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ORIGINAL PAPER

Making natural sparkling wines with non-*Saccharomyces* yeasts

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Abstract
The usage of alternative non-*Saccharomyces* yeasts might provide desirable characteristics to white and red sparkling wines. In this study, second fermentation in the bottle was carried out by *Saccharomyces cerevisiae* as control and two non-*Saccharomyces* species: *Saccharomycodes ludwigii* and *Schizosaccharomyces pombe*. The second fermentations of white base wine made from *Vitis Vinifera* cv. Airén grapes and red base wine made from *Vitis Vinifera* cv. Tempranillo grapes, in the bottle were followed by aging on lees during 4 months at 12 °C. Finally, physicochemical properties were analyzed and a sensory evaluation was held. Significant differences were detected among sparkling wines produced with the studied yeasts in acidity parameters and non-volatile compounds. The pyranoanthocyanin content and color intensity was higher with the use of *Schizosaccharomyces pombe* in red sparkling wines. The total amount of volatile compounds was similar among treatments, but in certain compounds, individual variations in concentration were seen. Total amount of biogenic amines decreased in all the samples after the treatment. Differences were also detected in sensory evaluation; the sparkling wines produced with *Saccharomyces cerevisiae* showed different aromatic profile in comparison to sparkling wines produced with *Schizosaccharomyces pombe*, considering the parameters of limpidity, aroma intensity, aroma quality, flowery, fruity, buttery and reduction aromas in white samples; color intensity, limpidity, aroma intensity, herbal, buttery, yeasty, and oxidation aromas in red samples. Usage of non-*Saccharomyces* yeasts for sparkling wine production with traditional method can be furtherly studied to change specific characteristics of sparkling wines without decreasing their overall quality and obtain differentiation.

Keywords Sparkling wine · *Saccharomyces cerevisiae* · *Saccharomycodes ludwigii* · *Schizosaccharomyces pombe* · Aging on lees

Introduction

The traditional method of sparkling wine production consists of two fermentations; the first one to produce the base wine from the must, the second one in the bottle, and it is followed by a period of aging on lees. For the second fermentation, a tirage liqueur which contains saccharose and yeast starter is added to each bottle, to produce the required amount of CO_2 which is between 5 and 6 bars. During the aging on lees period, the bottles remain in horizontal position for maximum wine—sediment contact. This period is ended by riddling process, which helps to collect the yeast sediments on the neck of the bottle, before their removal [1].

The second fermentation in the bottle does not provide favorable conditions for the yeasts, since it takes place in a hostile place with alcohol strength of 11% by volume, low pH, and presence of SO_2. Furthermore, the fermentation occurs in a closed tight container which causes increase of the carbon dioxide content inside of the wine. The yeasts settle down to the bottom of the bottle and they become suspended because of the carbon dioxide concentration in the bottle. Because of these reasons, the second fermentation in the bottle requires additional characteristics of the yeast which will be used, such as resistance to high concentration of ethanol, resistance to high pressures generated by carbon dioxide, ability to ferment in low temperatures, ability of autolysis after fermentation and transfer its components to the medium, good precipitation which will facilitate movement of the lees once the

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fermentation completed, and suitable organoleptic properties with the type of wine elaborated [2].

Saccharomyces cerevisiae is typically used in second fermentation of sparkling wines due to its favorable characteristics as high fermentative power (can ferment up to alcohol strength of 13–15% by volume), alcohol tolerance, and low production of volatile acidity [3].

Saccharomycodes ludwigii is tolerant to high concentration of ethanol until alcohol strength of 12–14% by volume and high concentrations of SO_2 until 200 mg/L [4]. These resistances give this yeast species a better adaptability to stress conditions in unfavorable environments [5]. Although *Saccharomycodes ludwigii* is believed to be a wine spoilage yeast, lately the studies have been focusing on the non-*Saccharomyces* yeasts including *Saccharomycodes* genus, as they have interesting oenological properties in terms of production of ethanol and secondary metabolites, as they produce β-glucosidases enzymes involved in the release of aromatic compounds from non-aromatic precursors in the grapes and they might, therefore, facilitate development of a varietal character and thus increase the wine aroma complexity [3, 6].

Schizosaccharomyces pombe is a currently studied yeast species with an increasing interest in modern oenology [7]. It is used in this experiment for its strong fermentative power, malic dehydrogenase activity and correlated biological deacidification [8], suitability for over-lees aging by reduction of the aging time, since yeast cell wall polysaccharides and mannoproteins show rapid autolytic release [9], high urease activity [10] that can reduce urea concentration and consequently reduce the ethyl carbamate contents of the wines, which is an interest of wine safety [11] and production of pyruvic acid and pyranoanthocyanins: Production of elevated amounts of pyruvic acid may be interesting in terms of color stability [7], since a strong correlation is found between amount of pyruvic acid and the pyranoanthocyanin called vitisin A [12].

The purpose of this study is to investigate the chemical and organoleptic characteristics that might occur during second fermentation and over-lees aging in the bottle by two species of non-*Saccharomyces* yeasts: *Saccharomycodes ludwigii* and *Schizosaccharomyces pombe*. The prementioned characteristics of these two non-*Saccharomyces* yeast species have the potential to have an impact on the compounds that have qualitative effects on the final product.

Materials and methods

Base wine and yeast inoculum used in second fermentation in bottle

White bulk wine made from *Vitis Vinifera* cv. Airén grapes and red bulk wine made from *Vitis Vinifera* cv. Tempranillo grapes (both produced in Ciudad Real, Spain) were used as base wine in the experiment. The reason of choosing bulk wines is to minimize the effect of varietal characteristics from the base wine, so that the influence of the yeast species could be reinforced and distinguished better. The white base wine had alcohol strength of 9.5% by volume, volatile acidity of 0.31 g/L as tartaric acid, total acidity of 5.4 g/L as tartaric acid and pH 3.36; while the red base wine had alcohol strength of 9.7% by volume, volatile acidity of 0.48 g/L as tartaric acid, total acidity of 5.2 g/L as tartaric acid and pH 3.36.

Three yeast strains, namely, *Saccharomyces cerevisiae* (7VA), *Saccharomycodes ludwigii* (979), and *Schizosaccharomyces pombe* (938) from the Chemistry and Food Technology Department of Universidad Politécnica de Madrid were used for the experiment. The liquid inoculum was obtained by growing these three yeast strains in YEPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) using a stepwise scale up method to generate sufficient amount of yeast biomass.

Second fermentation in bottle and over-lees aging

The second fermentation and aging on lees took place in 0.75 L sparkling wine bottles. Three bottles of wine were inoculated for each yeast species and for both white and red base wines. Saccharose (21 g/L), sand (3 g per bottle) as abrasive agent to speed over-lees aging [13, 14], and bentonite (0.3 g/L) were added into each bottle which contains 0.70 L base wine, and the bottles were closed airtightly with crown caps. In total, 18 bottles of base wine were inoculated to perform the second fermentation and aging on lees in laboratory conditions for 4 months. The temperature of the environment was between 11 and 12 °C during fermentation and over-lees aging. During this period, the bottles were placed horizontally and moved rotationally three times a week to increase the contact area of lees with wine.

Ethanol

Ethanol was analyzed by liquid chromatography with refractive index detection (LC-RI) using a Waters e2695 apparatus (Milford, Massachusetts, USA) equipped with a 2414 Refractive Index Detector. Analyses were performed using a reverse-phase Phenosphere XDB C18 column (4.6 mm × 150 mm, 5 µm particle size) (Phenomenex, Torrance, CA, USA). The solvent was Milli-Q water (used in isocratic mode) with a flow rate of 0.4 mL/min. The temperature was set at 30 °C in the column and detector. Calibration was performed using an external ethanol standard (Panreac, Barcelona, Spain). Samples were injected after filtration through 0.45 µm cellulose methyl ester membrane

filters (Tecknokroma, Barcelona, Spain). The injection volume was 2 µL.

Total acidity and pH

Total acidity and pH were analyzed using OenoFOSS™ analyzer that makes rapid analysis of ethanol, acidity, and sugar parameters. The analyzer uses Fourier transform infrared (FTIR) technology which involves infrared scanning of wine samples; light is absorbed in the wine sample according to wine constituents and then the absorption is converted through the Fourier transform mathematical model to a prediction of the concentrations within 60 s. The calibration range of the total acidity is 2–5 g/L as tartaric acid and that of pH is 2.6–4.0.

Sugars, organic acids, and glycerol

Glucose and fructose, L-lactic acid, L-malic acid, acetic acid, glycerol, and pyruvic acid were determined using a Y15 enzymatic auto-analyzer (Biosystems S.A, Barcelona, Spain) and its proper kits.

Anthocyanins

The anthocyanins were analyzed using high-performance liquid chromatography (HPLC) in an Agilent Technologies series 1100 (Palo Alto, CA) chromatograph equipped with a diode array detector and a quadrupole mass spectrometer with an electrospray interface. Solvent A (water/formic acid, 95:5, v/v) and solvent B (methanol/formic acid, 95:5, v/v) were used in a reverse-phase Kinetex C18 column (Phenomenex, Torrance, CA, USA) (100 mm × 4.6 mm; particle size 2.6 µm) with a flow rate of 0.8 mL/min. The gradient rate was as follows: 20–50% B linear increase from 0 to 27 min, 50% B from 27 to 28 min, 50–20% B linear decrease from 28 to 29 min, and re-equilibration of the column from 29 to 30 min. Detection was performed by scanning in the 500–600 nm range. Quantification was performed by comparison against an external standard at 525 nm and expressed as a function of the concentration of malvidin-3-*O*-glucoside (Extrasynthèse, Genay, France). The different anthocyanins were identified by their retention times with respect to the majority anthocyanin malvidin-3-*O*-glucoside and by comparing the UV–visible and mass spectra with data in the literature [15]. The electrospray ionization parameters were: drying gas (N_2) flow, 10 mL/min; temperature 350 °C; nebulizer pressure 380 kPa (55 psi); and capillary voltage 4000 V. Mass spectrometry was performed in positive mode scanning, from 100 to 1000 m/z, using a fragmenter voltage of 150 V from 0 min to 23 min. 100 µL of previously filtered (0.45 µm membrane filters made of cellulose methylic esters) (Tecknokroma, Barcelona, Spain) samples were injected into the HPLC apparatus. The detection limit was 0.1 mg/L.

Color measurements

The absorbance in 420, 520, and 620 nm of the red wines were measured using a spectrophotometer (V-530, JASCO, Tokyo, Japan) and a 1 mm path-length quartz cell, following the procedure of Glories [16, 17]. Color intensity was calculated as the sum of absorbance at the three wavelengths, while hue was calculated as the ratio between the absorbance at 420 and 520 nm.

Volatile compounds

The volatile compounds were analyzed using an Agilent Technologies 6850 gas chromatograph (Network GC System) equipped with an integrated flame ionization detector (GC-FID) (Hewlett Packard, Palo Alto, CA, USA). A DB-624 column (60 m × 250 µm × 1.40 µm) was employed to perform the separation. All volatile compounds were quantified using external calibration standards ($r^2 > 0.999$) and 4-methyl-2-pentanol as internal standard at the concentration of 50 mg/L (Fluka, Sigma–Aldrich Corp., Buchs SG, Switzerland). The injector temperature was 250 °C, and the detector temperature was 300 °C. The column temperature was 40 °C for the first 5 min, rising linearly by 10 °C/min until it reached 250 °C; this temperature was then maintained for 5 min. Hydrogen was used as the carrier gas; this was provided by a hydrogen generator (LNI Schmidlin SA, Geneva, Switzerland). The flow rate in the column was 2.2 L/min, the injection split ratio was 1:10, and the detection limit was 0.1 mg/L. 100 µL of internal standard (concentration 500 mg/L) was added to 1 mL test samples and filtered through syringe membrane filters (0.45 µm pore size) (Teknokroma, Barcelona, Spain) before injection. Wine samples were then placed in 2 mL glass vials sealed using a PTFE/silicon septum (Teknokroma, Barcelona, Spain) and 1 µL of this filtrate was injected into the GC apparatus.

Amino acids

The studied amino acids were analyzed using a Jasco (Tokyo, Japan) UHPLC chromatograph series X-LCTM, equipped with a Fluorescence detector 3120-FP. Gradients of solvent A (methanol/acetonitrile, 50:50, v/v) and B (sodium acetate/tetrahydrofuran, 99:1, v/v) were used in a C18 (HALO, USA) column (100 mm × 2.1 mm; particle size 2.7 µm) as follows: 90% B (0.25 mL/min) from 0 to 6 min, 90–78% B linear decrease (0.2 mL/min) from 6 to 7.5 min, 78% B from 7.5 to 8 min, 78–74% B linear decrease (0.2 mL/min) from 8 to 8.5 min, 74% B (0.2 mL/min) from 8.5 to 11 min, 74–50% B linear decrease (0.2 mL/min)

from 11 to 15 min, 50% B (0.2 mL/min) from 15 to 17 min, 50–20% B linear decrease (0.2 mL/min) from 17 to 21 min, 20–90% B linear increase (0.2 mL/min) from 21 to 25 min, and re-equilibration of the column from 25 to 26 min. Detection was performed by scanning in the 340–455 nm range. Quantification was performed by comparison against external standards of the studied amino acids. The different amino acids were identified by their retention times.

Biogenic amines

The analysis of biogenic amines (histamine, tyramine, phenylethylamine, putrescine, and cadaverine) was done using a Jasco (Tokyo, Japan) UHPLC chromatograph series X-LCTM, equipped with a Fluorescence detector 3120-FP. Gradients of solvent A (methanol/acetonitrile, 50:50, v/v) and B (sodium acetate/tetrahydrofuran, 99:1, v/v) were used in a C18 (HALO, USA) column (100 mm × 2.1 mm; particle size 2.7 µm) as follows: 60% B (0.25 mL/min) from 0 to 5 min, 60–50% B linear decrease (0.25 mL/min) from 5 to 8 min, 50% B from 8 to 9 min, 50–20% B linear decrease (0.2 mL/min) from 9 to 12 min, 20% B (0.2 mL/min) from 12 to 13 min, 20–60% B linear increase (0.2 mL/min) from 13 to 14.5 min, and re-equilibration of the column from 14.5 to 17 min. Detection was performed by scanning in the 340–420 nm range. Quantification was performed by comparison against external standards of the studied amines. The different biogenic amines were identified by their retention times.

Sensory evaluation

Sensory evaluation of the wines was done by a panel of 11 experienced tasters (age range from 27 to 57 years, four women and seven men) in the tasting room of Chemistry and Food Technology Department, Universidad Politécnica de Madrid, provided with fluorescent lighting and presenting samples in random order. The wines (20 mL/tasting glass) were served at 8–10 °C in six different standard wine-tasting glasses (three for the white and three for the red sparkling wines) at the same time to make comparative blind tasting. In addition, a glass of water was provided to panelists for palate cleansing between samples. The tasting was done according to the evaluation sheet which consists of 15 attributes (three visual, nine aromas, two mouth-feels, and one overall) and each attribute was examined on a 1–5 scale to rate the intensity perceived (0 = attribute not perceptible, 5 = attribute strongly perceptible).

Statistical analyzes

After obtaining the raw data, it was stored in Excel forms. Furthermore, it was statistically examined using PC Statgraphics v.5 software (Graphics Software Systems, Rockville, MD, USA). The statistical analysis was done to compare the white and red sparkling wines that were fermented with the studied yeast species and the white and red base wines. Three values were obtained from the analyzes performed on three different bottles, which were the repetitions of each three yeast species. The mean values and standard deviations were calculated and one-way analysis of variance (ANOVA) and least significant difference (LSD) tests were performed. Significance level was set at 5%, if the p value was equal to or greater than 0.05; it means that there was no statistically significant difference between dependent variable and the factor.

Results and discussion

Ethanol, sugars, organic acids, and acidity

The studied yeast species were able to increase the alcohol strength of approximately 1% by volume (Table 1); this indicates the fermentative power of the used non-*Saccharomyces* yeasts. However, ethanol amounts of the white and red sparkling wines did not show any significant differences among the studied yeast species. In white samples, higher amounts of residual sugar were left in the sparkling wines produced with *Schizosaccharomyces pombe* (2.0 ± 0.5 g/L), in comparison to other sparkling wines (Table 1). However, no significant differences were detected in red sparkling wines, with less than 0.2 g/L of glucose and fructose in all the samples. The produced sparkling wines (both white and red) have significantly higher amounts of glycerol in comparison to starting base wines (Table 1). In white sparkling wines, those produced with *Saccharomycodes ludwigii* have significantly higher amounts of glycerol (4.95 ± 0.04 g/L) in comparison to those produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Nevertheless, in red sparkling wines, no significant differences were detected regarding this compound.

Both in white and red sparkling wines, those produced with *Schizosaccharomyces pombe*, showed significantly lower amounts of malic acid (0.13 ± 0.03 and 0.06 ± 0.01 g/L, respectively) in comparison to the wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Table 1), due to its ability to metabolize, almost all the malic acid present with its transformation into ethanol [8]. In this study, during the second fermentation, *Schizosaccharomyces pombe* metabolized approximately 78% of the malic acid present in the white and red base wines. Lactic acid was detected in both white and red base wines, and consequently in the produced sparkling wines. In the red sparkling wines, no significant difference was observed among yeast species; however, in the white

Table 1 Ethanol, glucose and fructose, glycerol, total acidity, malic acid, lactic acid and acetic acid of the samples expressed as g/L, ethanol in % v/v, and pyruvic acid in mg/L and pH, with their mean values ± standard deviations of three replicates (except base wines that were only measured once)

Parameters	White wines				Red wines			
	S. cerevisiae	*S. ludwigii*	*S. pombe*	Base wine	*S. cerevisiae*	*S. ludwigii*	*S. pombe*	Base wine
Ethanol	10.75 ± 0.50^{b}	10.52 ± 0.27^{b}	10.43 ± 0.24^{b}	9.49^{a}	10.76 ± 0.27^{b}	10.67 ± 0.17^{b}	10.49 ± 0.37^{b}	9.74^{a}
Glucose-Fructose	0.08 ± 0.01^{a}	0.14 ± 0.01^{b}	2.00 ± 0.50^{c}	0.36^{c}	0.12 ± 0.10^{a}	0.15 ± 0.05^{a}	0.16 ± 0.04^{a}	2.21^{b}
Glycerol	4.57 ± 0.10^{b}	4.95 ± 0.04^{c}	4.67 ± 0.03^{b}	4.38^{a}	4.89 ± 0.15^{b}	5.12 ± 0.23^{b}	5.02 ± 0.14^{b}	4.61^{a}
Malic acid	0.68 ± 0.07^{b}	0.63 ± 0.04^{b}	0.13 ± 0.03^{a}	0.61^{b}	0.32 ± 0.04^{c}	0.32 ± 0.01^{c}	0.06 ± 0.01^{a}	0.26^{b}
Lactic acid	1.17 ± 0.01^{c}	$1.16 \pm 0.02^{b,c}$	1.09 ± 0.03^{a}	1.14^{b}	1.41 ± 0.08^{a}	1.39 ± 0.10^{a}	1.40 ± 0.04^{a}	1.40^{a}
Acetic acid	0.36 ± 0.00^{b}	0.41 ± 0.01^{c}	0.35 ± 0.03^{b}	0.31^{a}	0.45 ± 0.01^{a}	0.52 ± 0.03^{b}	0.46 ± 0.02^{a}	$0.48^{a,b}$
Pyruvic acid	48.00 ± 4.58^{b}	75.00 ± 3.18^{c}	115.00 ± 4.58^{d}	29.00^{a}	53.67 ± 6.35^{a}	67.00 ± 2.00^{b}	100.00 ± 7.81^{c}	48.00^{a}
Total acidity	5.17 ± 0.06^{b}	5.40 ± 0.00^{c}	4.87 ± 0.06^{a}	5.40^{c}	5.03 ± 0.06^{b}	5.17 ± 0.06^{c}	4.83 ± 0.06^{a}	5.20^{c}
pH	3.39 ± 0.01^{b}	3.39 ± 0.01^{b}	3.41 ± 0.01^{b}	3.36^{a}	3.38 ± 0.01^{b}	3.39 ± 0.01^{b}	3.40 ± 0.01^{b}	3.36^{a}

Values in the same line with the same letter are not significantly different ($p < 0.05$)

White and red wines treated statistically separately

sparkling wines, those produced with *Schizosaccharomyces pombe* showed significantly lower amount of lactic acid (1.09 ± 0.03 g/L) in comparison to the samples produced with *Saccharomyces cerevisiae* and *Saccharomycodes ludwigii*. Moreover, the white and red sparkling wines produced with *Saccharomycodes ludwigii* showed significantly higher values of acetic acid (0.41 ± 0.01 and 0.52 ± 0.03 g/L, respectively) in comparison to the sparkling wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Table 1); this can be related to its common consideration as spoilage yeast [18]. However, these concentrations of acetic acid are still below the sensory threshold in dry wines 0.7–1.0 g/L [19]. The amount of pyruvic acid, both in white and red sparkling wines, showed a similar pattern (Table 1). The sparkling wines showed significant differences among the yeasts, with amounts of pyruvic acid listed from higher to lower as, *Schizosaccharomyces pombe, Saccharomycodes ludwigii*, and *Saccharomyces cerevisiae*. These results agreed with Benito et al. [8] where some non-*Saccharomyces* yeast including *Schizosaccharomyces* were described as higher producers of pyruvic acid.

Regarding the total acidity, as tartaric acid, the sparkling wines also showed significant differences among the yeasts, with amounts of total acidity listed from higher to lower as, *Saccharomycodes ludwigii, Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* (Table 1). Lower values of total acidity of *Schizosaccharomyces pombe* were expected, since it is a yeast species which is recommended by the International Organization of Vine and Wine (OIV) [20] for deacidification processes due to its malic dehydrogenase activity. Although there was a significant increase of pH values after second fermentation and aging in bottles, in comparison to base wine, no significant difference was obtained among neither white nor red sparkling wines.

Anthocyanins, pyranoanthocyanins, color intensity, and tonality

Considering the total amount of anthocyanins, all the red sparkling wines showed a significant decrease in comparison to the red base wine (Table 2). The red sparkling wines produced with *Schizosaccharomyces pombe* showed significantly lower amount of total anthocyanins (7.60 ± 0.78 mg/L) in comparison to red sparkling wines produced with *Saccharomyces cerevisiae* and *Saccharomycodes ludwigii*. This can be explained by adsorption of anthocyanins during fermentation, of which amount depends on the yeast species which are employed [21].

Pyranoanthocyanins which were detected in the red samples were vitisins (vitisin A and vitisin B) and also a vinylphenolic derivative, malvidin-3-*O*-glucoside-4-vinylphenol. The red sparkling wines produced with *Schizosaccharomyces pombe* showed significantly higher values of total pyranoanthocyanins (1.07 ± 0.16 mg/L) in comparison to the red sparkling wines produced with *Saccharomycodes ludwigii* and *Saccharomyces cerevisiae* (Table 2). In a study conducted by Morata et al. [22], a comparison was done between the yeasts *Schizosaccharomyces pombe* (strains: 935, 936, 938 and 2139), *Saccharomyces cerevisiae* (7VA) and *Saccharomyces uvarum* (S6U) along the fermentation of a red must and *Schizosaccharomyces pombe 938* showed highest total pyranoanthocyanin content, which was further related with the more intense color of the sample which analyzed by wine absorbance at 420, 520 and 620 nm. Vitisin A is a pigment which originates from a condensation reaction between pyruvic acid and malvidin-3-*O*-glucoside [23]. Similarly, vitisin B is a pigment derived by a condensation reaction between acetaldehyde and malvidin-3-*O*-glucoside which is the majority anthocyanin in the grape must [23].

Table 2 Anthocyanin content (mg/L), color intensity (AU), and tonality (adimensional) of red sparkling wines

Parameters	Red wines			
	S. cerevisiae	*S. ludwigii*	*S. pombe*	Base wine
M3G	0.34 ± 0.00^{a}	0.34 ± 0.02^{a}	0.29 ± 0.07^{a}	0.49^{b}
Total anthocyanins	11.74 ± 0.24^{b}	11.16 ± 0.24^{b}	7.60 ± 0.78^{a}	20.00^{c}
Vitisin A	0.73 ± 0.02^{a}	0.75 ± 0.01^{a}	0.91 ± 0.14^{b}	$0.83^{a,b}$
Vitisin B	0.05 ± 0.00^{b}	0.05 ± 0.01^{b}	0.13 ± 0.01^{c}	0.03^{a}
M3G4Vph	0.09 ± 0.00^{c}	0.02 ± 0.00^{a}	0.03 ± 0.00^{b}	0.11^{d}
Total pyranoanthocyanins	0.87 ± 0.02^{a}	0.82 ± 0.01^{a}	1.07 ± 0.16^{b}	$0.97^{a,b}$
Color intensity	0.30 ± 0.03^{a}	$0.30 \pm 0.01^{a,b}$	0.35 ± 0.04^{b}	0.42^{c}
Tonality	0.89 ± 0.00^{b}	0.89 ± 0.02^{b}	0.87 ± 0.01^{a}	$0.88^{a,b}$

Means ± SD of three replicates, except base wine that was measured only once

Values in the same row with the same letter are not significantly different ($p < 0.05$)

M3G malvidin-3-*O*-glucoside, *M3G4Vph* malvidin-3-*O*-glucoside-4-vinylphenol

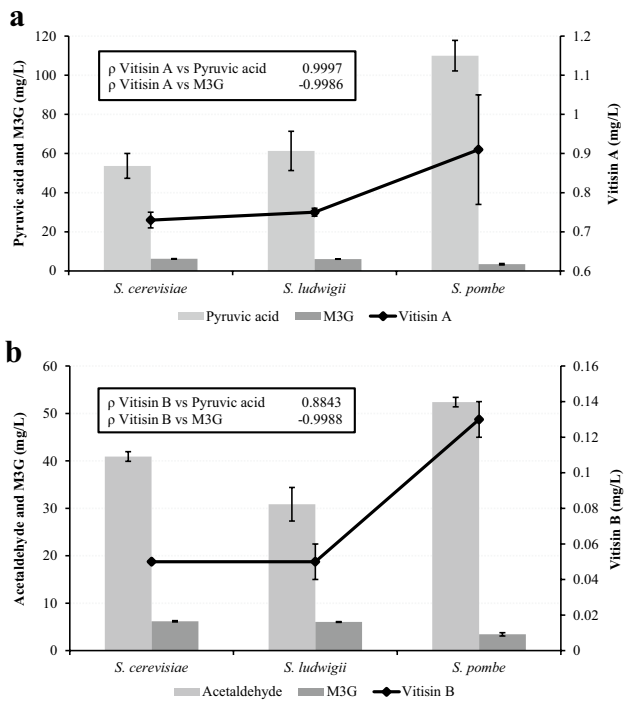

Fig. 1 Correlations between each vitisin A (**a**) and vitisin B (**b**) and its corresponding precursors. All concentrations (mg/L) were measured in the sparkling wines produced after the second fermentation in bottle

Figure 1a, b shows the correlations between all the compounds involved in vitisins formation. The red sparkling wines produced with *Schizosaccharomyces pombe* showed significantly higher values of vitisin A (0.91 ± 0.14 mg/L) and vitisin B (0.13 ± 0.01 mg/L), in comparison to the red sparkling wines produced with *Saccharomycodes ludwigii* and *Saccharomyces cerevisiae*. The similar tendency was also seen in the previously mentioned study done by Morata et al. [22], where *Schizosaccharomyces pombe* (938) showed significantly largest quantity of vitisin A (11.03 ± 0.82 mg/L), in comparison with *Saccharomyces cerevisiae* and *Saccharomyces uvarum*. Moreover, the red sparkling wines produced with *Schizosaccharomyces pombe* showed the highest amount of pyruvic acid; these results obtained about vitisin A and vitisin B is reinforced by the conclusion made by Morata et al. [12] that the yeast species with suitable pyruvic acid and acetaldehyde productions can be used to increase the formation of stable pigments during red wine fermentation.

Regarding color parameters, the red sparkling wines produced with *Schizosaccharomyces pombe* have the highest color intensity (0.35 ± 0.04 AU); however, a lower tonality (0.87 ± 0.01) in comparison to the red sparkling wines produced with *Saccharomycodes ludwigii* and *Saccharomyces cerevisiae* (Table 2). In a study conducted by Benito et al. [10], a comparison between four strains of *Schizosaccharomyces pombe* (935, 936, 938 and 2139), *Saccharomyces cerevisiae* (7VA), and *Saccharomyces uvarum* (S6U) was done after fermentation of a red grape must. *Schizosaccharomyces pombe* (938) showed significantly higher color intensity in the end of the fermentation process, similar to this study for the second fermentation in bottle. Over-lees aging was performed in a study conducted by Palomero et al. [9], in red wine with five different yeast species including also *Saccharomyces cerevisiae* (G37), *Schizosaccharomyces pombe* (936), and *Saccharomycodes ludwigii* (980). After 100 days of aging, non-*Saccharomyces* yeasts showed significantly higher color intensity in comparison to *Saccharomyces cerevisiae*. However, in this study, the red sparkling wines produced with *Saccharomycodes ludwigii* and *Saccharomyces cerevisiae* showed similar color intensities.

Volatile compounds

The amount of total volatile compounds of the sparkling wines produced with studied yeasts did not show any

significant differences among them (Table 3). However, there were significant differences when the compounds were considered individually and in groups; as esters, acetoin metabolites, and higher alcohols.

In the white samples, the sparkling wines produced with *Saccharomycodes ludwigii* showed significantly lower amounts of acetaldehyde (54.40 ± 3.65 mg/L) in comparison to sparkling wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Table 3). According to a

Table 3 Volatile compound amounts (mg/L) of the samples with their mean values ± standard deviations ($n = 3$; except base wines that were only measured once)

White wines					Red wines			
Compounds	*S. cerevisiae*	*S. ludwigii*	*S. pombe*	Base Wine	*S. cerevisiae*	*S. ludwigii*	*S. pombe*	base wine
Acetaldehyde	72.32 ± 2.02^{c}	54.40 ± 3.65^{b}	75.03 ± 0.75^{c}	26.72^{a}	40.93 ± 1.03^{b}	30.87 ± 3.54^{a}	52.40 ± 1.00^{c}	31.56^{a}
Methanol	59.60 ± 8.65^{a}	53.47 ± 3.32^{a}	57.00 ± 2.13^{a}	55.84^{a}	79.80 ± 2.10^{a}	77.66 ± 3.70^{a}	86.31 ± 0.97^{b}	87.33^{b}
Ethyl acetate	39.47 ± 7.34^{b}	55.93 ± 3.69^{c}	33.90 ± 1.60^{b}	31.97^{a}	47.03 ± 1.27^{b}	72.03 ± 4.20^{c}	36.60 ± 3.88^{a}	39.96^{a}
Ethyl butyrate	1.82 ± 0.10^{b}	1.68 ± 0.01^{a}	1.80 ± 0.07^{b}	1.69^{a}	1.70 ± 0.02^{b}	2.27 ± 0.39^{c}	1.97 ± 0.16^{b,c}	0.00^{a}
Ethyl lactate	98.68 ± 3.48^{c}	78.43 ± 7.21^{b}	84.23 ± 5.45^{b}	67.66^{a}	114.27 ± 3.14^{a}	110.07 ± 4.72^{a}	124.34 ± 1.79^{c}	119.97^{b}
Isoamyl acetate	3.67 ± 0.23^{b}	2.40 ± 0.09^{a}	2.63 ± 0.15^{a}	2.62^{a}	3.33 ± 0.47^{c}	2.07 ± 0.08^{b}	2.13 ± 0.22^{b}	1.93^{a}
Phenylethyl acetate	5.07 ± 0.06^{a}	6.30 ± 0.09^{c}	6.70 ± 0.10^{d}	6.04^{b}	6.27 ± 0.04^{c}	5.43 ± 0.46^{a}	5.37 ± 0.35^{a}	6.16^{b}
Total esters	148.71 ± 11.21^{c}	144.74 ± 11.09^{bc}	129.26 ± 7.37^{b}	73.70^{a}	172.60 ± 4.94^{a}	191.87 ± 9.85^{b}	170.41 ± 6.40^{a}	168.02^{a}
Diacetyl	1.93 ± 0.25^{b}	2.43 ± 0.15^{c}	2.20 ± 0.10^{bc}	0.00 ^{a}	1.80 ± 0.10^{a}	2.20 ± 0.23^{b}	2.23 ± 0.27^{b}	1.88^{ab}
Acetoin	5.59 ± 0.44^{a}	12.53 ± 4.35^{d}	9.37 ± 0.42^{c}	7.60^{b}	6.07 ± 0.42^{a}	16.47 ± 1.87^{d}	10.10 ± 1.29^{c}	8.67^{b}
2,3-butanediol	441.21 ± 5.65^{d}	421.66 ± 0.77^{c}	411.50 ± 3.75^{b}	384.14^{a}	499.64 ± 7.90^{c}	435.47 ± 4.42^{a}	489.54 ± 0.23^{b}	517.78^{d}
Total acetoin metabolites	448.73 ± 6.34^{d}	436.62 ± 5.27^{c}	423.07 ± 4.27^{b}	391.74^{a}	507.51 ± 8.42^{b}	454.14 ± 6.52^{a}	501.87 ± 1.79^{b}	528.33^{c}
1-propanol	26.83 ± 4.06^{ab}	22.87 ± 1.50^{a}	28.17 ± 0.56^{b}	23.81^{a}	23.03 ± 0.76^{b}	21.10 ± 0.75^{a}	24.20 ± 1.92^{b}	21.56^{a}
Isobutanol	27.07 ± 4.10^{a}	24.37 ± 1.16^{a}	25.83 ± 0.58^{a}	24.38^{a}	31.97 ± 1.14^{a}	32.60 ± 0.52^{a}	30.90 ± 2.78^{a}	34.08^{b}
1-butanol	4.80 ± 0.34^{a}	4.50 ± 0.14^{a}	4.73 ± 0.09^{a}	4.79^{a}	4.20 ± 0.15^{a}	4.27 ± 0.14^{a}	4.20 ± 0.10^{a}	4.70^{b}
2-Methyl-1-butanol	133.71 ± 1.95^{c}	111.30 ± 3.81^{a}	118.87 ± 2.08^{b}	110.10^{a}	115.87 ± 4.17^{a}	120.90 ± 5.66^{a}	122.52 ± 3.18^{a}	128.81^{b}
3-Methyl-1-butanol	30.23 ± 3.73^{a}	37.01 ± 1.47^{b}	35.70 ± 0.60^{b}	30.31^{a}	35.87 ± 2.30^{a}	47.60 ± 4.74^{c}	37.43 ± 3.63^{a}	41.44^{b}
Hexanol	3.93 ± 0.09^{a}	4.03 ± 0.06^{a}	4.53 ± 0.38^{b}	4.34^{b}	4.23 ± 0.15^{a}	4.30 ± 0.07^{a}	4.33 ± 0.08^{a}	4.24^{a}
Phenylethyl alcohol	21.93 ± 1.10^{c}	22.47 ± 1.86^{c}	19.27 ± 0.81^{b}	17.85^{a}	21.37 ± 1.26^{a}	22.33 ± 0.93^{a}	24.83 ± 1.08^{b}	25.56^{b}
Total higher alcohols	248.50 ± 15.37^{c}	226.55 ± 10.00^{b}	237.1 ± 5.10^{bc}	215.58^{a}	236.54 ± 9.93 ^{a}	253.10 ± 12.81^{ab}	248.41 ± 12.77^{ab}	260.39^{b}
Total volatiles	977.52 ± 99.19^{b}	876.16 ± 84.86^{ab}	921.36 ± 14.39^{b}	799.86^{a}	1037.35 ± 21.19^{a}	1007.73 ± 17.15^{a}	1036.78 ± 12.06^{a}	1075.61^{b}

Values in the same line with the same letter are not significantly different ($p < 0.05$)

White and red wines treated statistically separately

study conducted by Benito et al. [10], in which volatile compounds were detected after fermentation of white wine (performed at 25 °C) with *Schizosaccharomyces pombe* (938) and *Saccharomyces cerevisiae Cru Blanc* (CB) as sole yeasts and also combined, *Schizosaccharomyces pombe* produced significantly higher amount of acetaldehyde in comparison to *Saccharomyces cerevisiae*. However, this tendency was not seen significantly in this study.

In the case of red samples, the sparkling wines produced with *Saccharomycodes ludwigii* showed significantly lower amount of acetaldehyde (30.87 ± 3.54 mg/L) in comparison to sparkling wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, being the red sparkling wines produced with *Schizosaccharomyces pombe* the ones with the highest amount of acetaldehyde (52.40 ± 1.00 mg/L). In the studies of Benito et al. [24], fermentations are performed at 25 °C in red musts with *Schizosaccharomyces pombe* (938) and *Saccharomyces cerevisiae* (796), alone and combined, and volatile compounds were detected afterwards. Unlike this study, no significant differences were detected in acetaldehyde production between the sole fermentations of *Schizosaccharomyces pombe* (938) and *Saccharomyces cerevisiae* (796). In previously mentioned study of Palomero et al. [9], no significant differences were seen among studied yeasts considering acetaldehyde amounts, unlike this study where differences were detected.

There is a legal limit of methanol amount of 250 mg/L for white and rosé wines, and 400 mg/L for red wines [25]. In none of the samples, the legal limit was exceeded (57–65 mg/L in white, 78–87 mg/L in red sparkling wines) (Table 3). In white sparkling wines, no significant difference was detected between any of the samples while in the red samples, the sparkling wines produced with *Schizosaccharomyces pombe* showed significantly higher methanol amounts (86.31 ± 0.97 mg/L) in comparison to the sparkling wines produced with *Saccharomyces cerevisiae* and *Saccharomycodes ludwigii*.

Esters are important compounds which are produced by yeast during alcoholic fermentation and mostly contribute to a pleasant smell to wine, especially fruity character [19]. In white samples, the sparkling wines produced with *Saccharomyces cerevisiae* showed significantly higher amount of total esters (148.71 ± 11.21 mg/L) in comparison to the sparkling wines produced with *Schizosaccharomyces pombe*; however, it was not significantly different from the amount in the sparkling wines produced with *Saccharomycodes ludwigii* (Table 3). In red samples, the sparkling wines produced with *Saccharomycodes ludwigii* showed significantly higher amount of total esters (191.87 ± 9.85 mg/L) in comparison to the sparkling wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

Regarding total acetoin metabolites, in white samples, the sparkling wines produced with *Saccharomyces cerevisiae* showed significantly higher content (448.73 ± 6.34 mg/L) in comparison to the sparkling wines produced with studied non-*Saccharomyces* yeasts (Table 3). While in red samples quite the opposite, the sparkling wines produced with *Saccharomyces ludwigii* showed significantly the lowest amount of total acetoin metabolites (454.14 ± 6.52 mg/L).

Finally, the total concentration of higher alcohols in all the sparkling wines remained below the maximum level which can contribute general complexity to wine, which is 300 mg/L [26].

Amino acids and biogenic amines

The content of the amino acids of the base wine changed during the second fermentation in bottle (Table 4). A trend to increase was observed in the total amino acids content for the sparkling wines produced with *Saccharomycodes ludwigii* and *Schizosaccharomyces pombe*. Decreases in amino acids such as aspartic acid, serine, arginine, or tryptophan, especially for *Saccharomyces cerevisiae* can be related to the fact that are considered as preferentially assimilated [27] by fermentative yeast. Nevertheless, this fact was not observed in all cases for *Saccharomycodes ludwigii* and *Schizosaccharomyces pombe*. Increases such as the one detected for alanine in every case, arginine, lysine, and methionine for *Saccharomycodes ludwigii* and *Schizosaccharomyces pombe*, tryptophan for *Saccharomycodes ludwigii* or aspartic acid, asparagine, serine, phenylalanine look to be related to release processes and different yeast species structure composition [9]. The higher values reported for *Saccharomycodes ludwigii* in threonine were related to lower levels in 1-propanol. A similar effect but less intense was observed for valine and isobutanol. Likewise for *Saccharomycodes ludwigii* and *Schizosaccharomyces pombe* related to isoleucine and 2-methyl-1-butanol but only in white wines. This effect was not observed in the case of leucine and 3-methyl-1-butanol. Other authors have reported similar relations between nitrogen composition and aroma profile [28–30] for the case of *Saccharomyces cerevisiae*.

Increase in biogenic amines precursors [31] was detected after fermentations (Table 4). The sparkling wines produced with studied non-*Saccharomyces* yeasts showed higher amounts in histidine in comparison to the sparkling wines produced with *Saccharomyces cerevisiae*. The sparkling wines produced with *Schizosaccharomyces pombe* showed higher values in tyrosine in comparison to the sparkling wines produced with *Saccharomyces cerevisiae*, while the sparkling wines produced with *Saccharomyces cerevisiae* showed the highest levels in ornithine. Nevertheless, any other increase in their corresponding biogenic amines was reported as the second fermentation process was performed under control conditions. Biogenic amines' production is mainly related to lactic bacteria

Table 4 Amino acids and biogenic amines (mg/L) of white and red samples with their mean values ± SD ($n = 3$; except base wines that were only measured once)

Group	Compounds	White wines				Red wines			
		S. cerevisiae	*S. ludwigii*	*S. pombe*	Base wine	*S. cerevisiae*	*S. ludwigii*	*S. pombe*	Base wine
Amino acids	Aspartic acid	4.75 ± 0.52^{a}	6.63 ± 0.24^{b}	15.02 ± 1.02^{d}	9.39^{c}	3.97 ± 0.09^{a}	6.71 ± 0.10^{b}	16.20 ± 0.52^{d}	9.71^{c}
	Asparagine	12.57 ± 1.49^{a}	13.74 ± 0.51^{a}	19.43 ± 1.67^{b}	12.93^{a}	11.98 ± 0.28^{a}	14.52 ± 0.77^{b}	20.71 ± 0.67^{c}	15.01^{b}
	Serine	2.02 ± 0.30^{a}	3.61 ± 0.40^{b}	6.95 ± 0.87^{d}	4.35^{c}	1.80 ± 0.13^{a}	4.53 ± 0.92^{b}	7.81 ± 0.35^{c}	5.23^{b}
	Histidine	72.08 ± 5.14^{a}	94.11 ± 3.10^{c}	81.93 ± 4.75^{b}	83.16^{b}	69.10 ± 2.13^{a}	89.05 ± 6.59^{c}	79.69 ± 2.11^{b}	81.71^{b}
	Glycine	37.94 ± 1.52^{ab}	36.12 ± 2.75^{a}	35.21 ± 1.18^{a}	39.39^{b}	26.02 ± 0.18^{c}	23.85 ± 2.10^{ab}	22.58 ± 1.14^{a}	24.66^{b}
	Arginine	33.57 ± 1.22^{a}	49.09 ± 3.15^{c}	52.03 ± 0.83^{c}	40.56^{b}	35.19 ± 0.96^{a}	50.57 ± 3.30^{c}	53.43 ± 1.58^{c}	42.27^{b}
	Threonine	29.67 ± 0.87^{b}	39.48 ± 3.43^{d}	31.31 ± 0.29^{c}	19.58^{a}	36.40 ± 0.02^{b}	45.39 ± 3.94^{d}	38.00 ± 1.47^{c}	26.64^{a}
	Alanine	38.58 ± 1.98^{b}	47.63 ± 2.56^{c}	40.96 ± 1.50^{b}	34.85^{a}	39.40 ± 1.08^{b}	47.28 ± 2.78^{c}	41.67 ± 1.05^{b}	35.98^{a}
	Tyrosine	12.54 ± 0.76^{c}	11.13 ± 0.35^{b}	12.61 ± 0.58^{c}	9.22^{a}	9.68 ± 1.06^{a}	9.78 ± 0.62^{a}	12.48 ± 0.36^{b}	9.18^{a}
	Valine	2.76 ± 0.58^{a}	3.33 ± 0.72^{a}	2.67 ± 0.08^{a}	2.61^{a}	2.98 ± 0.25^{a}	4.03 ± 0.10^{c}	3.49 ± 0.14^{b}	3.00^{a}
	Tryptophan	0.67 ± 0.17^{b}	1.13 ± 0.20^{c}	0.38 ± 0.08^{a}	0.84^{b}	0.71 ± 0.11^{a}	1.37 ± 0.09^{c}	0.59 ± 0.17^{a}	1.01^{b}
	Phenylalanine	10.17 ± 1.52^{a}	10.15 ± 0.35^{a}	12.82 ± 6.64^{b}	10.83^{a}	7.46 ± 0.24^{a}	8.94 ± 0.69^{b}	12.73 ± 0.32^{d}	9.75^{c}
	Isoleucine	3.74 ± 0.62^{a}	7.63 ± 0.28^{c}	10.98 ± 0.54^{d}	4.72^{b}	2.84 ± 0.17^{a}	6.90 ± 0.43^{c}	10.37 ± 0.53^{d}	4.28^{b}
	Leucine	7.19 ± 0.77^{a}	9.27 ± 0.56^{b}	14.73 ± 0.40^{d}	10.88^{c}	4.43 ± 0.08^{a}	7.95 ± 0.83^{c}	14.54 ± 0.74^{d}	9.92^{b}
	Ornithine	31.15 ± 0.79^{d}	22.56 ± 1.36^{c}	17.59 ± 0.94^{b}	15.89^{a}	30.70 ± 0.26^{d}	24.97 ± 1.50^{c}	20.63 ± 0.71^{b}	19.32^{a}
	Lysine	18.87 ± 1.88^{a}	34.26 ± 2.28^{c}	42.07 ± 0.22^{d}	27.76^{b}	18.35 ± 0.79^{a}	36.71 ± 2.64^{c}	45.10 ± 1.87^{d}	31.13^{b}
	Methionine	3.52 ± 0.35^{a}	5.31 ± 0.21^{b}	5.37 ± 0.32^{b}	3.44^{a}	2.83 ± 0.14^{a}	4.83 ± 0.27^{c}	5.32 ± 0.24^{d}	3.26^{b}
	Total	321.19 ± 20.14^{a}	395.18 ± 21.76^{b}	398.75 ± 13.94^{b}	330.39^{a}	303.85 ± 5.97^{a}	387.39 ± 26.84^{c}	405.34 ± 7.88^{c}	332.06^{b}
Biogenic amines	Histamine	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10^{a}	1.43 ± 0.06^{a}	1.43 ± 0.15^{a}	1.53 ± 0.06^{a}	1.70^{b}
	Tyramine	0.43 ± 0.23^{a}	0.43 ± 0.23^{a}	0.87 ± 0.12^{c}	0.70^{b}	1.87 ± 0.06^{a}	1.97 ± 0.15^{ab}	1.93 ± 0.06^{a}	2.10^{b}
	Putrescine	2.90 ± 0.00^{b}	2.90 ± 0.00^{b}	2.60 ± 0.00^{a}	3.10^{c}	6.77 ± 0.06^{b}	6.47 ± 0.40^{ab}	6.10 ± 0.20^{a}	7.80^{c}
	Total	3.43 ± 0.25^{a}	3.43 ± 0.23^{a}	3.57 ± 0.15^{a}	3.90^{b}	10.07 ± 0.12^{b}	9.87 ± 0.67^{ab}	9.57 ± 0.21^{a}	11.60^{b}

Values in the same line with the same letter are not significantly different ($p < 0.05$)

White and red wines treated statistically separately

metabolism [32, 33]. Indeed, the sparkling wines produced with *Schizosaccharomyces pombe* as they contain the lowest levels of malic acid (Table 1), they would run a lower risk of increasing the levels of biogenic amines [34].

Biogenic amines have been proved to be harmful for human health [35, 36] which negatively influences wine quality in nowadays market [37]. The total amounts of the biogenic amines in white sparkling wines were significantly lower in comparison to the white base wine. In the red samples, a significant difference was seen between the sparkling wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which might be explained by different adsorption characteristics depending on the type of lees during the aging on lees process or during the fermentation [38]. The maximum reduction observed for total biogenic amines was 2.03 mg/L (Table 4) in red sparkling wine for *Schizosaccharomyces pombe*. Other authors have reported higher decreases up to 2.22 mg/L in histamine for the non-*Saccharomyces* species *Hanseniaspora vineae* [39]. However, other studies have reported increases in biogenic amines after aging on lees processes [40].

Sensory evaluation

The results of sensory evaluation of the white and red sparkling wines can be seen in Fig. 2A, B, respectively. In both white and red samples, the sparkling wines produced with the studied non-*Saccharomyces* yeasts showed significantly more limpidity in comparison to the sparkling wines produced with *Saccharomyces cerevisiae*. No significant difference was seen in effervescence of the white sparkling wines, however, in the red samples, the sparkling wines produced with *Saccharomycodes ludwigii* were found to have higher effervescence in comparison to red sparkling wines produced with other studied yeasts. Considering the color intensity, no significant difference was seen in white sparkling wines. Conversely, in the red samples, the sparkling wines produced with *Schizosaccharomyces pombe* were rated with the highest color intensity.

As for aroma quality, the white sparkling wines produced with *Saccharomyces cerevisiae* were found to have significantly higher aroma quality in comparison to the white sparkling wines produced with the studied non-*Saccharomyces* yeasts. However, no significant difference was detected

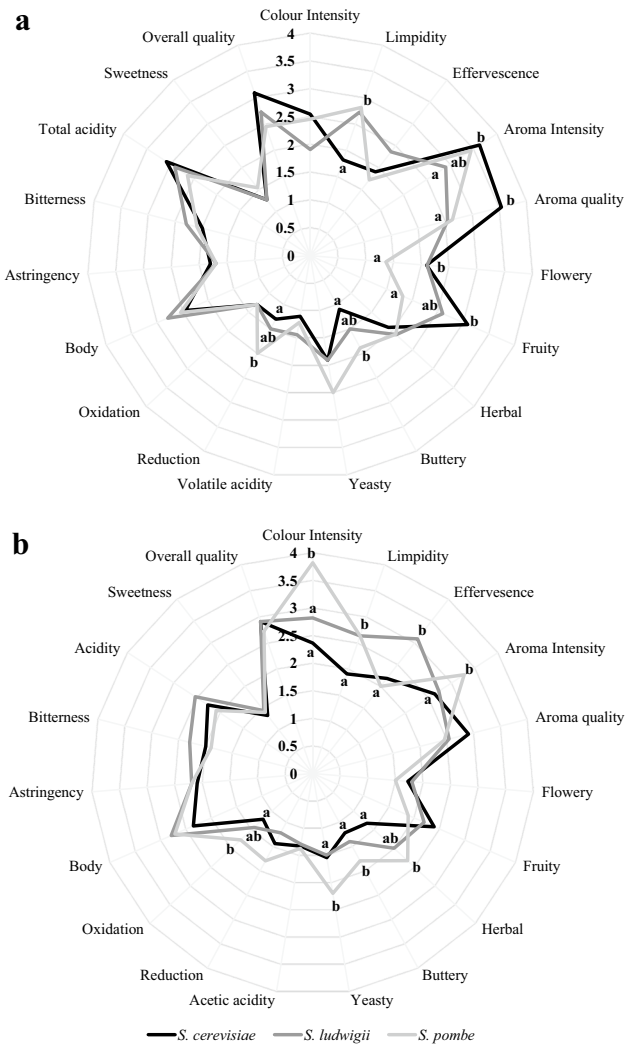

Fig. 2 Sensory evaluation of white (**a**) and red (**b**) sparkling wines produced after second fermentation in bottle

in the case of red sparkling wines. Significantly different aromatic profiles were seen in the white and red sparkling wines produced with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The white sparkling wines produced with *Saccharomyces cerevisiae* showed higher flowery and fruity aromas, but lower buttery and reduction aromas in comparison to the white sparkling wines produced with *Schizosaccharomyces pombe*. Moreover, the red sparkling wines produced with *Saccharomyces cerevisiae* showed lower herbal, buttery, yeasty, and oxidation aromas in comparison to the red sparkling wines produced with *Schizosaccharomyces pombe*. However, despite of these differences found at aromatic level, the sparkling wines produced with *Schizosaccharomyces pombe* had a good balance in nose and no aromatic defect was perceived.

Considering the gustatory aspects, no significant difference was detected in neither of the attributes such as acidity or sweetness. Similarly, no significant difference was detected among the sparkling wines considering overall quality.

Conclusion

In this study, the assessed non-*Saccharomyces* yeasts were used in sparkling wine production with the traditional method. According to the results, none of the studied non-*Saccharomyces* yeasts had a problem to finish the second fermentation in the bottle, to increase alcohol strength of 1% by volume, and to ferment the base wines until dryness. Moreover, no significant differences were detected concerning the overall quality of the final products among the three studied yeasts. The ability to change the characteristics of final product such as color, acidity, volatile compounds, and biogenic amines of the studied non-*Saccharomyces* yeasts, and the differences detected during the sensory evaluation of the sparkling wines showed the possibility to further study and examine the usage of the non-*Saccharomyces* yeast in the traditional method of sparkling wine production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human participants and/or animals This article does not contain any studies with human or animal subjects.

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