum of isoamyl esters of medium-long chain carboxylic acids and the sum of methyl esters. The grape variety had a significant influence on all sum parameters except the sum of esters of branched carboxylic acids. Finally, the yeast selection × grape variety interaction was significant for the sum of esters of higher alcohols and medium-length chain carboxylic acids, the sum of major ethyl esters and the sum of higher alcohols.

### 3. Sensory analysis

Figure 3 shows the PCA of the sensory evaluation. The raw CATA data can be found in Table S6. Together, F1 and F2 explain 69.38 % of the variance (a good value). F1 explains the differences between the two vintages, while F2 explains the differences between the variants. The 2020 wines have higher values for black pepper (2020: 71; 2021: 41), while the 2021 wines have more yellow fruit (2020: 41; 2021: 68), tropical fruit (2020: 45; 2021: 58), floral aromas (2020: 33; 2021: 47) and nutty aromas (2020: 0; 2021: 19). The data suggest small sensory characteristics of the different yeasts, with the Var1 and Var2 wines showing lower scores for tropical fruit (Var 1: 24; Var 2: 26; Var 3: 43 judgments) and black pepper (Var 1: 35; Var 2: 32; Var 3: 45 judgments), while Var1 also showed low scores for the floral attribute. Overall, the Var1 and Var2 wines show fewer attribute mentions than the Var3 wines.

## DISCUSSION

### 1. Do the data support the hypothesis?

Based on the results, the hypothesis that fermentation with *St. lactis-condensi* intensifies and positively influences the aroma of dry white GV wine cannot be confirmed. Table 5 shows a summary of the results with the rates of increase in the most important aroma groups. The sesquiterpene rotundone, which is important for the typicality of GV wines and is responsible for their spicy, peppery taste (Nauer *et al.*, 2018), was rather negatively influenced by fermentation with *St. lactis-condensi*. On average, the varieties fermented with *S. cerevisiae* had approximately 40 % more rotundone than the wines fermented with *St. lactis-condensi*. In addition, the levels of fruity thiols, which are responsible for the typical fruity, tropical flavour of the wines (Philipp *et al.*, 2024b), were even more significantly reduced (up to 100 %) in the wines fermented with *St. lactis-condensi*. However, the results for the fermentation aromas were less clear. While the major

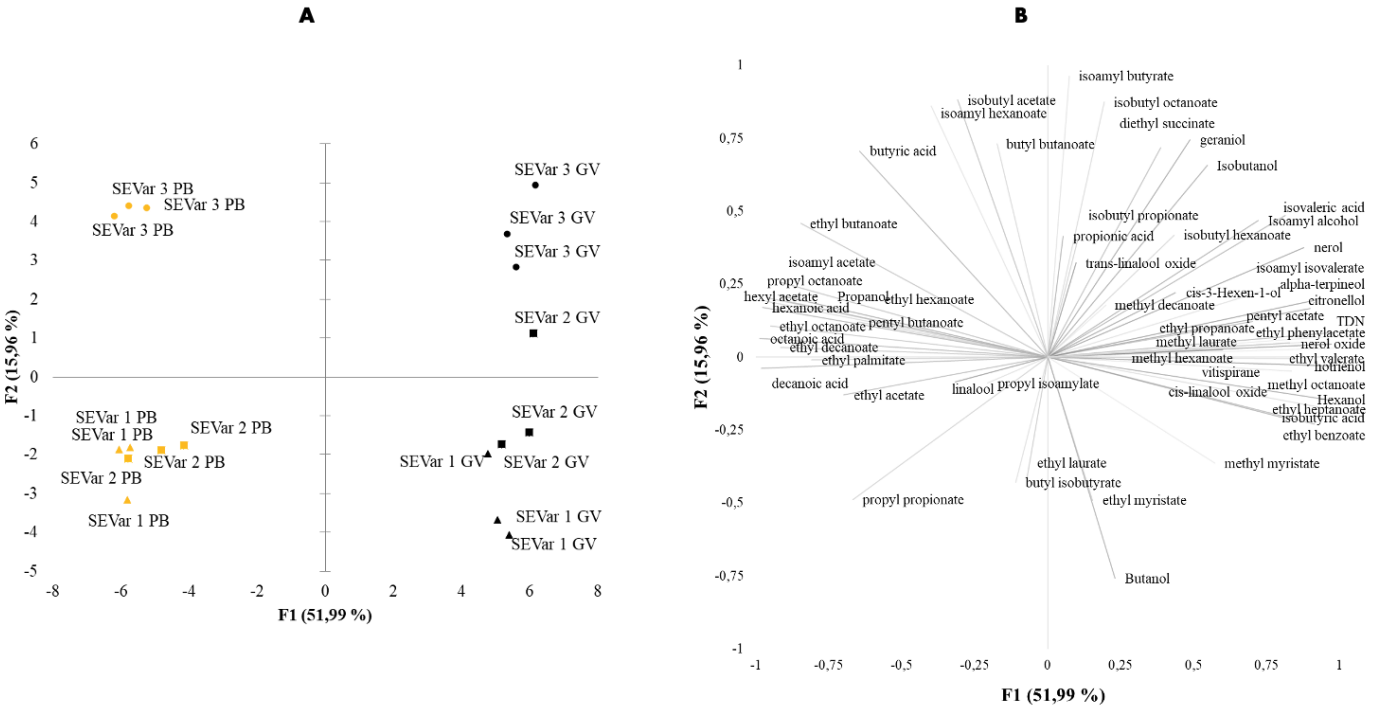



**FIGURE 2.** Principal component analysis of the small-scale experiment, showing the (A) scatter plot and (B) loading plot. The concentrations of all significant and not significant volatile compounds in the experimental wines of the vintages 2020 and 2021 were analysed for the scatter plot and the loading plot. Abbreviations: Var 1 (*St. lactis-condensi* alone), Var 2 (*St. lactis-condensi + S. cerevisiae*), Var 3 (*S. cerevisiae control*).

**TABLE 4.** The sum parameter for the small-scale experiment.

| | SE GV Var1 2020 | SE GV Var2 2020 | SE GV Var3 2020 | SE PB Var1 2020 | SE PB Var2 2020 | SE PB Var2 2020 | Yeast | Cultivar | Yeast × Cultivar |
|---|---|---|---|---|---|---|---|---|---|
| Sum of free monoterpenes (µg/L) | 22.0a ± 1.1 | 27.3b ± 2.5 | 28.8b ± 1.7 | 14.1a ± 0.9 | 15.8a ± 1.0 | 20.0b ± 0.7 | * | * | |
| Sum of C6 compounds (mg/L) | 1a ± 0.0 | 1a ± 0.0 | 1a ± 0.0 | 0.31a ± 0.02 | 0.32a ± 0.02 | 0.25a ± 0.01 | | * | |
| Sum of higher alcohols (mg/L) | 167.57a ± 7.20 | 199.26a ± 18.62 | 330.30b ± 23.28 | 143.34a ± 6.50 | 131.96a ± 5.90 | 143.17a ± 3.82 | * | * | * |
| Sum of carbon acids (mg/L) | 17.96a ± 1.43 | 17.22a ± 0.81 | 17.67a ± 0.88 | 24.68a ± 0.50 | 23.71a ± 0.71 | 25.58a ± 1.20 | | * | |
| Sum of aromatic esters (µg/L) | 9.7a ± 0.8 | 10.9a ± 1.2 | 10.9a ± 0.2 | 2.6a ± 0.1 | 3.1a ± 0.4 | 3.2a ± 0.3 | | * | |
| Sum of major ethyl esters (mg/L) | 45.75b ± 1.95 | 40.67b ± 3.99 | 37.55a ± 0.26 | 43.47a ± 1.92 | 51.69b ± 1.51 | 50.82a ± 1.71 | * | * | * |
| Sum of acetate esters of higher alcohols (µg/L) | 955.4a ± 104.2 | 1098.3a ± 115.3 | 1247.7a ± 93.4 | 1743.3a ± 187.8 | 1585.0a ± 71.3 | 1754.0a ± 27.1 | | * | |
| Sum of ethyl esters of carboxylic acids with an even number of carbon atoms (µg/L) | 3596.8a ± 354.9 | 3235.2a ± 253.0 | 3189.5a ± 101.7 | 5061.6a ± 61.6 | 4642.2a ± 373.0 | 5508.8a ± 290.9 | | * | |
| Sum of esters of higher alcohols and medium-length chain carboxylic acids (µg/L) | 28.6a ± 0.3 | 28.5a ± 0.4 | 29.6a ± 0.5 | 31.4b ± 0.1 | 31.8c ± 0.2 | 29.6a ± 0.0 | | * | * |
| Sum of ethyl esters of carboxylic acids with an odd number of carbon atoms (µg/L) | 3.0a ± 0.1 | 3.1a ± 0.3 | 3.0a ± 0.1 | 2.3a ± 0.1 | 2.4a ± 0.1 | 2.4a ± 0.1 | | * | |
| Sum of ester of branched carboxylic acids (µg/L) | 8.2a ± 0.3 | 7.9a ± 0.7 | 7.9a ± 0.2 | 8.2a ± 0.3 | 7.9a ± 0.3 | 7.9a ± 0.1 | | | |
| Sum of isoamyl ester of medium-long chain carboxylic acids (µg/L) | 2.1a ± 0.1 | 2.4a ± 0.3 | 2.9a ± 0.2 | 2.5a ± 0.0 | 2.4a ± 0.1 | 3.4b ± 0.1 | * | * | |
| Sum of methyl esters (µg/L) | 8.0a ± 0.5 | 7.1a ± 0.1 | 7.9a ± 0.2 | 7.3b ± 0.1 | 6.6a ± 0.1 | 6.5a ± 0.0 | * | * | |
| Sum of minor esters (µg/L) | 4611.9a ± 459.2 | 4393.4a ± 259.9 | 4499.5a ± 109.8 | 6859.2a ± 164.2 | 6281.4a ± 374.7 | 7315.6a ± 272.4 | | * | |
| Sum of C13-norisoprenoids (µg/L) | 3.7a ± 0.1 | 3.9a ± 0.0 | 3.9a ± 0.0 | 0.7a ± 0.0 | 0.7a ± 0.0 | 0.3a ± 0.0 | | * | |

Significant differences are indicated by different letters in the line (based on Tukey's *B*-test with the Benjamin–Hochberg correction, $\alpha = 0.05$ for three degrees of freedom), with the results for the grape varieties (Grüner Veltliner [GV] and Pinot blanc [PB]) marked separately. Significant differences in the multivariate analysis are marked with an asterisk (Benjamin–Hochberg correction, $\alpha = 0.05$).

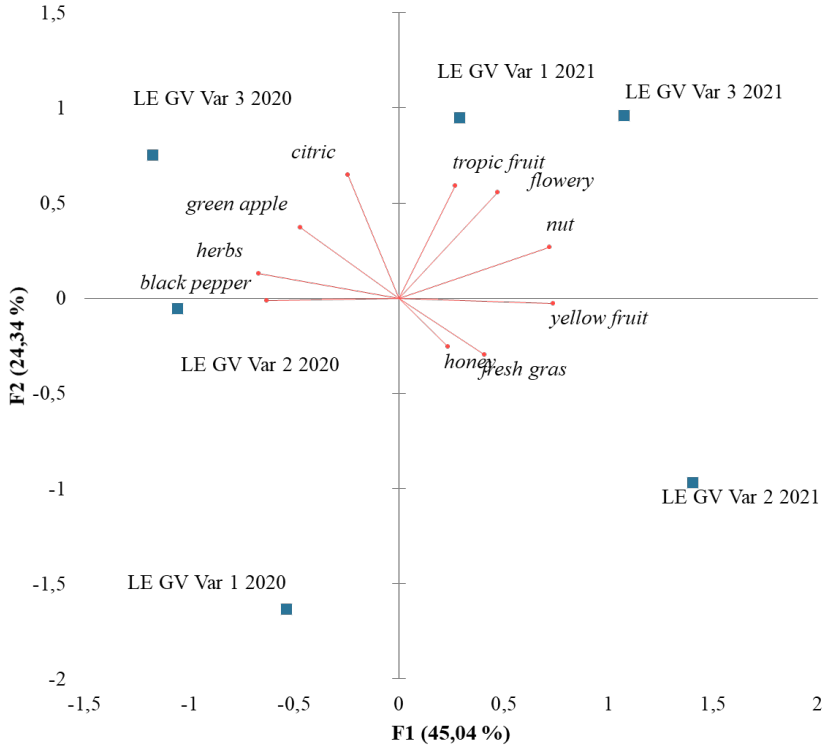



**FIGURE 3.** Biplot of the principal component analysis of the sensory data of the large-scale experiment. The biplot is based on the sensory CATA analysis of the LE. The raw CATA data can be found in supplementary data Abbreviations. Var 1 (*St. lactis-condensi* alone), Var 2 (*St. lactis-condensi + S. cerevisiae),* Var 3 *(S. cerevisiae control).*

ethyl esters were highest in the varieties fermented with both *St. lactis-condensi* and *S. cerevisiae*, except for the GV wines in the SE, the varieties fermented with *S. cerevisiae* always achieved the highest concentrations of minor esters, although the differences were rather smaller. Except for the GV wines from the SE, the wines fermented with *S. cerevisiae* also had the highest monoterpene concentrations, with some showing an increase of up to 78 %. The analytical results are also supported by the sensory results. Although the sensory analysis was only carried out for the LE, the variants fermented with *S. cerevisiae* received a higher number of attribute mentions in both vintages. Therefore, the hypothesis can be rejected based on the sensory and analytical results.

## 2. Distinction between the large- and small-scale experiments

The results of the LE are valuable for three reasons, and the results of the SE also serve to fully address the hypothesis. The first reason is that the pasteurised must was used in the LE to show the real effect of the yeast, as the naturally present yeast population was significantly reduced (Rouwen & Hühn, 2023). This is evident in the very low variance of the individual results and clearly visible in the comparison of the PCA of the LE (Figure 1) with the PCA of the SE (Figure 2). Co-inoculation, on the other hand, shows that the interaction of different yeasts does not necessarily lead to an additive effect: complex interactions lead to complex results (King *et al.*, 2008; Vilela, 2020), which is why pasteurisation was so important. Second, at a 200 L scale, it is easier to reduce the influence of oxidation in comparison with 34 L containers and thus produce results that are relevant to practice. Third, the analysis was quite complex. The main aroma compounds of these wines were fully analysed and a sensory test was also carried out. The SE was only used to check whether the results from the LE could also be seen in unpasteurised must and with less complex analysis and no sensory evaluation. This was only partially successful as there were only small differences between the three variants. A comparison of the aroma compounds analysed in Grüner Veltliner small scale and Grüner Veltliner large scale clearly shows that the influence of *St. lactis-condensi* in unpasteurised must is lower than in pasteurised must. While in the Grüner Veltliner small scale, only three of the 59 individual compounds analysed were significant, in the comparable Grüner Veltliner large scale 2020 there were 13 compounds and in 2021 even 15 individual compounds. Nevertheless, the interpretation of such studies with unpasteurised grape must is complicated by the complex interactions with the spontaneous microflora, which raises questions about their validity.

## 3. Integration of the results

### 3.1. Rotundone

Rotundone is responsible for the peppery aroma of wine. GV, the leading grape variety in Austria, is known for its spicy, peppery aroma. Mattivi *et al.* (2011) reported a rotundone concentration of 66–266 ng/L in GV wine, while in a large-scale study (> 100 wines from different vintages) the range was 9–85 ng/L (Nauer *et al.*, 2018). In the latter

**TABLE 5.** Comparison of the main results for each experiment.

| Cultivar and Vintage | Parameter | Rate of increase from the lowest to highest concentration | Var1 | Var2 | Var3 |
|---|---|---|---|---|---|
| Grüner Veltliner 2020 (large scale) | Rotundone | +39.0 % | *a* | b | **c** |
| | Thiols (sum of thiols) | +100.0 % | *a* | b | **c** |
| | Esters (sum of minor esters) | +6.5 % | *a* | a | **a** |
| | Esters (sum of major ethyl esters) | +10.1 % | b | **b** | *a* |
| | Monoterpenes (sum of monoterpenes) | +6.5 % | **a** | *a* | a |
| | Sensory results | +12.3% | *3* | 2 | **1** |
| Grüner Veltliner 2021 (large scale) | Rotundone | +44.2 % | *a* | a | **a** |
| | Thiols (sum of thiols) | +70.4 % | *a* | b | **c** |
| | Esters (sum of minor esters) | +19.1 % | *a* | a | **a** |
| | Esters (sum of major ethyl esters) | +40.3 % | b | **b** | *a* |
| | Monoterpenes (sum of monoterpenes) | +78.0 % | *a* | a | **b** |
| | Sensory results | +26.7 % | 2 | *3* | **1** |
| Grüner Veltliner 2020 (small scale) | Esters (sum of minor esters) | +5.0 % | **a** | *a* | a |
| | Esters (sum of major ethyl esters) | +21.8 % | **b** | b | *a* |
| | Monoterpenes (sum of free monoterpenes) | +30.9 % | *a* | b | **b** |
| Pinot blanc 2020 (small scale) | Esters (sum of minor esters) | +16.5 % | a | *a* | **a** |
| | Esters (sum of major ethyl esters) | +18.9 % | *a* | **b** | a |
| | Monoterpenes (sum of free monoterpenes) | +41.8 % | *a* | a | **b** |

The same letter indicates no significant difference; a indicates the lowest content. The sensory analyses are ranked according to the number of mentions, with 1 indicating the fewest mentions: lowest marked italics, biggest marked bold.

study, the authors found that in very dry, warm vintages, the rotundone content fell below the assumed perception threshold of 15 ng/L (Nauer *et al.*, 2018). Based on this finding, researchers have tested the influence of conventional and unconventional winemaking practices—including prolonged skin contact before fermentation, the addition of $SO_2$, the addition of yeast nutrients, the addition of whole grapes and/or vine leaves during fermentation as well as skin fermentation—on the rotundone concentrations in GV wines (Nauer *et al.*, 2021; Philipp *et al.*, 2023; Philipp *et al.*, 2024a; Philipp *et al.*, 2024b). They found that conventional oenological practices had little effect on the rotundone concentration. Must fermentation and fermentation with the addition of whole grapes had a positive effect on the rotundone concentration. However, the overall quality of the wines was unsatisfactory due to the leaching of undesirable aromas and phenolic compounds (Philipp *et al.*, 2024a). The present study clearly demonstrated the strong influence of the vintage on the rotundone concentration. The cool and wet autumn of 2020 resulted in a rotundone concentration of around 30 ng/L, while the concentration in the 2021 vintage was around 7 ng/L, well below the assumed perception threshold of 15 ng/L. The significant influence of yeast selection on the rotundone concentration in 2020 and the average 40 % higher rotundone concentration in the 2020 and 2021 vintages were not expected. Current knowledge suggests that, unlike other aroma compounds derived from odourless precursors or formed during fermentation, rotundone is extracted directly from the grape skins during winemaking (Geffroy *et al.*, 2020). Therefore, yeast selection should not influence the rotundone concentration. This statement is probably true for red wines at least, but the extraction effect of alcohol during maceration could mask other possible influencing factors, so the much smaller influence of yeast may not yet have been observed. Another possible explanation, to be confirmed by additional studies, could be that rotundone is converted to a small extent into other compounds by fermentation with *St. lactis-condensi*. In any case, different *S. cerevisiae* strains should be tested to assess their influence on rotundone accumulation or depletion, as it cannot be completely ruled out that there could be changes due to commercial yeasts, which could be a key factor for GV in times of climate change.

### 3.2. Fruity thiols

Due to their very low odour threshold, thiols play a key role in the primary aroma of white wines. The smell of boxtree, grapefruit, citrus fruits and passion fruit comes from these compounds (Tominago *et al.*, 1998). These thiols are released from their odourless cysteine or glutathione precursors. Three free thiols—3-SH, 3-SHA and 4-MSP—play a key role in the varietal aroma of Sauvignon blanc (Coetzee *et al.*, 2012), but are also of the utmost importance for GV (Philipp *et al.*, 2024b). It is not surprising that yeast selection influences the concentrations of thiols (Coetzee *et al.*, 2012). The variant fermented with *S. cerevisiae* consistently had a higher thiol expression than the variants fermented with *St. lactis-condensi*. This means

that the lyase activity of *S. cerevisiae* was higher than that of *St. lactis-condensi*. This study is the first to provide data regarding the thiol release behaviour of *St. lactis-condensi*.

### 3.3. Monoterpenes

Monoterpenes are a very broad aroma group in wine and are responsible for the varietal characteristics of several grape varieties (Black *et al.*, 2015). The monoterpene content in GV and PB is rather low (Philipp *et al.*, 2024b; Rapp, 1995), which was also the case in the wines analysed in the present study (the sum of free monoterpenes was < 31 µg/L). Monoterpenes are present either in a free form or bound to sugars (Stahl-Biskup *et al.*, 1993). Yeasts can release precursors by cleaving glycosidic bonds (Romano *et al.*, 2022). The *S. cerevisiae* strain used in the present study seems to have more potential in this respect: in three out of four experiments (BE GV 2021, SE GV, SE PB), the monoterpene content in the variant fermented with *S. cerevisiae* was significantly higher than the variants fermented with *St. lactis-condensi*. There are no comparative published results in this context.

### 3.4. Fermentation aromas

Esters are important compounds for the fruitiness of wine (Ferreira *et al.*, 2022). While the main ethyl esters were significantly affected by the yeast selection in all four experiments, there was no significant effect on the sum of minor esters. It is interesting to note that in three of the four experiments (LE GV 2020, LE GV 20 21 and SE GV), co-inoculation with *S. cerevisiae* and *St. lactis-condensi* significantly increased the main ethyl ester concentrations compared with fermentation with only *S. cerevisiae*, and in the case of SE PB, co-inoculation produced significantly higher concentrations than fermentation with *S. cerevisiae* or *St. lactis-condensi* alone. There were no consistent results for the other esters. Of note, in several cases, the *S. cerevisiae* variants showed significantly higher concentrations in the individual minor ester groups and individual compounds, but these results varied between the experiments. The ester content can be indirectly influenced by the must composition in addition to the fermentation conditions (including yeast selection, fermentation temperature and yeast nutrition; (Styger *et al.*, 2011), so the variability of the ester content can be attributed to the variability of the amino acid and acid contents of the different grape samples (Antalick *et al.*, 2010), to which the different yeasts reacted differently. The same applies to higher alcohols and carboxylic acids, for which inconsistent results were also obtained, although there were a few significant influences.

### 3.5. Sensory results

CATA analysis was used to check for the presence of typical descriptors of the wines. For the GV wines, the tasters reported an increased spiciness in the 2020 vintage, which is clearly related to the higher rotundone content (Wood *et al.*, 2008). Interestingly, the variants fermented with *St. lactis-condensi* received fewer attribute mentions, which is also consistent with the results of the aroma analysis. Francesca *et al.* (2024) showed that *St. lactis-condensi* has a positive effect on fruitiness and floral notes in sweet wines, which cannot be confirmed for dry white wines based on the results of the present study.

## CONCLUSION

The present study demonstrated that *St. lactis-condensi* is capable of fermenting pasteurised GV must into a dry wine; however, the resulting wines exhibited reduced aromatic properties. In the unpasteurised must, there were minimal differences when fermented with *St. lactis-condensi* or *S. cerevisiae*. Although the effects observed were less pronounced in field trials conducted without pasteurisation, the hypothesis that the non-commercial yeast *St. lactis-condensi* has an advantage over *S. cerevisiae* in producing particularly aromatic dry white wines can be rejected. Further research is recommended, especially regarding the influence of yeast selection on the rotundone content of GV, which has been documented for the first time. In general, it is worthwhile to continue the yeast selection to promote flavour diversity, even if not every non-*Saccharomyces* yeast performs better than *S. cereviseae* in this context.

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