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Chemometric Differentiation of White Wines from a Low-Aromatic Grape Obtained by Spontaneous Fermentation, Enriched with Non-*Saccharomyces*, or with a High-Glutathione-Producing *Saccharomyces* Yeast

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Abstract: This work focuses on the establishment of analytical and sensory differences between young wines obtained from the same grape must fermented using different strategies. The main purpose is to provide winemakers with objective criteria to help them to choose the best fermentation method to obtain the desired wine characteristics. The effects of four strategies were tested: a spontaneous fermentation with wild yeasts (WYs) and the addition of starter cultures of *Lachancea thermotolerans* (LT), *Metschnikowia pulcherrima* (MP), and a *Saccharomyces cerevisiae* strain that is an overproducer of glutathione (SC) in different batches of the same must of the Pedro Ximénez white grape. The analytical results obtained show as the LT wine has the highest glutathione content, while the lowest ethanol concentrations. The use of chemometric tools applied to the volatile compounds allowed to differentiate the four wines by a principal component analysis (PCA) and the identification of 27 key compounds. The four wines did not show statistical differences in their smell or taste attributes and only the LT wine was visually differentiated from the rest.

Keywords: spontaneous fermentation; active dry yeast; wine; chemometric; glutathione; volatile compounds; non-*Saccharomyces*

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1. Introduction

Wine can be considered as a hydroalcoholic solution containing several hundreds of chemical compounds of natural biological origin in a wide range of concentrations. Some of them come directly from the grape itself and others are produced by the action of the microorganisms involved in the transformation of grape must into wine. Wine researchers propose the use of *Saccharomyces* yeast strains for their production of bioactive compounds or non-*Saccharomyces* yeasts with high enzymatic activity and secondary metabolite production as a realistic innovation in this sector [1]. Nevertheless, winemakers from areas covered by the Protected Designation of Origin (PDO) trademark are reluctant to embrace these innovations due to their unknown effects on the quality of wines traditionally elaborated in their PDO area. In the wineries of these areas and in organic wineries, the winemaking process is usually carried out using spontaneous fermentation or the "pied de cuve" technique, which aim to select the natural indigenous yeasts (*Saccharomyces* and non-*Saccharomyces*) provided by the grape itself. However, this methodology can lead to sluggish or arrested fermentations and a lack of reproducibility in the analytical and sensory characteristics of wines from different harvests. In this regard, although the presence of non-*Saccharomyces* yeasts was traditionally associated with undesirable aromas and defective wines, at present, it is known that these yeasts contribute with the production of key metabolites that impact

on the flavor and style of wines [2,3]. These properties make these yeasts powerful biotechnological tools to address the challenges in the chemical and microbiological composition of grape musts caused by climate change, especially in warm grape-growing regions.

Pedro Ximénez is a low-aromatic white grape variety that is well adapted to growing in the warm climates of southern Spain. However, the increase in the average temperatures during the ripening period causes an accelerated phenological development and an unbalanced chemical composition of the ripened grapes, with a high sugar content and high pH values. This results in wines with a high alcohol content and low acidity, causing significant problems with their microbiological and color stabilities [4]. In addition, these wines are at odds with the current consumer preference for low-alcoholic-content wines. Traditionally, winemakers have addressed the sugar increase through several accepted practices, ranging from vineyard treatments (canopy and vine management) to winemaking processes (partial de-alcoholization). Acidity is restored by adding tartaric acid or using ion exchange resins [5], and color stability is improved by increasing the SO_2 content or, more recently, by adding glutathione in its reduced form (GSH) to avoid browning problems in white wines [6].

Experts in wine microbiology suggest, as an innovation source for winemakers, the use of *Saccharomyces* and/or non-*Saccharomyces* yeast strains as starter cultures, so that these yeasts can dominate over the indigenous yeast microbiota. These cultures are commercially available as active dry yeasts (ADYs) and add interesting organoleptic properties to the wine compared to spontaneous fermentation [3,7]. Fermentations with selected ADYs that overexpress some attributes are becoming an emerging trend in winemaking [8] to increase the diversity of wines, as well as the use of yeast immobilization techniques [2]. As the market is constantly changing, the criteria for selecting and developing new yeast strains are also in a constant evolution and, at present, about 42 commercial products based on non-*Saccharomyces* yeasts are available for winemaking, of which 52% are based only on *Torulaspora delbrueckii*, *Lachancea thermotolerans* (LT), and *Metschnikowia pulcherrima* (MP) species [9].

Some of these ADY strains contribute to wines' sensory profiles, receiving positive feedback from consumers, and synthesize enzymes that can improve wine production processes, including filtration and clarification [10]. In this context, LT is noteworthy for its production of lactic acid, which enhances wine acidity and microbial stability by controlling spoilage microorganisms [11]. Another non-*Saccharomyces* yeast strain that has gained importance in recent years is MP. Its β-glucosidase activity and ability to enhance the production of esters, terpenes, fatty acids, and higher alcohols make it ideal for improving wines made from Verdejo white grapes and Shiraz red grapes [8,9,12,13].

Among the bio-active metabolites produced by yeasts during alcoholic fermentation, glutathione, a natural antioxidant, has special relevance. Glutathione is a sulfur-containing tripeptide composed of L-glutamyl-L-cysteinyl-glycine. It is predominantly presented in its reduced form (GSH), although it can also be oxidized (GSSG) or bound to other molecules [6]. This tripeptide is present in certain grape varieties and plays an important role in preventing oxidative reactions in musts and wines, thus avoiding their deterioration. GSH plays multiple roles in cells, including reactions with potentially toxic electrophilic compounds, being a cofactor for several enzymes, being involved in protein disulfide bond rearrangement, and the reduction of peroxides [14]. Adding GSH in enological processes is an authorized practice that can be achieved through various methods, one of which involves the addition of pure glutathione. However, this strategy results in increased costs for wineries. Another option is to add inactive dry yeasts enriched with this compound to white musts at the beginning of the fermentation process to prevent oxidation and maintain the sensory quality of wines that tend to brown [15]. Additionally, highly GSH-producing non-*Saccharomyces* yeasts or *S. cerevisiae* strains that have been improved for GSH production through an adaptive evolutionary technique can be used. The increase in glutathione levels in wine may be a promising method to partially diminish the addition of

sulfur dioxide (SO_2), thereby addressing the growing consumer preference for healthier and sustainable products and procedures [6].

There are two recommended strategies for using non-*Saccharomyces* yeasts as starter cultures. The first one involves the co-inoculation of non-*Saccharomyces* yeasts at a high cell concentration with *S. cerevisiae* to avoid stuck fermentations and achieve significant changes in the analytical and sensory properties of the wines [3,8]. Another strategy is sequential inoculation, in which non-*Saccharomyces* yeasts are initially inoculated at a high-cell population and allowed to ferment independently for a set period, prior to the inoculation of *S. cerevisiae* to complete the alcoholic fermentation. This enables the non-*Saccharomyces* yeast to better showcase its metabolic footprint without the stress of competition [16,17]. Contrary to the above recommendations, winemakers from traditional areas, who produce high-quality wines, have always used spontaneous fermentation or the "pied de cuve" technique as a strategy for must fermentation. They reject proposals to add ADYs as starter cultures, believing that it will alter the characteristics of traditional wines. However, the use of selected ADYs can be helpful to increase the levels of bioactive compounds and the sensory quality of wines to meet current consumer trends and create new wines.

This work focuses on exploring the differences in the chemical composition (enological variables and glutathione, major and minor volatile compounds, respectively) and the sensory attributes of young white wines from Pedro Ximénez grapes, using two commercial non-*Saccharomyces* yeasts (*L. thermotolerans* and *M. pulcherrima*), a glutathione-overproducing strain of *S. cerevisiae*, and the spontaneous fermentation of must as a control. Chemometric tools are applied to the analytical dataset, including multiple variable analysis (MVA) and principal component analysis (PCA), with the aim of establishing objective criteria for winemakers to select the most appropriate enological strategy to improve the traceability, quality, and diversity of their wines.

2. Materials and Methods

2.1. Winemaking Conditions

Must from Pedro Ximénez white grapes, grown in the warm-climate Montilla-Moriles winemaking region (Córdoba, southern Spain) and characterized by a density of 1088 gL^{-1} (21.4 °Bx) and a pH value of 3.78, was subjected to pre-fermentative corrections by the addition of 1.5 g L^{-1} tartaric acid and 100 mg L^{-1} potassium meta-disulfite ($K_2S_2O_5$). The must was then homogenized and divided into eight 2 L Pyrex glass cylinders, each containing 1.75 L, in order to obtain four different fermentation strategies, which were tested in duplicate. One of them was carried out following the spontaneous fermentation method, with the indigenous (wild) yeasts of the grapes (further referenced as WY). The other three fermentations were carried out by adding starter cultures of the following commercial ADYs: Glutaferm one®, Primaflora® VB, and Laktia™. Glutaferm one® is a *Saccharomyces cerevisiae* (SC) strain from AEB (Stuttgart-Möhringen, Germany), which has been improved by adaptative evolution for high GSH production and low H_2S production. Primaflora VB (from AEB) and Laktia from Lallemand® (Ontario, Canada) are non-*Saccharomyces* yeasts: *Metschnikowia pulcherrima* (MP) and *Lachancea thermotolerans* (LT), respectively. Each ADY was rehydrated following the manufacturer's instructions and precultured in a synthetic medium containing 50 g L^{-1} glucose, 2.8 g L^{-1} tartaric acid, 2.4 g L^{-1} potassium bitartrate, and 200 mg L^{-1} diammonium hydrogen phosphate (DAP). Aliquots of each preculture were added to the respective must to obtain a yeast population of 2×10^6 cells mL^{-1}. The eight cylinders were covered with hydrophobic cotton and placed in a water bath at the constant temperature of 20 °C until the fermentations were concluded (density < 1000 g L^{-1}).

2.2. Chemical Analysis

The enological variables of ethanol, titratable acidity, volatile acidity, pH, and reducing sugars were determined according to the OIV (2021) [18] protocols. Lactic and malic acids were quantified by reflectometry in a Reflectoquant™ (Merck®, Darmstadt, Germany). The

absorbances at 280, 420, 520, and 620 nm were measured in an Agilent Cary 60 UV-Vis spectrophotometer (Agilent technologies, Santa Clara, CA, USA). Glutathione was quantified by ultra-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in an Acquity H-Class UPLC (Waters, Mildford, MA, USA) and QTRAP 5500 Mass Detector (Sciex, Concord, ON, Canada), respectively.

Major volatile compounds and polyols were analyzed by gas chromatography using an Agilent 6890 GC (Agilent technologies, Santa Clara, CA, USA) equipped with a Flame Ionization Detector (FID) and a capillary column CP-WAX 57 CB (60 m, 0.25 mm, and 0.4 μm of film thickness) by the direct injection of the wine. Briefly, 10 mL of wine sample was added with 1 mL of a 1.018 gL^{-1} 4-methyl-2-pentanol (CAS 108-11-2) solution as the internal standard and 0.2 g solid calcium carbonate. This mixture was then stirred for 30 s in an ultrasonic bath and centrifugated at 5000 rpm for 10 min (2 °C), and lastly, 0.7 μL of the supernatant was injected into the gas chromatograph inlet. The absolute quantification of methanol, higher alcohols (1-propanol, isobutanol, 2-methyl- and 3-methyl-1-butanol, and 2-phenylethanol), acetaldehyde, acetoin, ethyl acetate, and the polyols glycerol and 2,3-butanodiol (*levo* and *meso* forms) was performed by a calibration table built with the standard solutions, containing a known concentration of the compounds and subjected to the same treatment as the samples [19].

Minor volatile compounds were analyzed using the platform SBSE-TD-GC-MS (Stir Bar Sorptive Extraction–Thermal Desorption–Gas Chromatography–Mass Spectrometry) composed by an Agilent-7890A GC coupled to a MSD 5975C (Wilmington, DE, USA) and a Multi-Purpose Sampler (MPS) from Gerstel (GmbH & Co. KG—Mülheim an der Rhur, Germany). The software ChemStation v. 02. 02. 1431 from Agilent and Maestro from Gerstel were used as a chromatographic data processing and platform control, respectively. The minor volatile compounds were extracted using the SBSE technique, using a Twister (10 mm long and 0.5 mm thick film) coated with polydimethylsiloxane (PDMS). Briefly, the procedure was: A total of 1 mL wine sample, 0.1 mL internal standard solution (0.4116 g L^{-1} hexyl butyrate in absolute ethanol), and 8.9 mL solution 12% (*v*/*v*) ethanol, containing 2.6 g L^{-1} tartaric acid and 2.2 g L^{-1} potassium bitartrate (pH 3.5), were added to a 10 mL vial. Then, the twister was placed in the vial and stirred at 1200 rpm and 20 °C for 120 min to favor the adsorption of the compounds in a Variomag Multipoint 15 magnetic stirrer (Thermo Fisher Scientific, Waltham, MA, USA). The twister was removed, water-rinsed, dried, and placed in a desorption tube to be transferred by MPS to the Thermal Desorption Unit (TDU) from Gerstel, where the volatiles were desorbed and transferred to the GC system. A HP-5MS-fused silica capillary column (60 m × 0.25 mm i.d. and 0.25 μm film) from Agilent Technologies was used along with an initial oven temperature of 50 °C (2 min), which was then increased at a rate of 4 °C/min to 190 °C for 10 min. The MSD operated at 70 eV in the electron impact mode (EI), with a mass range of 35–550 Da at a temperature of 150 °C. All samples were analyzed in triplicate. The quantification of the minor volatile compounds was conducted in a semiquantitative way as the mass equivalent to the internal standard by using its response factor.

All major and minor volatiles described in this work were identified and confirmed by GC-MS in the same Agilent 7890-MSD 5975C described before and using the same capillary column and settings for the temperature and carrier helium gas programs used for their analysis. Compound identification was performed by comparing the peak data of the compounds with the mass spectra libraries NIST08 and Wiley7 and consulting the NIST database from the Web of Chemistry. A second identification of the analyzed compounds was performed by subjecting a mixture of commercially available pure compounds to the same analytical conditions as those of the samples. Reagents and pure chemical compounds for the identification and quantification were provided by Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany), respectively.

2.3. Sensorial Analysis

The four wines were evaluated by a tasting panel composed of eight experienced judges (five males and three females) from the Department of Agricultural Chemistry, Edaphology, and Microbiology of the University of Córdoba, Spain. The panel used the OIV (2021) [18] official tasting sheet, which evaluates the attributes for sight (limpidity and aspects other than limpidity), smell (genuineness, positive intensity, and quality), and taste (genuineness, positive intensity, harmonious persistence, and quality). Furthermore, the judges were asked to issue a score for the general quality from 0 to 100 points, considering the previously scored attributes. All samples were stored for 24 h at 4 °C before the analysis. The wine samples (30 mL) were presented to the tasters at room temperature (20 °C) in standardized wine glasses (NF V09-110 AFNOR, 1995), according to the requirements by ISO 3591 norms [18]. Wines were poured in a random order, labeled with four-digit codes and with 1 min breaks between the samples.

2.4. Statistical Analysis

The analytical data were analyzed using the Statgraphics statistical software package (Centurion XVI v. 16. 1. 11). Multiple analysis of variance (MANOVA) and multiple variable analysis (MVA) were carried out to establish the significant differences between the four wines obtained. The dataset containing the concentration of major volatile compounds and polyols of wines was subjected to a principal component analysis (PCA) and the data matrix of the relative response obtained for the selected minor volatile compounds was also subjected to a PCA. Both analyses were performed using the PLS_toolbox v. 8. 5. 2 of MATLAB R2016a v. 9. 0. 0. 341360 (Natick, MA, USA).

3. Results and Discussion

3.1. Fermentation Progress and Enological Variables of the Wines

Figure S1 in the Supplementary Materials shows the fermentation progress according to the mean of the absolute density. This value decreased from 1088 to 990 g L^{-1} after 11 days for musts fermented with SC, while WY, MP, and LT reached values around 990 g L^{-1} after 16 days. The fermentation carried out with MP was the slowest, following a similar behavior to WY fermentations. The fermentations with LT and SC displayed a parallel evolution, with having LT a lower rate and ending with the highest density value. Thus, two different profiles were observed. One of them was shown by WY and MP, and was related to the presence of non-*Saccharomyces* yeasts in the must that initiate the fermentation process and to the *Saccharomyces* species that take over when the alcohol content is around 4% (*v*/*v*). This effect is shown in Figure S1 and can be explained by the competitive effect of MP on the *Saccharomyces* yeasts present in the must. Another profile was observed in the LT and SC fermentations, which show a faster fermentation rate than those of WY and MP, although the LT density decreased more gradually than that of SC. In this respect, it is known that non-*Saccharomyces* yeasts have limits in their fermentation potential, as they compete with other yeasts for nutrients and are sensitive to high alcohol contents. For these reasons, most of non-*Saccharomyces* yeasts are not able to dominate wild yeasts during fermentation [11].

The results obtained from quantifying the general enological variables in wines are summarized in Table 1. The ethanol content ranged from 12 ± 0.3 in LT to 14 ± 0.2% (*v*/*v*) in WY, SC, and MP. The pH value, total acidity (in g L^{-1} of tartaric acid), and volatile acidity (g L^{-1} of acetic acid) are variables closely related to the total content of acids in wines and to the wines' analytical and organoleptic qualities [20]. MP, WY, and SC wines had pH values around 3.18–3.29, while LT wines showed pH values of 3.39. The total acidity ranged from 6.30 to 7.7 g L^{-1} for SC, MP, and WY, increasing significantly in LT to 9.5 g L^{-1}. Regarding the volatile acidity, WY and MP wines showed the same mean value, while SC and LT reported 0.46 g L^{-1} and 0.39 g L^{-1}, respectively. Acceptable volatile acidity values for white wines should be lower than 0.5 g L^{-1}. These four enological variables, with the exception of the volatile acidity with three homogeneous groups (HGs), displayed four

HGs with a significance level of $p \leq 0.05$ for the four wines and, generally, those of LT were distinguished as the most different. All the obtained wines had a reducing sugar content below 1 g L^{-1}, indicating that the alcoholic fermentation process was completed. As their content is lower than 4 g L^{-1}, they are classified as dry wines.

Table 1. Enological variables of wines. Mean values and standard deviations. Different letters in the same row indicate different homogeneous groups at a significance level of $p \leq 0.05$. Wine samples: WY: obtained by spontaneous fermentation; SC: obtained using the glutathione-overproducing *Saccharomyces cerevisiae* strain; MP: wines using *Metschnikowia pulcherrima*; LT: wine using *Lachancea thermotolerans* yeast.

	WY	SC	MP	LT
Ethanol (% *v*/*v*)	13.95 ± 0.05^{cd}	13.75 ± 0.27^{b}	14.10 ± 0.00^{d}	12.30 ± 0.33^{a}
pH	3.23 ± 0.01^{b}	3.29 ± 0.00^{c}	3.18 ± 0.01^{a}	3.39 ± 0.03^{d}
Volatile acidity (g L^{-1})	0.27 ± 0.01^{a}	0.46 ± 0.00^{c}	0.27 ± 0.03^{a}	0.39 ± 0.00^{b}
Total acidity (g L^{-1})	7.70 ± 0.05^{c}	6.30 ± 0.08^{a}	7.17 ± 0.04^{b}	9.53 ± 0.21^{d}
Reducing sugars (g L^{-1})	0.17 ± 0.00^{a}	0.14 ± 0.00^{a}	0.22 ± 0.00^{a}	0.59 ± 0.20^{b}
IPT (Absorbance at 280 nm)	5.7 ± 0.2^{c}	5.7 ± 0.2^{c}	5.5 ± 0.1^{b}	4.32 ± 0.05^{a}
Absorbance at 420 nm	0.138 ± 0.001^{b}	0.137 ± 0.003^{b}	0.130 ± 0.014^{b}	0.090 ± 0.004^{a}
Absorbance at 520 nm	0.034 ± 0.001^{b}	0.033 ± 0.003^{b}	0.039 ± 0.013^{b}	0.024 ± 0.005^{a}
Lactic acid (g L^{-1})	0.23 ± 0.02^{a}	0.18 ± 0.00^{a}	0.25 ± 0.01^{a}	4.70 ± 0.50^{b}
Malic acid (g L^{-1})	0.88 ± 0.07^{c}	0.84 ± 0.02^{bc}	0.81 ± 0.01^{b}	0.44 ± 0.02^{a}
Gluthatione (mg L^{-1})	0.64 ± 0.04^{b}	1.99 ± 0.19^{c}	0.25 ± 0.01^{a}	6.61 ± 0.12^{d}

The absorbance measurements at 280 nm are related to the content in the compounds with conjugated double bonds (mainly polyphenols in wines), while the absorbances at 420 and 520 nm are related to yellow-brown and yellow-reddish colors and are considered indicators of wine browning. Wines produced using LT had the lowest values for these absorbances by comparison to the remaining inoculum tested at a significance level of $p \leq 0.05$ (Table 1). The values for A280 nm are considered common for these wine types, which usually range from 4 to 10. These results are aligned with those reported by Vaquero et al. (2022) [21] for LT wines.

Lastly, all wine samples showed low lactic acid concentrations, with the exception of LT, which had a value of about 5.0 g L^{-1}. By contrast, the malic acid content in LT wines was the lowest compared to that of the other samples, with SC wines having the highest content. Increases in the lactic and acetic acid contents, which are weaker acids than tartaric acid, affect the pH value, buffer capacity, and consequently the total acidity, increasing their values due to changes in the salification balance. One of the most interesting results obtained in this work is the content of glutathione. Higher glutathione values were expected in SC wines; however, their content (about 2 mg L^{-1}) was exceeded by that of LT, reaching over 6 mg L^{-1}. These results confirm those obtained by Vicente et al. (2021) [22] and Binati et al. (2022) [6], where the authors reported glutathione production by some non-*Saccharomyces* yeasts.

The use of non-*Saccharomyces* yeasts as starter cultures, particularly LT and MP, is recommended by their ability to make alcoholic beverages with a lower ethanol content [23,24]. In this context, LT had a slower kinetic growth and metabolized sugars at a lower rate than *S. cerevisiae*, leading to a lower production of ethanol during fermentation and a high production of glycerol and also lactic acid [21] (Tables 1 and 2). The use of MP as a starter culture had little impact on the analyzed enological variables in comparison to those obtained with WY fermentation. MP has a low fermentation potential compared to other non-*Saccharomyces* yeasts [24], and our results indicate that competition and interactions with the indigenous microbiota of grapes during fermentation limited the expected effects regarding the reduction in the ethanol content of wines [25]. By contrast, this yeast produced less volatile acidity in monocultures [26] and succinic, malic, and lactic acids during sequential inoculation [8]. Lastly, SC fermentations had a higher sugar consumption rate

than the rest and provided wines with similar values for the enological characteristics to those obtained using non-inoculated musts (WY), but a higher glutathione content and volatile acidity (Table 1).

Table 2. Mean values and deviations of the major volatile compounds and polyols (mg L^{-1}) in wines. $^{a–d}$ Different letters indicate different homogeneous groups (HGs) at a significance level of $p \leq 0.05$. WY: obtained by spontaneous fermentation. SC: obtained using the glutathione-overproducing *Saccharomyces cerevisiae* strain. MP: wines using *Metschnikowia pulcherrima*. LT: wines produced using *Lachancea thermotolerans*. CAS: identification number assigned by the Chemical Abstracts Service.

Compounds	CAS	WY	SC	MP	LT	HG
Methanol	67-56-1	41 ± 3^{a}	60 ± 9^{b}	45 ± 2^{a}	40 ± 2^{a}	2
1-Propanol	71-23-8	22 ± 1^{b}	18.7 ± 0.7^{a}	24 ± 1^{b}	69 ± 2^{c}	3
Isobutanol	78-83-1	65 ± 4^{b}	31.8 ± 0.9^{a}	93 ± 1^{d}	75 ± 3^{c}	4
2-Methyl-1-butanol	137-32-6	54 ± 2^{b}	47 ± 1^{a}	77 ± 1^{d}	73 ± 2^{c}	4
3-Methyl-1-butanol	123-51-3	313 ± 7^{c}	274 ± 7^{a}	301 ± 3^{b}	317 ± 10^{c}	3
2-Phenylethanol	60-12-8	57 ± 13^{ab}	50 ± 3^{a}	62 ± 4^{b}	82 ± 7^{c}	3
Acetaldehyde	75-07-0	68 ± 9^{ab}	95 ± 8^{b}	64 ± 4^{a}	200 ± 40^{c}	3
1,1-Diethoxyethane	105-57-7	0^{a}	0^{a}	0^{a}	8 ± 3^{b}	2
Acetoin	513-86-0	36 ± 3^{b}	30 ± 2^{a}	32 ± 2^{ab}	145 ± 7^{c}	3
Ethyl acetate	141-78-6	58.7 ± 0.6^{c}	42 ± 2^{b}	37 ± 2^{a}	86 ± 6^{d}	4
Ethyl lactate	97-64-3	17 ± 2^{a}	16.6 ± 0.6^{a}	21 ± 1^{a}	95 ± 9^{b}	2
Diethyl succinate	123-25-1	12 ± 3^{c}	7.9 ± 0.6^{b}	8 ± 1^{b}	0^{a}	3
2,3-Butanediol *levo*	24347-58-8	448 ± 149^{b}	378 ± 27^{ab}	460 ± 49^{b}	328 ± 28^{a}	2
2,3-Butanediol *meso*	5341-95-7	177 ± 70^{b}	115 ± 7^{a}	166 ± 14^{b}	126 ± 9^{a}	2
Glycerol (g L^{-1})	56-81-5	12 ± 2^{bc}	8.2 ± 0.5^{a}	12.5 ± 0.9^{b}	15 ± 2^{c}	3

As a summary, the tested starter cultures affect their respective wines in different ways. It is worth highlighting the lower ethanol content of those of LT wines, the lower malic acid content, higher volatile acidity, and the higher pH values related to the high total acidity (due to the higher content in lactic acid). Also, it is worth highlighting the high glutathione content related to the decrease in absorbances at 280, 420, and 520 nm when this yeast is inoculated in must. These results are aligned with those obtained by Vicente et al. (2021) [22] regarding the antioxidant effect of LT for preventing detrimental changes in color and oxidative aromas. Despite the considerable number of studies investigating the beneficial effects of glutathione, there are contradictory results regarding its impact on the organoleptic properties of wines. Therefore, further research is required to explore the antioxidant efficacy of GSH after wine fermentations as well as its role in the wine preservation, aging, and bottling processes [15].

3.2. Effects on the Major Volatile Compounds and Polyols

It is widely known that the wine aroma increases during the alcoholic fermentation process, mainly as a result of the secondary metabolism of *Saccharomyces* and non-*Saccharomyces* yeasts involved. Volatile metabolites whose concentration is about 10 mg L^{-1} or higher are known as major volatile compounds and are constituted by higher alcohols, some carbonylic compounds, and ethyl esters of acetic, succinic, and lactic acids. Other secondary and low volatile metabolites synthesized by yeasts are the polyols 2,3-butanediol and glycerol. The production of these compounds depends on several factors, such as the contents in fermentable sugars, assimilable nitrogen, fermentation temperature, yeast-derived products, and yeast species and strains [27].

Higher alcohols are those with more than two carbon atoms, and they are synthetized from the keto-acid pool, mainly via the Ehrlich pathway. They are volatile compounds influencing the intensity and quality of the wine overall aroma, although there is evidence of negative effects if their concentration is higher than 400 mg L^{-1} [28]. Among the five higher alcohols quantified, only 2-methyl-1-butanol and isobutanol showed four HGs (homogeneous groups), with WY and SC reporting the lowest values while non-*Saccharomyces* yeasts reported the highest (Table 2). The alcohols 1-propanol, 3-methyl-1-

butanol, and 2-phenylethanol showed three HGs, with SC wines containing the lowest values (Table 2). LT is the largest producer of 1-propanol and, according to Escribano et al. (2018) [26] and Vaquero et al. (2021) [29], its content is directly proportional to the amount of lactic acid produced. The only higher alcohol with a pleasant odor, recalling rose flowers, is 2-phenylethanol, which shows a higher concentration in LT wines, in accordance with the results of Nisiotou et al. (2019) [3]. Methanol showed its highest content in SC wines, while the remaining wines had similar values.

Carbonylic compounds, such as acetaldehyde and its derivative acetoin, displayed three homogeneous groups, having LT wines values twice than the remaining wines. In addition, LT wines were the only one that contained 1,1-diethoxyethane, a chemical combination of acetaldehyde with ethanol, mainly due to their high content of acetaldehyde. These two compounds are relevant and characteristic of velum wines subjected to biological aging in Jerez and Montilla-Moriles (Spain) and are also described as important metabolites of some major lactic-acid-producing strains [29].

Esters are also an important family of secondary metabolites, and the ethyl esters of acetic, lactic, and succinic acids have the highest contents in wines. Only ethyl acetate had four HGs, which correspond to the four wines; ethyl lactate and diethyl succinate showed two and three HGs, respectively. Ethyl lactate and ethyl acetate had higher concentrations in LT wines compared to the rest. In this regard, values higher than 60 mg L^{-1} in ethyl acetate could lead to varnish-like aromas. The concentration of this ester in the remaining wines was lower and could provide pleasant fruity aromas (pineapple). One should take into consideration that ethyl lactate has a concentration of around 100 mg L^{-1} in LT wines, which creates sweet, lactic acid, and yogurt aromas [29]. All samples contained 8–10 mgL^{-1} diethyl succinate, except for LT wines, with no quantifiable concentration.

The contents of the polyol 2,3-butanediol (*levo* and *meso* forms) showed small but significant differences at $p \leq 0.05$ between *Saccharomyces* and non-*Saccharomyces* yeasts. As for glycerol, it was observed that the SC yeast produced a lower concentration than the rest. Escribano et al. (2018) [26] indicated that LT and MP generated a glycerol overproduction in wines, thus confirming this result. The increase in glycerol contents affects the production of ethanol because the metabolic pathways for the formation of both have several common steps and intermediate products.

Lastly, 2,3-butanediol (*l* and *m*) and diethyl succinate in wines from spontaneous fermentation showed the highest deviations in their averaged contents.

3.3. Principal Component Analysis of GC-FID-Quantified Compounds

A principal component analysis (PCA) was performed using the data matrix built using the content of 15 compounds quantified in two biological replicates and analyzed in triplicate for each of the four wines (24 columns for 90 data each). The results are presented in Figure 1. The first two components accounted for 80.27% of the total variance (56.63% for PC1 and 23.64% for PC2). PC1 was mainly influenced by a positive contribution of 1-propanol, 1,1-dietoxyethane, ethyl lactate, ethyl acetate, 2-phenylethanol, acetaldehyde, glycerol, and 2- and 3-methylbutanol, while the most influential variables for PC2 were 2,3-butanediol (*l* and *m*), isobutanol, and methanol (see loadings in Table S1 in the Supplementary Materials). These two PCs established different groups among the four wines tested and demonstrated a lack of homogeneity in the WY wines obtained without the addition of a culture starter in comparison to those obtained with it. The heterogeneity of WY is explained by the differences in PC2 scores. LT wines were located to the right of the origin of coordinates, in the lower quadrant, with positive scores for PC1 and negative for PC2, indicating that these wines were different from the others. The remaining wines were to the left of PC1, with negative scores. Thus, SC had the lowest negative values for PC2, while WY and MP had higher values. WY wines showed two groups, corresponding to the two biological replicates. MP wines were grouped in PC2 with positive values, between the two WY previously cited. These results indicate that the wines obtained without inoculation were not easily distinguishable from those of MP and were also closer to the

fermentations carried out with a single inoculation of *S. cerevisiae* in the study of Binati et al. (2020) [10]. Some authors reported the presence of *M. pulcherrima* at the beginning of the fermentations carried out without additional inoculation [26], which could support the similarities between the WY and MP fermentations in our study.

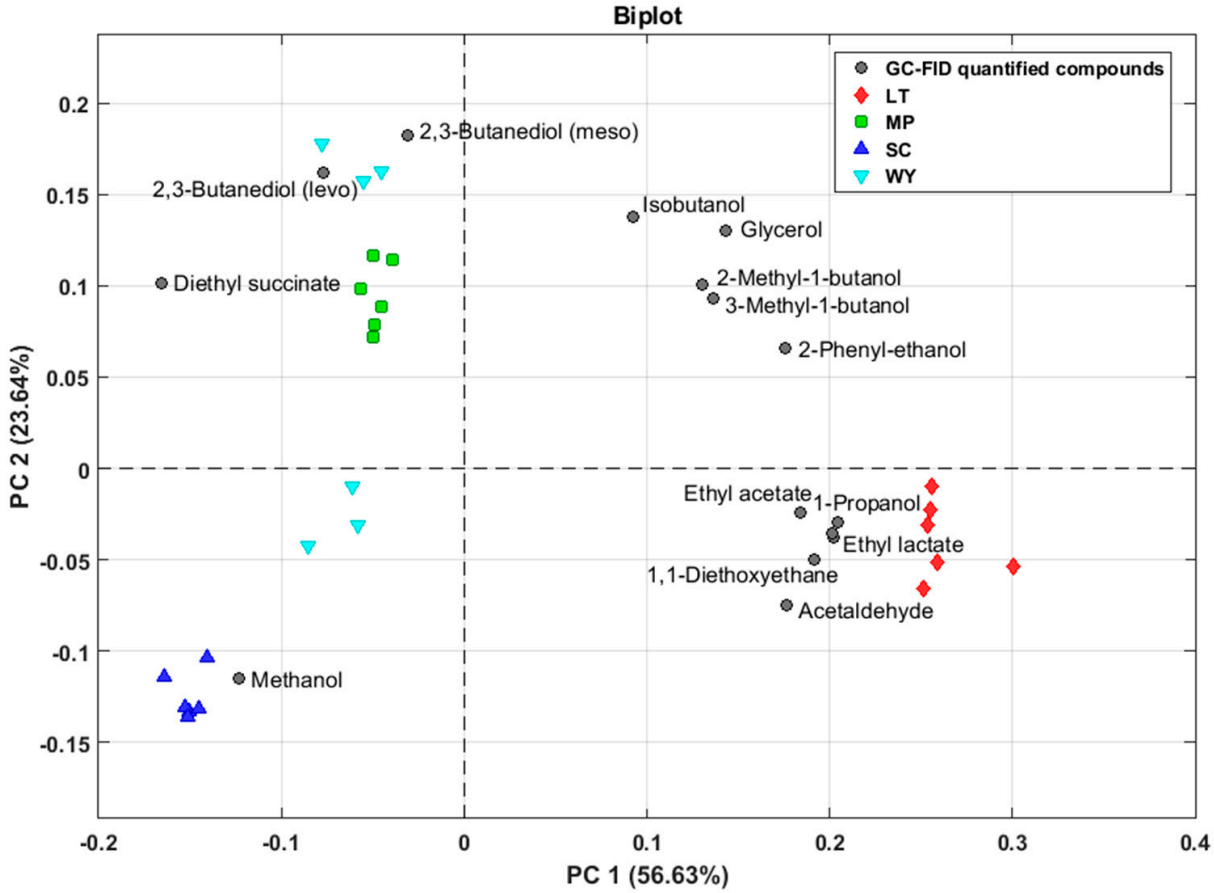

Figure 1. Principal component analysis biplot built using the absolute contents in 15 compounds quantified by GC-FID in the wines. Circles represent the variables and their coordinates the contribution to each component. Other geometric figures represent the scores of the wines in the two components. Abbreviations: WY: spontaneous fermentation; SC: glutathione-overproducing *Saccharomyces cerevisiae* strain; MP: *Metschnikowia pulcherrima*; LT: *Lachancea thermotolerans*.

3.4. Pattern Recognition Using the Semiquantitative SBSE-GC-MS Minor Volatile Compound Dataset

Table 3 shows the linear retention index (LRI) values, calculated using C7–C25 hydrocarbons according to Van Den Dool and Kratz, and those tabulated in the NIST Web of Chemistry. This information is important for proper compound identification when compared to the LRI values obtained using the same type of column and the same or similar chromatographic conditions. Unfortunately, this information is not available for all compounds identified under the conditions used in this work, but the values shown in Table 3 may help other researchers if using the same or similar analytical conditions.

Table 3. Mean values and standard deviations for the relative responses of minor volatile compounds identified by SBSE-GC-MSD in wines. WY: wine obtained by spontaneous fermentation. SC: wine using starter cultures of a glutathione-overproducing strain of *Saccharomyces cerevisiae*. MP: wines obtained using *Metschnikowia pulcherrima* as the starter culture. LT: wines obtained using *Lachancea thermotolerans*. Compounds families: I: Higher alcohols; II: Fatty acids; III: Ethyl esters of short, medium, and long fatty acids; IV: Acetates and other esters; V: Terpenes and derivatives; VI: Miscellaneous compounds. CAS: identification number assigned by the Chemical Abstracts Service. LRI: Linear retention index calculated and tabulated in the NIST Web of Chemistry. ID: Identification of the compound by mass spectrometry (MS) and by the addition of a pure chemical standard (S). NF: Not found in the NIST Web of Chemistry. * indicate values in a HP5 30 m column length. The different letters indicate homogeneous groups which significantly differ statistically in the parameters between wines ($p < 0.05$, F-test).

Families	Volatile Compound	CAS	ID	LRI_{cal}	LRI_{NIST}	WY	SC	MP	LT	HG
I (4)	2-Furanmethanol	98-00-0	MS, S	944.1	856.0	0^{a}	2.1 ± 0.6^{b}	2.6 ± 0.9^{b}	0^{a}	2
	1-Hexadecanol	36653-82-4	MS	2231.2	1883.5	0^{a}	1.6 ± 0.3^{b}	0^{a}	0^{a}	2
	1-Tetradecanol	112-72-1	MS	2065.4	1680.6	1.5 ± 0.6^{b}	0^{a}	0^{a}	0^{a}	2
	2-Ethyl-1-hexanol	104-76-7	MS	1045.9	1031.0	6.9 ± 0.5^{a}	6.7 ± 0.4^{a}	8 ± 1^{b}	$7.2 \pm_{ab} 0.7$	2
II (2)	Octanoic acid	124-07-2	MS, S	1348.2	1170.0	5 ± 2^{b}	8 ± 2^{c}	8 ± 3^{c}	0^{a}	3
	Decanoic acid	334-48-5	MS, S	1521.5	1380.0	0^{a}	42 ± 13^{c}	21 ± 8^{b}	0^{a}	3
III (11)	Ethyl isobutyrate	97-62-1	MS, S	840.7	774.0	0^{a}	0^{a}	0^{a}	4.1 ± 0.7^{b}	2
	Ethyl butanoate	105-54-4	MS, S	804.2	793.0	7 ± 1^{a}	6.6 ± 0.8^{a}	9 ± 1^{b}	6 ± 1^{a}	2
	Ethyl hexanoate	123-66-0	MS, S	1001.0	1001.0	29 ± 4^{b}	31 ± 3^{b}	31 ± 1^{b}	8 ± 3^{a}	2
	Ethyl octanoate	106-32-1	MS, S	1203.7	1196.0	$18.7 \pm_{c} 0.5$	20 ± 3^{c}	14 ± 3^{b}	10 ± 2^{a}	3
	Ethyl 9-decenoate	67233-91-4	MS	2304.8	1386.0	4.5 ± 0.7^{c}	3.7 ± 0.4^{b}	0^{a}	0^{a}	3
	Ethyl decanoate	110-38-3	MS, S	1402.9	1393.0	8.0 ± 0.6^{b}	9 ± 2^{b}	8.1 ± 0.5^{b}	6.6 ± 0.3^{a}	2
	Ethyl dodecanoate	106-33-2	MS, S	1602.7	1593.0	1.7 ± 0.1^{a}	0.9 ± 0.9^{b}	$1.85 \pm_{a} 0.08$	1.8 ± 0.4^{a}	2
	Ethyl 3-hydroxytridecanoate	107141-15-1	MS	1569.4	1539.0	0^{a}	13 ± 2^{b}	0^{a}	0^{a}	2
	Ethyl tetradecanoate	124-06-1	MS, S	1803.6	1782.0	$2.3 \pm_{ab} 0.4$	3 ± 2^{b}	$2.1 \pm_{ab} 0.5$	1.7 ± 0.3^{a}	2
	Ethyl hexadecanoate	628-97-7	MS, S	2004.5	1997.0	$5.8 \pm_{ab} 0.7$	6 ± 4^{ab}	8 ± 2^{b}	3.9 ± 0.7^{a}	2
	Ethyl 4-ethoxybenzoate	23676-09-7	MS	1563.3	1521.0	0^{a}	0^{a}	0^{a}	0.8 ± 0.1^{b}	2
IV (8)	3-Methyl-1 butanol acetate	123-92-2	MS, S	1129.2	876.0.	87 ± 6^{d}	74 ± 8^{c}	46 ± 4^{a}	55 ± 12^{b}	4
	2-Methyl-1 butanol acetate	624-41-9	MS, S	1197.9	879.0	7 ± 2^{a}	5.6 ± 0.4^{a}	6.5 ± 0.9^{a}	12 ± 3^{b}	2
	Octyl acetate	112-14-1	MS, S	1216.5	1209.0	2.8 ± 0.2^{a}	2.9 ± 0.2^{a}	2.8 ± 0.1^{a}	2.8 ± 0.1^{a}	1
	Butyl 2-methylbutanoate	15706-73-7	MS, S	1197.9	1047.0	3.1 ± 0.2^{a}	2.9 ± 0.4^{a}	3.1 ± 0.4^{a}	3.2 ± 0.2^{a}	1
	Dihydro methyl jasmonate	24851-98-7	MS	1820.9	1650.0	1 ± 1^{b}	2.0 ± 0.4^{c}	0^{a}	0^{a}	3
	Methyl hydrojasmonate	39924-52-2	MS	1802.8	1614.0	0.8 ± 0.9^{b}	0^{a}	2.0 ± 0.1^{c}	1.6 ± 0.2^{c}	3
	1H-Indole-3-ethanol, acetate	13137-14-9	MS	1925.0	1926.0	4.7 ± 0.8^{b}	0^{a}	0^{a}	0^{a}	2
	δ-Dodecalactone	713-95-1	MS, S	1733.3	1679.0	0^{a}	0^{a}	0^{a}	5 ± 2^{b}	2
V (5)	β-Pinene	127-91-3	MS	971.2	980.0	3.0 ± 0.6^{b}	0^{a}	0^{a}	0^{a}	2
	D-Limonene	5989-27-5	MS, S	1054.5	1032.0	118 ± 5^{b}	87 ± 7^{a}	85 ± 16^{a}	88 ± 4^{a}	2
	Nerolidol	142-50-7	MS, S	1778.6	1535.0	0^{a}	0^{a}	2.2 ± 0.3^{b}	0^{a}	2
	Geranyl acetone	689-67-8	MS	1554.3	1455.0	2.0 ± 0.3^{b}	2.4 ± 0.3^{c}	0^{a}	0^{a}	3
	Farnesol	4602-84-0	MS, S	1750.0	1658.0	3.3 ± 0.7^{b}	$1.97 \pm_{a} 0.09$	7 ± 1^{c}	1.3 ± 1.4^{a}	3

Table 3. *Cont.*

Families	Volatile Compound	CAS	ID	LRI_{cal}	LRI_{NIST}	WY	SC	MP	LT	HG
VI (7)	Benzophenone	119-61-9	MS, S	1704.8	1621.0	0 ^{a}	0.9 ± 0.1 ^{b}	0 ^{a}	0 ^{a}	2
	2,4-Di-tert-butylphenol	96-76-4	MS	1524.7	1513.0 *	42 ± 6 ^{ab}	34 ± 3 ^{a}	48 ± 10 ^{bc}	56 ± 15 ^{c}	3
	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	1620-98-0	MS	2280.3	1774.0 *	1.9 ± 0.2 ^{a}	2.1 ± 0.1 ^{a}	2.3 ± 0.5 ^{a}	2.3 ± 0.6 ^{a}	1
	Decanal	112-31-2	MS, S	1211.2	1207.0	8.3 ± 0.7 ^{a}	9.4 ± 0.5 ^{a}	9.2 ± 0.8 ^{a}	11 ± 1 ^{b}	2
	Cyclododecane	294-62-2	MS	1768.6	NF	0 ^{a}	9 ± 1 ^{b}	0 ^{a}	9 ± 1 ^{b}	2
	1-Decene	872-05-9	MS	1873.1	993.0 *	9 ± 1 ^{b}	0 ^{a}	9.8 ± 0.5 ^{b}	0 ^{a}	2
	2,5-Cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-	6738-27-8	MS	1680.7	NF	0 ^{a}	0 ^{a}	1.8 ± 0.2 ^{b}	0 ^{a}	2
	Number of compounds					28	27	26	25	

The mean values, standard deviation, and HGs at a significance level of $p \leq 0.05$ obtained for the relative response of the determined 37 minor volatiles are shown in Table 3. This way of expressing the results of the SBSE-GC-MS analysis is faster and easier to obtain than the construction of expensive calibration tables with commercially available pure compounds. Moreover, it is useful to identify key compounds that allow the classification of wines obtained with different strategies of fermentation. Seven chemical groups or families were detected: 4 alcohols, 2 medium chain fatty acids, 11 ethyl esters of medium- and long-chain organic acids, 8 other esters, 5 terpenes, and 7 compounds classified as miscellaneous. Among these compounds, 1 had four HGs, 10 had three HGs, 23 compounds had two HGs, and only 3 volatiles (octyl acetate, butyl 2-methylbutanoate, and 3,5-di-tert-butyl-4-hydroxybenzaldehyde) had one HG. Seven compounds showed higher levels or were only detected in WY and SC wines. Only two compounds (nerolidol and 2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-) were reported in detectable amounts in MP wines and three (ethyl isobutyrate, ethyl 4-ethoxybenzoate, and δ-dodecalactone) in LT wines, although the latter showed the lowest number of compounds (**22**). The compounds *β*-pinene and 1-tetradecanol were detected only in WY and 1-hexadecanol, ethyl-3-hydroxytridecanoate, and benzophenone in SC wines.

All seven families of volatile compounds contribute to the aroma of the wines. Higher alcohols are formed during alcoholic fermentation and contribute to the secondary aromas of wine [8]. The presence of 2-Ethyl-1-hexanol in all wines contributes to fresh and citrus aromas, and its amount was slightly higher in wines obtained with non-*Saccharomyces* yeasts. Octanoic and decanoic acids showed the highest levels in SC wines. These acids are important metabolic by-products of *S. cerevisiae* yeast under hypoxic stress conditions and are associated with fat, rancidity, and soap-like flavors [19]. Ethyl esters are the most important group among the compounds detected, due to their contribution to the fruity aroma. According to Sgouros et al. (2020) [30] and Ge et al. (2022) [31], ethyl isobutyrate increases in fermentations with the presence of non-*Saccharomyces* yeasts, especially LT [30,32,33] and *Torulaspora delbrueckii* [34,35]. Solvent-like, fresh, and fatty are the general descriptors for ethyl 4-ethoxybeanzoate [36]. The ethyl esters of C4–C16 are powerful wine odorants with fruity odor descriptors [19], and LT wines had the lowest levels. Nevertheless, the lactone δ-dodecalactone, with coconut, creamy, and fruity aromas, was detected only in LT. This compound provides protection against various fungi and bacteria [37,38]. Terpenes are accountable key compounds in Muscat varieties and other aromatic grapes, and they can be released on their bound-glycosylated precursors via glucosidase action [10]. The most important one detected in this study was D-limonene, which results from the dehydroxylation and cyclization of nerol. This conversion can be catalyzed by enzymes during wine fermentation or in the presence of low pH values [39]. It provides a citrus and fresh orange aroma to the wine [40]. Lastly, among the miscellaneous compounds, the presence

of 2,4-di-tert-butylphenol and 3,5-di-tert-butyl-4hidroxybenzaldehyde stands out, which were produced by *S. cerevisiae* and was present in all wines.

3.5. Principal Component Analysis of the Data Matrix Obtained by GC-MS

The triplicate analysis carried out on the two biological replicates of the obtained wines provided a dataset of 37 rows by eight columns. However, three volatiles were discarded due to their low differentiating value since they presented only one HG. The resulting data matrix contained 816 data (eight columns for 34 compounds analyzed in triplicate) and was used for the detection of key compounds, which helped to identify significant differences among the wines. The performed PCA (Figure 2) resulted in three PCs with eigen values above 1 and accounted for 79.46% of the total variance (35.14% for PC1; 23.91% for PC2; and 20.41% for PC3). Table S2 in the Supplementary Materials contains the contribution (loadings) of each compound to these PCs. Figure 2 shows the sample scores for the four wines in the three selected PCs. This three-dimensional plot allows to visualize the reproducibility of the behavior of each yeast in the biological replicates for each fermentation strategy studied with regard to the volatiles determined. Secondly, the grouping of the four wines according to their scores in the three PCs obtained is interesting as it can be used to understand their characterization and traceability. Thus, the WY wine stands out as the most different from the others, having positive values for the three PCs. SC wines were located near the area defined as PC1–PC2 and exhibited positive scores for PC1 and PC3 and negative for PC2. The wines of MP were located in the plane opposite to that of SC, with negative values for PC1 and PC3 and positive values for PC2. Lastly, LT wines were located in the area opposite to that of WY, with negative values for PC1 and PC3 and positive values for PC2.

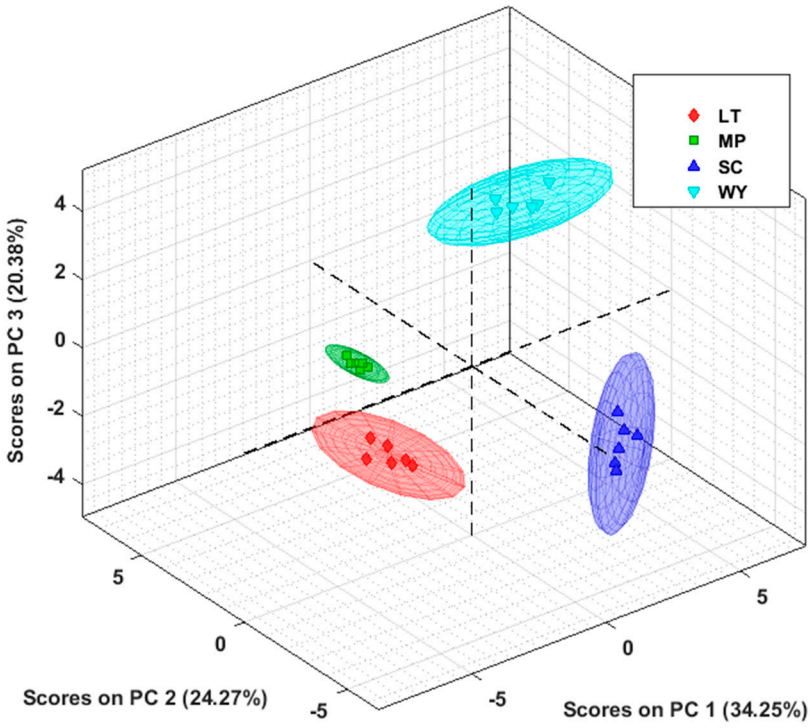

Figure 2. Plot of the principal component analysis (PCA) of the wines obtained with starter cultures using the selected 34 minor volatile compounds determined. The scores of each biological replicate, analyzed in triplicate, are shown. Abbreviations: WY: spontaneous fermentation; SC: glutathione-overproducing *Saccharomyces cerevisiae* strain; MP: *Metschnikowia pulcherrima*; LT: *Lachancea thermotolerans*.

Table S2 in the Supplementary Materials shows the volatiles that influence the three PCs. PC1 was affected mainly by 12 compounds with loads higher than 0.200 or lower than

−0.200, while seven volatiles contributed to PC2, with methyl hydrojasmonate being in common with PC1. PC3 was affected by 12 volatiles with a load higher than 0.200. The values of the obtained loads allows the selection of the most significant compounds influencing the PCs. PC1 was mainly affected by the ester family, showing values between 0.258 for ethyl octanoate and −0.230 for 1-butanol, 2-methyl acetate. Other compounds, such as geranyl acetone and δ-dodecalactone, contributed with loads of 0.255 and −0.231, respectively. PC2 was mainly affected by ethyl butanoate, the terpenes nerolidol and farnesol, and miscellaneous compounds, such as cyclododecane, 1-decene, and 2,5-cyclohexadien-1-one, 2,6-bis(1,1-dimethylethyl)-4-ethylidene-. Finally, PC3 was influenced by three higher alcohols, decanoic acid, ethyl-3-hidroxytridecanoate, and the acetate of 3-methyl-1-butanol, two terpenes (*β*-pinene and D-limonene), and other miscellaneous compounds.

3.6. Sensorial Analysis of the Wines

Table 4 shows that the MANOVA statistical treatment established only one HG for the smell, taste, general quality, and global score of the wines tested, which indicates that the tasters did not find significant differences in these attributes among the four wines. The tasters only perceived significant differences between the visual aspect of the LT wine, which had the highest score compared to the rest. This evaluation can be related to the lowest absorption values of LT wines and their highest glutathione contents, which agrees with the results obtained by Vaquero et al. (2021) [29], who found that LT produced less golden hues. The results obtained for the odor attribute show the complexity of its relationships with the aroma compounds identified in all wines, despite each of them having different contents for the major volatiles and different numbers and levels of the minor volatiles. Also, the difference of 1% v/v in ethanol content and the high lactic acid content (4.7 g L^{-1}) in LT wines compared to the other wines did not lead to a different evaluation by the tasters. In terms of the overall quality, none of the four wines was rated "unpleasant or unacceptable", and all were considered good wines (70–76 points), with the MP wine receiving the highest score (76 points).

Table 4. Mean scores, standard deviations, and homogeneous group values for the organoleptic attributes tested. Different letters in the same row indicate homogeneous groups with statistical differences at a significance level of $p \leq 0.05$. Identification of wine samples: WY: wine obtained by spontaneous fermentation. SC: obtained with a glutathione-overproducing strain of *Saccharomyces cerevisiae*. MP: wines produced using *Metschnikowia pulcherrima*. LT: wines produced using *Lachancea thermotolerans*.

Attributes	WY	SC	MP	LT	HGs
Sight	7.55 ± 0.69 [a]	7.09 ± 1.30 [a]	7.91 ± 1.14 [ab]	8.55 ± 0.82 [b]	3
Smell	14.82 ± 1.99 [a]	13.36 ± 2.25 [a]	14.64 ± 2.62 [a]	13.91 ± 1.81 [a]	1
Taste	27.73 ± 3.77 [a]	28.82 ± 3.68 [a]	30.09 ± 5.26 [a]	26.91 ± 3.24 [a]	1
Overall quality	21.36 ± 2.69 [a]	21.73 ± 2.76 [a]	23.73 ± 2.53 [a]	21.36 ± 3.04 [a]	1
Total points	71.45 ± 7.46 [a]	71.00 ± 8.07 [a]	76.36 ± 10.18 [a]	70.73 ± 6.92 [a]	1

4. Conclusions

The study of the chemical composition and the organoleptic evaluation of wines obtained by spontaneous fermentation with wild yeast (WY) and wines obtained from the same must fermented with the addition of starter cultures of the non-*Saccharomyces* yeasts *Lachancea thermotolerans* (LT) and *Metscnikowia pulcherrima* (MP) or a glutathione-producing strain of *Saccharomyces cerevisiae* (SC) allowed us to draw several conclusions.

The use of SC or MP does not affect values of the general enological variables used for wine characterization. Only the wines obtained with LT showed a lower ethanol content and absorbances at 420 and 520 nm and higher values of volatile acidity, total acidity, lactic acid, and glutathione than the others. All the wines show no significant differences in the smell and taste attributes, and only LT wines scored higher than the others in the visual aspect category. This better score may be related to the higher glutathione content of these

wines and the lower absorbance values at 520 nm. The chemometric analysis of the content of the major volatile compounds and polyols allowed us to distinguish among the SC, LT, and MP wines, but not between MP and spontaneously fermented wines. The principal component analysis performed on the dataset of 34 minor volatile compounds allowed the differentiation of the four wines obtained and the selection of 27 key compounds. The results obtained show the great potential of chemometric methods to differentiate wines obtained from the same must with different fermentation strategies. This is extremely important for the objective identification and the traceability of wines produced in the traditional way as opposed to those produced according to new trends.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9121023/s1, Table S1: Loadings obtained from the data matrix of major volatile compounds used as chemical variables to build the PCA of wines; Table S2. Loadings obtained from the data matrix of minor volatile compounds used as chemical variables to build the PCA of wines; Figure S1. Fermentation progress. Starter cultures supplied to grape must: WY spontaneous fermentation without starter addition. SC *Saccharomyces cerevisiae* glutathione over-producing. MP: *Mestchnikovia pulcherrima*. LT: *Lachancea termotolerans*.

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