fermentation

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The Effect of Yeast, Sugar and Sulfur Dioxide on the Volatile Compounds in Wine

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Abstract: This study compares three yeast strains: two wild *Saccharomyces cerevisiae* strains (Sc1 and Sc5) and a commercial strain (Lc). The objective is to assess their fermentation efficiency and volatile compound production. The factors examined are yeast strain, initial sugar concentration of the must, and the presence of sulfur dioxide. Volatile aroma compounds, determined via GC–MS, were categorized into aromatic series based on aroma descriptors. Out of the volatile compounds analyzed, the yeast strain influenced 39, while sugar content and sulfur dioxide affected 16 and 23 compounds, respectively. Twelve compounds displayed odor activity values exceeding unity, with notable contributions from ethyl esters, β-damascenone, and β-ionone, impacting fruit, floral, and herbal aromatic series. Overall, the Sc1 yeast strain exhibited higher values in the aromatic series compared to the Lc strain. Multivariate analysis revealed that the Sc1 strain highlighted green fruit, citrus, and spice series, while the Lc strain stood out for smoky and herbal aromas. Cluster and principal component analyses emphasized that the aromatic composition of wines produced with wild yeast strains is more influenced by sulfur dioxide than initial sugar content, whereas the opposite holds true for the commercial strain. The key aroma series distinguishing between yeast strains were fruity, green fruit, and citrus for Sc1, and herbal, floral, and smoky for Lc. In conclusion, the Sc1 wild yeast strain showed similar fermentation behavior to the commercial strain, resulting in increased aroma compound presence. The distinctive aromatic profiles contributed by each strain enable winemakers to leverage this diversity and create wines that emphasize specific aromas.

Keywords: fermentation efficiency; *Saccharomyces cerevisiae*; sugar concentration; sulfur dioxide; volatilome; wild yeast; wine

Citation: Martín-García, F.J.; Palacios-Fernández, S.; López de Lerma, N.; García-Martínez, T.; Mauricio, J.C.; Peinado, R.A. The Effect of Yeast, Sugar and Sulfur Dioxide on the Volatile Compounds in Wine. *Fermentation* **2023**, *9*, 541. https://doi.org/10.3390/fermentation9060541

Academic Editor: Maurizio Ciani

Received: 9 April 2023
Revised: 30 May 2023
Accepted: 1 June 2023
Published: 4 June 2023

1. Introduction

Spain is considered among the leading countries in wine production and marketing, and its international export levels are among the highest in the world [1]. Many wine regions are distributed throughout the Spanish geography which are dedicated to elaborate wine products of great variety and organoleptic quality [2]. Therefore, among the biggest concerns for the winemaking sector is the increasingly noticeable effect of climate change on vine crops, in addition to its consequences on the production and conservation of wines.

Weather conditions derived from climate change, such as the more irregular rainfall or longer periods of warm temperature each year, increase grapevine vulnerability to water stress and to high salinity levels in the soil [3–5]. Several authors have detailed the consequences of these effects on vine crops. Some of them [6–8] outline the decrease in grape weight, with consequent maturation problems, a notable sugar increase in the grape, and a high alcoholic degree in wines. In addition, a decrease in the contents of tartaric acid and malic acid is described, the second being the most essential in the acidity of the grape [9–11].

In addition, to achieve adequate phenolic maturity, red grapes are subjected to a longer maturation period, which entails a much higher final sugar content in grape. Additionally, the synthesis of aromas together with polyphenols can be inhibited at temperatures higher than 30 °C [9].

On the one hand, there are some studies about the modulation of structural and technical farm characteristics to mitigate injuries in vineyards [12]. Additionally, there are several viticultural practices to minimize the effect of climatic change: (i) minimizing excessive exposure of leaves and clusters, thereby reducing their temperature and shielding them against sunburn, by implementing sprawling trellis systems; (ii) employing late pruning to deliberately delay the phenological cycle by postponing pruning activities; (iii) adjusting the leaf-to-fruit ratio to postpone the harvest date, primarily by impeding the rapid accumulation of sugars and thus achieving a synchronization of phenolic and industrial ripeness; (iv) applying sun protectants to reflect a portion of the solar radiation reaching the plants, resulting in decreased plant temperature [13–16]. Additionally, studies highlight the genetic bases that regulate the resistance of the vine to these stress conditions, seeking to enhance that resistance. Similarly, desired genotypes in the cultivated varieties have been tested, given the greater genetic diversity they expose compared to wild varieties [6]. Nevertheless, despite these mechanisms, climate change continues to undesirable consequences not only for the plant, but for the product, by altering its organoleptic qualities as well.

Furthermore, wine fermentation by yeasts is conditioned by biotic and abiotic stress. The high sugar level could determine incomplete fermentations that may result in wines with a large amount of residual sugar content and organoleptic defects [17]. On the contrary, a complete fermentation could potentially determine an overly high alcoholic degree in the final product. Therefore, a recent research trend carries the isolation and selection of yeasts trained to ferment musts with solvency under extreme conditions, which could enable obtaining high-quality wines despite the climate change effects [18].

As described by several authors [19,20], the fermentation of must with a high sugar concentration presents numerous challenges for wine yeast. The elevated sugar levels subject the yeast to osmotic stress, which can alter its metabolism, and may also lead to fermentation difficulties and the potential for spoilage by undesirable microorganisms. Additionally, the high sugar concentration can alter the aroma profile of the resulting wines and, as a response to the osmotic stress acetic acid, may be produced in high concentrations [19], which could even hinder the commercialization of the resulting wine. Some research lines open horizons as well to the utilization of different *Saccharomyces* strains or non-*Saccharomyces* yeasts [21] to obtain good-quality wines under climate change conditions. In addition, some of those research trends focus on the utilization of these yeasts to produce wines containing lower ethanol levels [22,23].

To prevent the above-mentioned spoilage, winemakers increase the application of antimicrobial agents such as sulfur dioxide. It is well known that sulfur dioxide application needs to be adequately moderated, since high doses could alter the organoleptic qualities of the product as well as to produce toxicity to the consumer [24,25]. Nevertheless, the use of this compound has become very useful in the winemaking process, since it has multiple advantages such as its antiseptic, antioxidant capability [25].

The aim of this study is to compare two specific wild yeast strains with a commercial strain in terms of their efficiency in fermenting high-sugar musts in the presence and absence of sulfur dioxide. Additionally, the volatile composition of wines produced under these experimental conditions will be compared.

2. Materials and Methods

2.1. Chemical Standards

The identification and quantification of aroma compounds were carried out with standard solutions of pure compounds of analytical grade, purchased from Sigma-Aldrich (Darmstadt, Germany), Merck Darmstadt, Germany) and Fluka (Madrid, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, MA, USA).

2.2. Yeasts

Three *Saccharomyces cerevisiae* yeast strains were used in this work. Lalvin Clos® (Lc), a commercial strain known by its good fermentative capacity and its high tolerance to the presence of sulfur dioxide in the medium [26], was used as control. Its effect on the oenological parameters, and volatile composition of the resulting wine was compared with the strains, *S. cerevisiae* 1 and *S. cerevisiae* 5 (Sc1 and Sc5), which were isolated and selected by its capability to ferment completely must with high sugar concentration. The screening and selection of both yeasts was carried out by the Institut Català de la Vinya i el Vi (INCAVI), Barcelona, Spain.

Yeast inoculum was performed in flasks containing 150 mL of YPD medium (1% yeast extract, 2% peptone and 2% glucose in distilled water), and incubated at 28 °C for 48 h at 165 rpm. Fermentations were conducted in a 250 mL flask containing 150 mL of pasteurized grape must.

2.3. Fermentation Conditions

Pasteurized musts supplied by Baixas Lehnberg, Tarragona, Spain were used for this study. Sugar content was modified by adding sterilized glucose and fructose (equimolecular) to a final content of 220 and 250 g/L. Furthermore, the effects of the presence and absence of sulfur dioxide were studied in musts with 250 g/L sugar. Therefore, 75 mg/L of sulfur dioxide (as potassium metabisulfite) was added to the corresponding flasks.

Three flasks were inoculated for each strain and each experimental condition (220 g/L sugar, 250 g/L sugar, and 250 g/L sugar + 75 mg/L sulfur dioxide) with 1×10^6 cells/mL, resulting a total of 18 flasks. Fermentation was performed at 22 °C.

The evaluation of the fermentation kinetics was made by measuring the loss of weight of the flasks due to the release of CO_2 during fermentation [27,28]. Checkpoints were performed once every 24 h. Fermentation was considered finished when no difference in weight for a given flask was observed in 48 h. The kinetic values were obtained by representing the loss of mass per unit of time during the time of fermentation.

2.4. Determination of Oenological Parameters

Oenological parameters such as pH, titratable acidity, volatile acidity, ethanol and residual sugar content were determined according to the official EEC methods [29]. Glycerol was determined with the enzymatic test of R-Biopharm AG, (Darmstadt, Germany).

2.5. Volatile Compounds Determination

Volatile compounds in must and wines can be classified according to their contents in major volatile compounds (≥10 mg/L) and minor volatile compounds (<10 mg/L). Three biological replicates were used to undertake the analysis.

2.5.1. Major Volatile Compounds

Major volatile compounds and polyols were quantified in a gas HP 6890 Series II chromatograph, from Agilent Technologies (Palo Alto, CA, USA), equipped with the capillary column CP-WAX 57 CB (50 m in length, 0.25 mm in internal diameter and 0.4 µm), and a FID, according to the conditions described by Peinado et al. [30]. The 0.5 µL aliquots of 10 mL wine samples previously supplied with 1 mL of 4-methyl-2-pentanol as the internal standard (1024 mg/L) were injected. Tartaric acid in the wine was previously removed by precipitation with 0.2 g of calcium carbonate, followed by centrifugation at 300× *g*.

The chromatographic conditions were as follows: a split ratio of 30:1, an FID, and a temperature program involving an initial temperature of 50 °C (15 min), a 4 °C/min ramp, and a final temperature of 190 °C (35 min) were used. The injector and detector temperatures were 270 and 300 °C, respectively. The flow rate of the carrier gas (helium) was initially set at 0.7 mL/min (16 min) and followed by a 0.2 mL/min ramp to the final value (1.1 mL/min), which was held for 52 min. To identify and quantify the analyzed

compounds, standards were injected under the same conditions as the samples. Additional information about LRI to identify volatile compounds is detailed in Table S1.

2.5.2. Minor Volatile Compounds

These compounds were identified and quantified in a two-step process, both described previously in detail by López de Lerma et al. [31]. The first one consists of an extraction procedure using stir bars (film thickness 0.5 mm, 10 mm length, Gerstel GmbH, Mülheim an der Ruhr, Germany). These are placed in a vial containing 10 mL of 1:10 diluted sample and 0.1 mL of ethyl nonanoate (0.4464 mg/L) as the internal standard. After 100 min of stirring at 1500 rpm, the stir bars were removed and inserted into a desorption tube for chromatographic analysis.

The second phase consists of the determination of the volatile compounds in a GC–MS equipped with a Gerstel TDS 2 thermal desorption system. Desorption tubes, containing the stir bars, were heated at 280 °C with the aim of releasing the volatile compounds in a CIS 4 PTV cooling system programmed at 25 °C, which contains a Tenax adsorption tube. Lastly, the CIS is heated to release the volatiles in the GC–MS equipped with an Agilent-19091S capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The mass detector works in the scan mode at 1850 V and checks the mass from 39 to 300 amu.

To identify the volatile compounds, analytical standards were injected under the same chromatographic conditions as the samples and the retention times together with the Wiley spectral library was used. Quantification was made using calibration curves. Aditional information about LRI to identify volatile compounds is detailed in Table S1.

2.6. Calculation of Aroma Series

The odor activity values (OAV) of the volatile compounds were determined as the ratio between concentration and the odor perception threshold. An aromatic series includes the volatile compounds with similar aroma descriptors, and its value is obtained as the sum of the OAVs of the volatile compounds that integrate it. In this way, there were twelve aroma series, namely, chemistry, citrus, creamy, floral, fruity, green, green fruit, herbal, honey, smoky, spice, and waxy. Based on its aroma descriptors, a given volatile aroma compound can be included in one or several aromatic series.

2.7. Statistical Analysis

Multivariate analysis of variance (MANOVA) was carried out to test the differences among the oenological parameters, volatile aroma compounds and aromatic series of the obtained wines under the studied conditions. A footprint of the wines was obtained by multivariate analysis using the aromatic series. Lastly, such aromatic series were used to perform cluster and a principal component analyses. To this end, the statistical software Statgraphics Plus v. 2 from STSC, Inc. (Rockville, MD, USA) was used.

3. Results

3.1. Fermentation Conditions and Kinetics

As described in material and methods section two sugar concentrations (220 and 250 g/L) were chosen to test the assayed strains—220 g/L equals to 12.9% (*v*/*v*) to 13% (*v*/*v*) of ethanol, a normal concentration under typical climatic conditions during grape maturation. However, in recent years, increasing temperatures provoke a difference between industrial maturation and phenolic and aromatic maturation. To obtain an appropriate phenolic and aromatic maturation, winemakers, usually, delay grape harvest, which gives rise to increasing sugar contents of the grape (approximately 250 g/L). This high sugar concentration together with the use of sulfur dioxide, mainly as antimicrobial agent, makes it difficult to ensure proper completion of alcoholic fermentation.

Table S2 and Figure S1 show the fermentation kinetics of the three yeast studied. At 220 and 250 g/L of initial sugar content, the yeast Sc5 reaches the maximum amount of CO_2 released. No remarkable differences among yeasts were observed in the time needed

to reach the maximum, and as expected, fermentations finished in a shorter time when the initial sugar content was lower. However, Lc always finished in first place.

On the other hand, the presence of sulfur dioxide was a determining factor in the fermentation kinetics of the three yeasts. In the Sc1 and Sc5 strains, a delay in the beginning of the fermentation was observed in its presence, which did not seem to affect the fermentation rates of Lc as much. Furthermore, sulfur dioxide induced lower maximum rates of daily ethanol production.

3.2. Oenological Parameters

Prior to fermentation, the oenological variables of the must were analyzed providing the results: pH = 3.4 ± 0.1; titratable acidity (g tartaric acid/L) = 6.6 ± 0.1; volatile acidity (g acetic acid/L) = 0.08 ± 0.1.

Among the oenological variables, once the fermentation finished (Table 1), it is surprising that in wines produced by Lalvin Clos yeast (Lc), the residual sugars are above 5 g/L. This commercial yeast is recommended for the fermentation of musts with a high concentration of sugars, so that the inability to finish fermentation may be due to the low levels of nitrogen easily assimilated from the starting must (80 mg/L). However, the two wild yeasts (Sc1 and Sc5) finished the fermentation completely. The few differences observed in the ethanol content are because the conversion rate of sugars into ethanol can vary for each yeast, although it is accepted that 1% (*v*/*v*) of ethanol is produced for 17 g/L of sugars [32].

Table 1. Oenological parameters of the wines obtained under the studied conditions. TA: titratable acidity (g tartaric acid/L); VA: volatile acidity (g/L) (g acetic acid/L); RS: residual sugars (g/L). MANOVA: multivariate analysis of variance performed with the factors: yeast strain (yeast), initial sugar content (sugar) and the presence or absence of sulfur dioxide (SO_2).

	220 g/L of Initial Sugars			250 g/L of Initial Sugars			250 g/L of Initial Sugars and 70 mg/L of SO_2			MANOVA		
	Sc1	**Sc5**	**Lc**	**Sc1**	**Sc5**	**Lc**	**Sc1**	**Sc5**	**Lc**	**Yeast**	**Sugar**	**SO_2**
pH	3.17 ± 0.05	3.28 ± 0.04	3.20 ± 0.04	3.17 ± 0.03	3.28 ± 0.02	3.24 ± 0.02	3.22 ± 0.03	3.26 ± 0.04	3.20 ± 0.03	ns	ns	ns
TA	6.2 ± 0.2	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.2	6.25 ± 0.07	6.3 ± 0.1	6.3 ± 0.2	6.2 ± 0.1	ns	ns	ns
VA	0.47 ± 0.03	0.53 ± 0.02	0.30 ± 0.01	0.53 ± 0.01	0.72 ± 0.02	0.43 ± 0.03	0.96 ± 0.02	1.01 ± 0.02	0.43 ± 0.01	***	**	***
Glycerol (g/L)	6.2 ± 0.2	5.1 ± 0.2	4.6 ± 0.1	6.9 ± 0.3	5.6 ± 0.1	5.2 ± 0.1	7.6 ± 0.3	6.4 ± 0.2	5.8 ± 0.2	***	**	**
RS	1.8 ± 0.4	0.35 ± 0.07	6.3 ± 0.4	3.8 ± 0.4	1.8 ± 0.4	7.5 ± 0.7	4.3 ± 0.4	1.5 ± 0.7	8.0 ± 0.7	***	***	ns
Ethanol (% *v*/*v*)	12.8 ± 0.1	12.9 ± 0.1	12.9 ± 0.1	14.6 ± 0.1	14.8 ± 0.1	13.9 ± 0.1	14.6 ± 0.1	14.9 ± 0.1	13.9 ± 0.1	***	***	ns

** denotes significant differences at the 99% confidence level; *** denotes significant differences at the 99.9% confidence level; ns denotes no significant differences.

On the other hand, a relation between the initial sugar content of the must and the production of glycerol, as well as with the presence of sulfurous, with the Sc1 yeast being the one that produces the highest glycerol content, is observed. Additionally, it is noteworthy that the two wild yeasts produce higher amounts of acetic acid in wines with initial concentrations of sugar of 250 g/L. This fact was reported in wines produced from must of dried grapes [19]. It is well known that there is a relationship between the production of this compound and that of glycerol, being greater with a higher production of glycerol. Yeasts produce glycerol to protect themselves from osmotic stress and a greater amount of acetic acid is also produced to balance the redox potential [33].

3.3. Volatile Compounds

Table 2 shows the volatile compound concentration determined in wines under the assayed conditions, and the results of the multivariate analysis of variance performed considering the factors of variability. The concentration of a given compound can be influenced by the factors: (i) initial sugar concentration, (ii) yeast strain and (iii) presence of sulfur dioxide.

Table 2. Chemical families and volatile aroma compounds (μg/L, except where indicated), determined in wines obtained under the studied conditions. MANOVA: multivariate analysis of variance performed with the factors: yeast strain (yeast), initial sugar content (sugar) and the presence or absence of sulfur dioxide (SO_2).

	220 g/L of Initial Sugars			250 g/L of Initial Sugars			250 g/L of Initial Sugars and 70 mg/L of SO_2			MANOVA		
	Sc1	**Sc5**	**Lc**	**Sc1**	**Sc5**	**Lc**	**Sc1**	**Sc5**	**Lc**	**Yeast**	**Sugar**	**SO_2**
Acetate esters (mg/L)	13.8 ± 0.9	6.5 ± 0.4	13.0 ± 0.6	12.4 ± 0.4	6.2 ± 0.2	12.0 ± 0.7	16.2 ± 1.0	10.6 ± 0.3	11.4 ± 0.3	***	ns	***
Isoamyl acetate	112 ± 8	46 ± 3	189 ± 9	158 ± 5	35 ± 3	176 ± 11	81 ± 6	62 ± 2	189 ± 4	***	ns	ns
Ethyl acetate (mg/L)	13.4 ± 0.9	6.27 ± 0.38	12.5 ± 0.6	12.0 ± 0.4	5.94 ± 0.19	11.6 ± 0.7	16.0 ± 1.0	10.5 ± 0.3	11.0 ± 0.3	***	ns	***
2-Phenylethyl acetate	314 ± 21	218 ± 14	237 ± 15	295 ± 22	213 ± 9	217 ± 17	146 ± 9	86 ± 3	259 ± 2	**	ns	**
Hexyl acetate	0 ± 0	0 ± 0	0.62 ± 0.03	0 ± 0	0 ± 0	0.14 ± 0.01	0 ± 0	0 ± 0	0 ± 0	***	*	ns
Alcohols (mg/L)	260 ± 12	244 ± 15	220 ± 12	261 ± 1	254 ± 1	198 ± 16	114 ± 5	154 ± 3	240 ± 6	ns	ns	**
Isoamyl alcohols (mg/L)	176 ± 10	174 ± 10	183 ± 11	181 ± 6	176 ± 2	174 ± 1	88 ± 4	125 ± 3	204 ± 5	*	ns	**
2-Phenylethanol (mg/L)	80 ± 3	67 ± 5	32 ± 1	77 ± 6	75 ± 2	19 ± 17	23 ± 1	25 ± 0	32 ± 1	***	ns	***
Hexanol	1832 ± 91	2119 ± 85	2801 ± 97	1748 ± 32	2145 ± 66	2527 ± 84	1214 ± 90	1570 ± 48	2831 ± 93	***	ns	*
2-Ethyl-1-hexanol	1014 ± 92	1056 ± 29	1742 ± 69	451 ± 15	1133 ± 49	774 ± 53	1863 ± 114	1699 ± 115	1622 ± 99	ns	***	***
Dodecanol	45 ± 4	30 ± 2	20.8 ± 0.9	20 ± 1	38 ± 2	19.6 ± 0.6	46 ± 3	71 ± 5	23 ± 1	***	ns	***
Guaiacol	82 ± 4	72 ± 5	55 ± 3	95 ± 5	90 ± 3	61 ± 2	69 ± 5	96 ± 5	43 ± 1	***	**	**
4-Vinylphenol	24 ± 2	14.0 ± 0.7	294 ± 8	20 ± 2	21 ± 1	231 ± 10	12.0 ± 0.7	14.0 ± 0.6	276 ± 12	***	*	ns
2-Methoxy-4-vinylphenol	14 ± 1	11.0 ± 0.4	141 ± 3	10.5 ± 0.6	15.4 ± 0.9	102 ± 8	12.6 ± 0.7	16 ± 1	119 ± 6	***	**	ns
Carbonyl compounds	64 ± 2	55 ± 2	40 ± 2	51 ± 3	57 ± 2	35.8 ± 0.2	77 ± 4	43 ± 2	48 ± 2	***	ns	*
Heptanal	2.0 ± 0.2	1.06 ± 0.05	1.04 ± 0.09	1.15 ± 0.05	0.84 ± 0.07	0.81 ± 0.06	2.4 ± 0.2	1.04 ± 0.06	1.16 ± 0.06	***	***	***
Octanal	1.7 ± 0.1	1.3 ± 0.1	2.4 ± 0.2	1.34 ± 0.09	1.14 ± 0.09	1.91 ± 0.09	2.9 ± 0.2	1.66 ± 0.09	2.0 ± 0.1	***	ns	***
Nonanal	15 ± 1	2.4 ± 0.1	4.1 ± 0.2	12.7 ± 0.9	3.1 ± 0.2	3.0 ± 0.2	14.0 ± 0.7	3.5 ± 0.2	4.3 ± 0.2	***	*	**
Decanal	2.9 ± 0.1	0.84 ± 0.07	1.98 ± 0.09	0.79 ± 0.06	1.9 ± 0.1	0.23 ± 0.02	1.9 ± 0.2	2.4 ± 0.2	0.36 ± 0.02	*	*	ns
Benzaldehyde	30 ± 2	31 ± 2	23 ± 2	17.1 ± 0.8	44 ± 1	22.5 ± 0.5	25 ± 2	26 ± 2	34 ± 3	*	ns	ns
6-Methyl-5-hepten-2-one	12.4 ± 0.7	18.3 ± 0.5	7.1 ± 0.4	18 ± 2	6.5 ± 0.3	7.5 ± 0.3	31 ± 2	9.1 ± 0.4	5.2 ± 0.3	***	ns	ns
Ethyl esters	1435 ± 83	934 ± 25	1101 ± 26	1381 ± 25	968 ± 17	1070 ± 37	1429 ± 36	1390 ± 21	1067 ± 6	***	ns	**
Ethyl propanoate	142 ± 10	74 ± 4	108 ± 7	127 ± 10	108 ± 7	71 ± 2	86 ± 4	115 ± 5	71 ± 4	**	ns	ns
Ethyl butanoate	404 ± 26	426 ± 12	464 ± 11	357 ± 11	396 ± 8	447 ± 32	317 ± 8	386 ± 2	425 ± 9	***	**	*
Ethyl 3-methylbutanoate	36 ± 2	1.70 ± 0.09	4.7 ± 0.3	28.9 ± 0.9	4.2 ± 0.2	20.0 ± 0.6	23.7 ± 0.7	3.3 ± 0.1	25 ± 1	***	ns	ns
Ethyl 4-hydroxybutanoate	37 ± 1	18.5 ± 0.8	22 ± 2	47 ± 4	25 ± 1	2.1 ± 0.1	18 ± 1	16.1 ± 0.7	30 ± 2	*	ns	ns
Ethyl hexanoate	246 ± 24	141 ± 7	196 ± 3	337 ± 7	148 ± 2	190 ± 6	263 ± 7	240 ± 4	186 ± 1	*	ns	ns
Ethyl heptanoate	0.54 ± 0.04	1.48 ± 0.09	0 ± 0	0.68 ± 0.04	1.54 ± 0.01	0 ± 0	0 ± 0	0.38 ± 0.03	0 ± 0	***	ns	***
Ethyl octanoate	272 ± 13	189 ± 6	232 ± 6	301 ± 11	193 ± 1	264 ± 10	355 ± 19	254 ± 5	271 ± 2	***	**	***
Ethyl decanoate	223 ± 12	69.6 ± 0.6	51 ± 1	131 ± 10	70 ± 2	62 ± 3	220 ± 9	288 ± 5	46.4 ± 0.9	***	ns	**
Ethyl dodecanoate	68 ± 2	6.2 ± 0.2	4.6 ± 0.2	44 ± 3	13.6 ± 0.9	5.43 ± 0.06	125 ± 7	71 ± 3	3.16 ± 0.03	***	ns	***
Ethyl hexadecanoate	4.88 ± 0.09	7.6 ± 0.4	19.1 ± 0.5	7.1 ± 0.4	9.0 ± 0.4	8.0 ± 0.6	21 ± 2	16 ± 1	8.5 ± 0.5	ns	ns	**
Lactones (mg/L)	57 ± 1	34 ± 1	31 ± 0	41 ± 1	40 ± 0	37 ± 2	36 ± 1	34 ± 1	32 ± 1	***	ns	*
γ-Crotonolactone (mg/L)	33 ± 2	19 ± 1	21 ± 1	26 ± 1	27 ± 1	16 ± 1	27 ± 1	25 ± 1	17 ± 1	***	ns	ns
γ-Butyrolactone (mg/L)	24 ± 1	15 ± 0	9 ± 1	15 ± 1	13 ± 1	21 ± 2	9 ± 0	8 ± 0	15 ± 1	ns	ns	**
γ-Nonalactone	9.3 ± 0.7	9.3 ± 0.6	11.4 ± 0.6	9.2 ± 0.9	10.8 ± 0.6	7.6 ± 0.3	14.7 ± 0.6	11.1 ± 0.5	11 ± 1	*	ns	ns
Valerolactone	51 ± 4	23 ± 2	24 ± 2	47 ± 3	32 ± 2	15.2 ± 0.8	24 ± 2	25 ± 1	31 ± 2	***	ns	ns
Nor-isoprenoids	344 ± 14	295 ± 14	546 ± 34	279 ± 14	300 ± 13	363 ± 11	293 ± 25	227 ± 16	418 ± 10	***	***	ns
β-Damascenone	330 ± 13	286 ± 14	542 ± 34	269 ± 15	292 ± 13	356 ± 10	283 ± 25	221 ± 16	410 ± 10	***	***	ns
β-Ionone	1.47 ± 0.01	1.47 ± 0.03	1.50 ± 0.01	1.47 ± 0.01	1.47 ± 0.01	1.50 ± 0.01	1.46 ± 0.03	1.47 ± 0.01	1.48 ± 0.02	***	ns	ns
Vitispirane	12.7 ± 0.8	7.7 ± 0.5	3.2 ± 0.1	8.6 ± 0.4	6.6 ± 0.5	5.5 ± 0.5	8.8 ± 0.5	4.7 ± 0.4	6.5 ± 0.4	***	ns	ns
Terpenoids	104 ± 3	88 ± 4	118 ± 3	109 ± 5	101 ± 0.2	113 ± 3	113 ± 4	114 ± 5	120 ± 2	***	ns	*
Linalol	1.7 ± 0.2	1.34 ± 0.08	0.60 ± 0.06	1.6 ± 0.2	2.4 ± 0.2	0 ± 0	8.1 ± 0.6	0.50 ± 0.04	0.32 ± 0.03	**	ns	ns
Limonene	28 ± 1	23 ± 1	22.5 ± 0.7	22 ± 1	20 ± 1	24 ± 1	19.0 ± 0.7	23 ± 1	23 ± 1	ns	*	ns
β-Farnesene	6.0 ± 0.1	6.0 ± 0.2	11.8 ± 0.3	6.2 ± 0.3	6.3 ± 0.1	11.6 ± 0.1	5.8 ± 0.1	6.2 ± 0.3	11.7 ± 0.2	***	ns	ns
E-Nerolidol	17.9 ± 0.6	14.2 ± 0.8	15.8 ± 0.7	20.0 ± 0.9	15.7 ± 0.4	15.0 ± 0.2	21.8 ± 0.9	20.8 ± 0.7	17.4 ± 0.6	***	ns	***
Z-Dihydrofarnesol	17 ± 1	12.7 ± 0.6	21 ± 1	16.7 ± 0.9	14.2 ± 0.4	27 ± 1	26 ± 2	17 ± 1	24 ± 1	***	*	*
Farnesol 3	18.9 ± 0.7	18 ± 1	32.9 ± 0.3	26 ± 2	30.3 ± 0.8	23 ± 1	15.4 ± 0.8	33 ± 2	32.1 ± 0.9	**	ns	ns
Geranyl acetone	15 ± 1	13.0 ± 0.6	12.8 ± 0.2	16.2 ± 0.4	12.5 ± 0.6	12.0 ± 0.3	17 ± 1	13.6 ± 0.9	11.3 ± 0.9	***	ns	ns
Methyl esters	4.4 ± 0.2	5.6 ± 0.2	2.3 ± 0.2	5.1 ± 0.1	6.7 ± 0.2	5.3 ± 0.2	5.5 ± 0.3	7.5 ± 0.3	5.7 ± 0.2	***	***	*
E-Methyl dihydrojasmonate	4.4 ± 0.2	5.6 ± 0.2	2.3 ± 0.2	5.1 ± 0.1	6.7 ± 0.2	5.3 ± 0.2	5.5 ± 0.3	7.5 ± 0.3	5.7 ± 0.2	***	***	*

* Denotes significant differences at the 95% confidence level; ** denotes significant differences at the 99% confidence level; *** denotes significant differences at the 99.9% confidence level; ns denotes no significant differences.

Forty-seven volatile compounds were determined, and the chemical families with the highest contents were alcohols, acetate esters and lactones.

As products of the esterification between alcohols and acids during wine fermentation, esters are, in addition to alcohols, the compounds that contribute the most to the aroma of the alcoholic drinks [34]. According to their chemical nature, esters can be classified into acetate esters, ethyl esters and other esters. Altogether, esters confer wines with sweet and fruity-like aromas [31,34]. The influence of the yeast strain on the ester content has been described by many authors [35–37], although they also have a chemical origin in wine [38].

Ten ethyl esters and four acetate esters were detected, and the ethyl acetate content in wines fermented by Sc1 and Lc is greatest. Among the different esters families' acetate esters is the most representative. The levels of acetate esters in all the wines obtained, except for those obtained by Sc5 in the absence of sulfur dioxide, markedly exceeded the odor perception threshold (Table S3), which revealed their contribution to the wine aroma. Furthermore, they were present at lower than the undesirable level (>150 mg/L,) which would have provided the wines a nail polish, varnish and solvent-like aroma. A noticeable contribution to the wine aroma also comes from isoamyl acetate, ethyl propanoate, ethyl butanoate, ethyl 3-methylbutanoate, ethyl hexanoate and ethyl octanoate, due to their concentration above the perception thresholds. Regarding the influence of sulfur dioxide in the ester levels, a moderate increase was detected in the wines fermented by Sc5. These results are in accordance with those described by Morgan et al. [39]. Nevertheless, these results contrast with other works that described a decrease in the ester content due to the presence of sulfur dioxide [40].

Lactones are cyclic esters produced through the intramolecular condensation of carboxylic acid and alcohol groups. There has been limited research conducted to explore the factors influencing the production of unsubstituted lactones. However, it seems that butyrolactone is generated through yeast catabolism of glutamic acid [38]. Although lactones showed the second highest contents in wines in this study, greater than the contents of γ-crotonolactone and γ-butyrolactone, they were not representative since they do not exceed their odor thresholds. In general, the concentration of the analyzed lactones seems to be dependent only on the yeast strain.

Some alcohols and phenols are pre-existing in musts, and others are released during yeast metabolism [36]. The contents of alcohols and phenols were lower than 300 mg/L in all wines, so they contribute positively to the aroma complexity at these levels [38]. Eight different alcohols were detected, with isoamyl alcohols and 2-phenylethanol having the greatest influence. Isoamyl alcohols are described to confer burnt, alcohol scents to the wines, whereas 2-phenylethanol contributes with floral, rose, honey essences [31]. Both alcohols were present at highly above their thresholds, unlike the rest of the alcohols. Exceptionally, 4-vinylphenol showed appreciable levels in wines obtained by Lc, which could entail little chemical, phenolic nuances in their aromas. Overall, the results highlight that the presence of sulfur dioxide induced lower concentrations of some alcohols, such as isoamyl alcohols, 2-phenylethanol and hexanol, in wines fermented by Sc1 and Sc5. In this sense, Sun et al. [41] provided similar results in wines produced from strawberry fruit. In terms of the other factors, the concentration of the individual alcohols depends, to a high extent, on the yeast strain and, to a lower extent, on the initial sugar content.

Carbonyl compounds, and particularly aldehydes, constitute a significant group of aroma compounds that have the potential to influence the sensory attributes of the ultimate wine product. These compounds are produced during the yeast metabolism of sugars [42], and their production is affected by the presence of sulfur dioxide [43]. Most of the carbonyl compounds detected in the wines were below the odor threshold so they may not have much influence on wine aroma. Nonanal levels in wines fermented by Sc1 had the greatest effect, enriching the overall aroma of those wines with some citrus nuances. The contents of carbonyl compounds were influenced by the strain used, with the lowest levels obtained in wines produced by Lc.

Nor-isoprenoids are ubiquitous flavor compounds and arise from the enzymatic or chemical breakdown of carotenoid pigments. As is reported in the bibliography, β-damascenone has a low sensory threshold and is often reported to have the highest odor activity of any compound in wine [38]. However, it is not an important odorant and its aroma is rarely the dominant sensation perceived in a wine [44], although it can enhance fruitiness associated with esters [45]. Here, β-damascenone was the nor-isoprenoid with the highest concentration, influenced by the yeast strain (highlighting the contents produced by Lc) and the initial sugar concentration of the must.

Terpenoids, are present in the grape and released to the must during prefermentative and fermentative treatments. Their contents are mainly linked to grape variety, although some factors can contribute to change the terpenoid profile of the wine such as enzymatic and chemical transformation [38]. Additionally, yeast autolysis [31], can influence the final content of terpenoids. Seven terpenes were detected in this study, and the wines obtained by the Lc strain had the highest content. Neither the initial sugar concentration nor the presence of sulfur dioxide significantly impacts the majority of the analyzed terpenes.

3.4. Aroma Profile

The aroma of a wine is determined not only by the volatile compounds it contains, considered individually, but also by the interactions among them [46]. Theoretically, by calculating the odor activity value (ratio concentration vs. odor perception threshold), we can obtain information about the influence of a given compound on the wine's aroma. In this way, volatile compounds with an odor activity value above unity indicate a potential contribution to the wine's aroma. However, a way of taking into account all aroma compounds is constructing aromatic series as described in the materials and methods section. The ultimate result is obtaining a volatilome fingerprint, which reduces the number of variables to take into account when analyzing differences among oenological treatments. Based on the volatile compounds that show similar aroma descriptors (Table S3), twelve aromatic series were established (Table 3): fruity, green fruit, green, creamy, citrus, chemical, honey, waxy, spice, herbal, floral and smoky. As in the individual aroma compounds, here the influence of initial sugar concentration, yeast strain and the presence of sulfur dioxide were taking into account.

Table 3. Odorant activity values of the aromatic series determined in wines obtained under the studied conditions. MANOVA: multivariate analysis of variance performed with the factors: yeast strain (yeast), initial sugar content (sugar) and the presence or absence of sulfur dioxide (SO_2).

	220 g/L of Initial Sugars			250 g/L of Initial Sugars			250 g/L of Initial Sugars and 70 mg/L of SO_2			MANOVA		
	Sc1	**Sc5**	**Lc**	**Sc1**	**Sc5**	**Lc**	**Sc1**	**Sc5**	**Lc**	**Yeast**	**Sugar**	**SO_2**
Chemistry	23 ± 1	15.4 ± 0.6	23 ± 1	21.6 ± 0.4	15.1 ± 0.3	21.9 ± 0.8	22 ± 1	18.3 ± 0.4	22.2 ± 0.3	***	*	*
Citrus	12.4 ± 0.6	5.2 ± 0.2	6.9 ± 0.3	9.2 ± 0.6	5.9 ± 0.3	4.8 ± 0.2	12.5 ± 0.6	6.7 ± 0.3	5.5 ± 0.2	***	**	**
Creamy	1.93 ± 0.01	1.28 ± 0.04	1.26 ± 0.03	1.48 ± 0.05	1.51 ± 0.02	1.32 ± 0.05	1.51 ± 0.05	1.33 ± 0.05	1.30 ± 0.07	***	ns	ns
Floral	80 ± 2	71 ± 2	106 ± 5	71 ± 2	74 ± 2	79 ± 2	68 ± 4	58 ± 2	87 ± 2	***	**	ns
Fruity	124 ± 7	80 ± 2	103 ± 3	131 ± 2	84 ± 1	109 ± 3	127 ± 5	104 ± 2	113 ± 1	***	ns	*
Green	1.47 ± 0.07	1.30 ± 0.04	1.45 ± 0.03	1.30 ± 0.06	1.01 ± 0.03	1.33 ± 0.04	1.9 ± 0.1	1.07 ± 0.01	1.45 ± 0.02	***	*	**
Green fruit	30 ± 2	11.3 ± 0.5	15.6 ± 0.3	34 ± 1	12.6 ± 0.2	21 ± 1	27 ± 1	18.4 ± 0.3	21.7 ± 0.2	***	*	ns
Herbal	68 ± 2	62 ± 2	99 ± 5	60 ± 2	63 ± 2	73 ± 2	62 ± 4	53 ± 2	80 ± 2	***	***	ns
Honey	1.25 ± 0.08	0.87 ± 0.06	0.95 ± 0.06	1.18 ± 0.09	0.85 ± 0.04	0.87 ± 0.07	0.58 ± 0.04	0.34 ± 0.01	1.03 ± 0.01	**	ns	**
Smoky	1.49 ± 0.03	1.26 ± 0.06	4.28 ± 0.05	1.57 ± 0.07	1.61 ± 0.06	3.4 ± 0.2	1.25 ± 0.05	1.69 ± 0.08	3.6 ± 0.2	***	ns	ns
Spice	5.1 ± 0.4	2.3 ± 0.2	2.4 ± 0.2	4.7 ± 0.3	3.2 ± 0.2	1.52 ± 0.08	2.5 ± 0.2	2.5 ± 0.1	3.1 ± 0.2	***	ns	ns
Waxy	58 ± 3	39 ± 1	48 ± 1	62 ± 2	41 ± 1	53 ± 2	74 ± 4	54 ± 1	55 ± 1	***	*	***

* Denotes significant differences at the 95% confidence level; ** denotes significant differences at the 99% confidence level; *** denotes significant differences at the 99.9% confidence level; ns denotes no significant differences.

All the aromatic series depend on the yeast strain and, except for creamy, smoky and spice series, at least another factor. The chemistry, citrus green and waxy series significantly depend on the three studied factors.

The fruity series has the greatest influence. Values of this series were not influenced by the initial sugar although significantly dependent on the yeast strain and the presence of sulfur dioxide. Furthermore, the wines that showed the highest fruity OAVs were those fermented by Sc1, whereas those fermented by Sc5 showed the lowest levels. This relates directly to their ester levels, highlighting ethyl octanoate, the compound that contributed

the most, ethyl hexanoate and ethyl 3-methyl butanoate. All of them contribute with tropical fruit nuances. Sc1 also highlights the production of aromas related to green fruit notes. Opposite to the fruity series, their contents depend significantly on the initial sugar contents but not on sulfur dioxide. The floral series was the second series with the highest values, highlighting those reached by the Lc yeast strain. As in the case of the green fruit series, their contents are not influenced by the presence of sulfur dioxide.

Among the contribution of the individual aroma compounds to the aromatic series only twelve compounds show odor activity values above unity, and most of them are ethyl esters (ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl acetate) that contribute, except for ethyl acetate, to the fruity series. Ethyl hexanoate and ethyl octanoate also contribute to the green fruit and waxy series, respectively. Other series such as floral and herbal are mainly influenced by β-damascenone and β-ionone. Ethyl octanoate and β-damascenone are those that contribute the most to wine aroma and, as can be seen in Table 2, depend on the yeast strain and the initial sugar concentration; and in the case of ethyl octanoate, also on the sulfur dioxide.

As reported by other authors [47,48], yeast strain is among the most differentiating factor in relation to the aroma profile of the wines. Here, a limited number of series are affected by the presence of sulfur dioxide and the initial sugar concentration of the must, but all aromatic series are influenced by the yeast strain (Table 3).

3.5. Multivariate Analysis

Figure 1 shows the results of a ray graph obtained by means of multivariate analysis. To construct such a graph, the values of the aromatic series are standardized, so each variable receives equal weight in the visual impression. The unity represents the average value of a given aromatic series. Values above the unity indicate that for a given condition these wines show higher values than the average. As Chambers et al. found [49], by means of this procedure, we can determine the dominant series for a given observation. Figure 1a shows the results obtained when the initial sugar concentration was 220 g/L. The green fruit, citrus and spice series are highlighted in Sc1 whereas the smoky and herbal series stand out for Lc. In general, Sc5 shows values above the median.

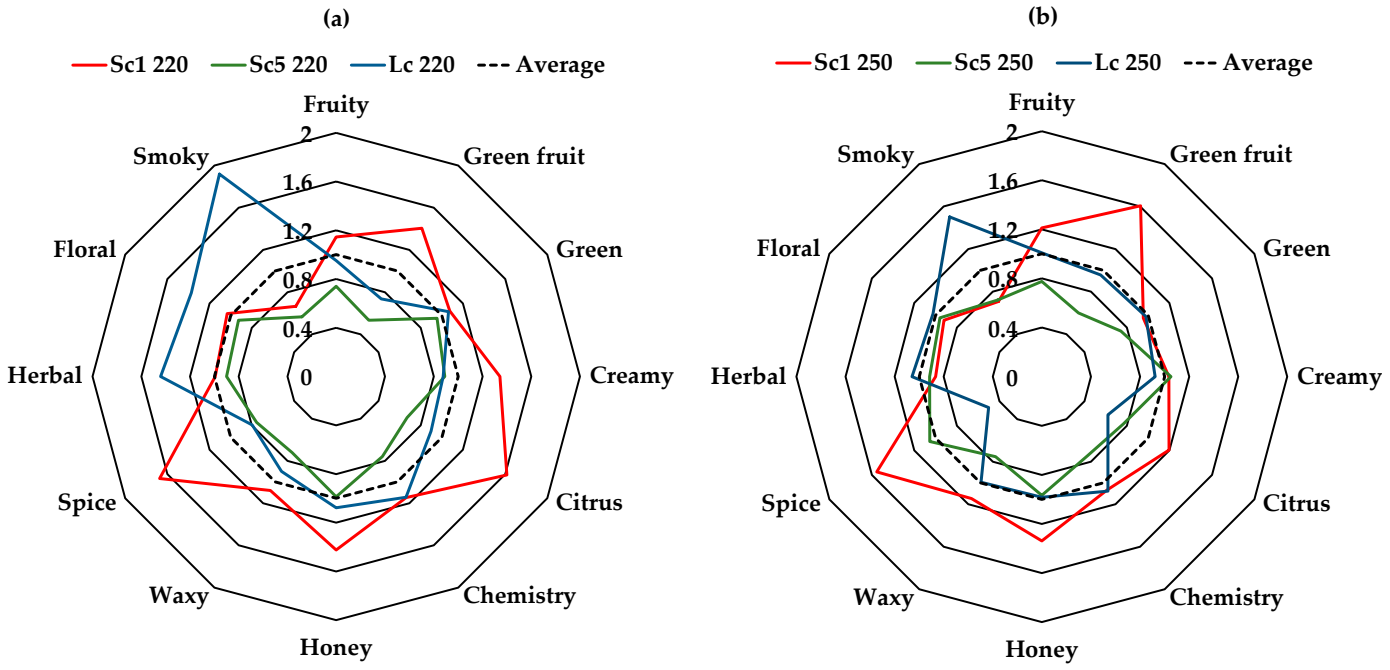

Figure 1. *Cont.*

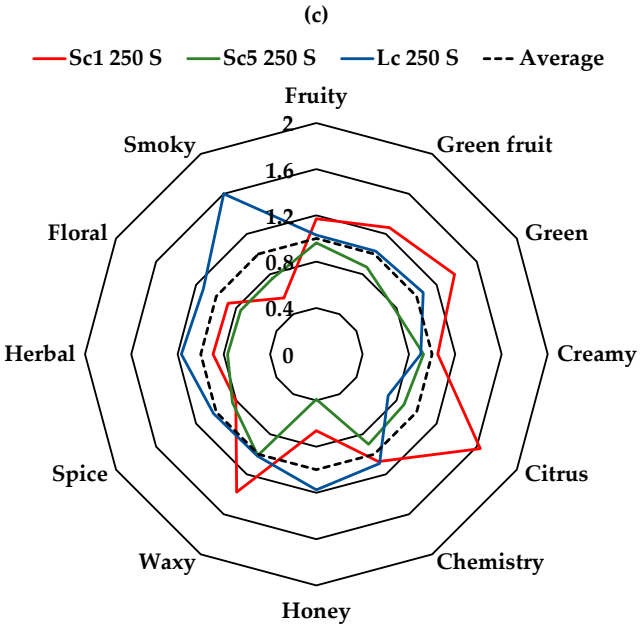

Figure 1. Star plot obtained by multivariate data analysis of aromatic series. (**a**) musts with 220 g/L w/o SO_2; (**b**) musts with 250 g/L w/o SO_2; (**c**) musts with 250 g/L w SO_2.

When the initial sugar concentration was 250 g/L (Figure 1b), the importance of the above-mentioned series diminished for both Sc1 and Lc yeast strains, except for the green fruit series. Additionally, the floral series was decreased for Lc. So, it can be assumed that a reduction in the wine aromas related to such series will be obtained.

On the other hand, the presence of sulfur dioxide (Figure 1c) slightly increases the values of the smoky series and, to a great extent, the spice series for Lc. This last series together with the honey and green fruit series were decreased, whereas the citrus and green series were increased, for Sc1. As in the previous conditions, Sc5 show the lowest values.

3.6. Cluster Analysis

Cluster analysis consists of a multivariate statistical technique with the purpose of grouping sets of samples according to their similarities. To this end, classifying variables are selected. The smaller the distance separating two clusters, the greater the similarity between the samples contained within these clusters. Here, cluster analysis according to Ward's method was carried out using the aromatic series as classifying variables (Figure 2).

On first sight, we can distinguish two well-differentiated clusters. One of the groups is wines produced by the Sc1 strain, and there is a small distance between wines obtained from musts containing 220 and 250 g/L of sugars. Nevertheless, a major distance is found between wines fermented from musts containing sulfur dioxide, so it can be assumed that sulfur dioxide is a differentiating factor for this yeast strain.

The other cluster groups the wines produced by the Sc5 and LC strains. In a similar way to those obtained by Sc1, wines fermented by Sc5 showed few differences due to the initial sugar contents, and considering the distance that separates the wines produced by Sc5 in the presence and absence of sulfur dioxide, these are more similar to each other than those produced by Sc1. Lastly, taking into account the distance between the different treatments, wines obtained with Lc showed the smallest differences among them. However, in this case, the differentiating factor was the initial sugar concentration.

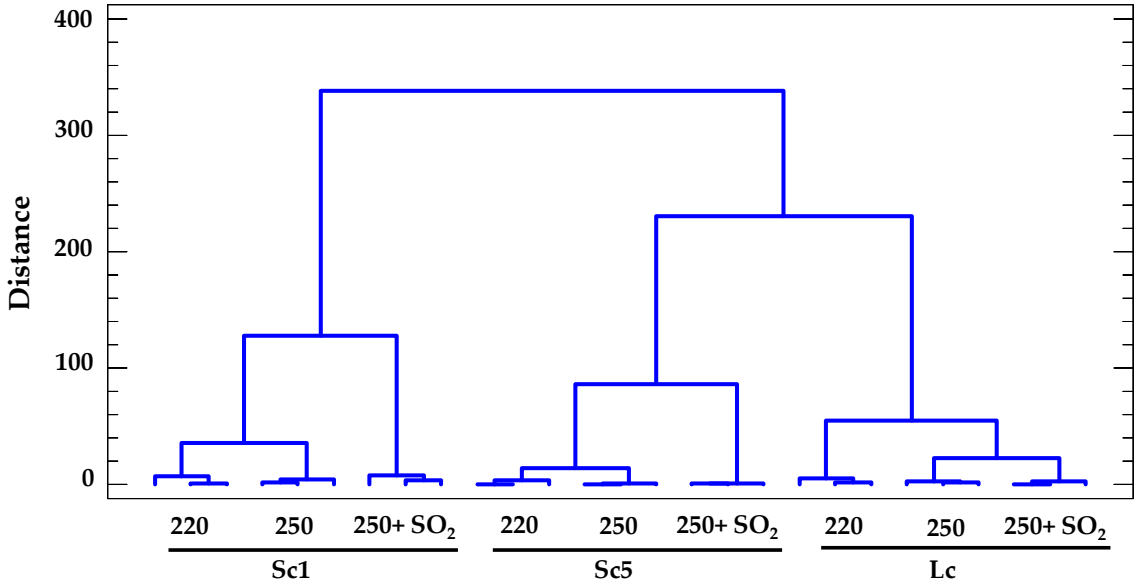

Figure 2. Cluster analysis of the wines obtained by the Sc1, Sc5 and Lc yeast strains under the study conditions. Musts conditions: 220: 220 g/L sugar; 250: 250 g/L sugar; 250 + SO_2: 250 g/L sugar with 75 mg/L SO_2.

3.7. Principal Components Analysis

Principal component (PCA) is a statistical technique used to minimize the number of variables while preserving as much information as possible. The new factors or components are formed by linearly combining the input variables. It is the responsibility of the analyst to interpret the obtained components. Here, a PCA was performed using the values of the aromatic series as classifying variables (Figure 3).

Three components were selected that together 88.6% of the observed variability. The first component (Figure 3a) explains 43.8% and clearly differentiates the strain Sc1, whereas the second component (29.4% of the variability) differentiates the Sc5 and Lc strains. The third component differentiates, in wild yeasts, the wines obtained with and without sulfur dioxide.

Principal component allows us to identify which variables have the greatest impact on the observed differences. The weight of the aromatic series is shown in Table S4. The first component is influenced by the aromatic series, fruit, green fruit and citrus, the second by the herbal, floral and smoky series, and the third component by the honey and spice series.

Regarding these results, in addition to those observed in the cluster analysis, it could be stated that increasing the sugar content in musts did not notably influence the aroma of the wines fermented by Sc1 and Sc5, while the presence of sulfur dioxide determined some differences in their aroma. On the other hand, wines fermented by Lc were not influenced by any of these conditions.

Some authors [39], using principal component analysis, found that the production of volatile metabolites was affected by both yeast strain and sulfur dioxide.

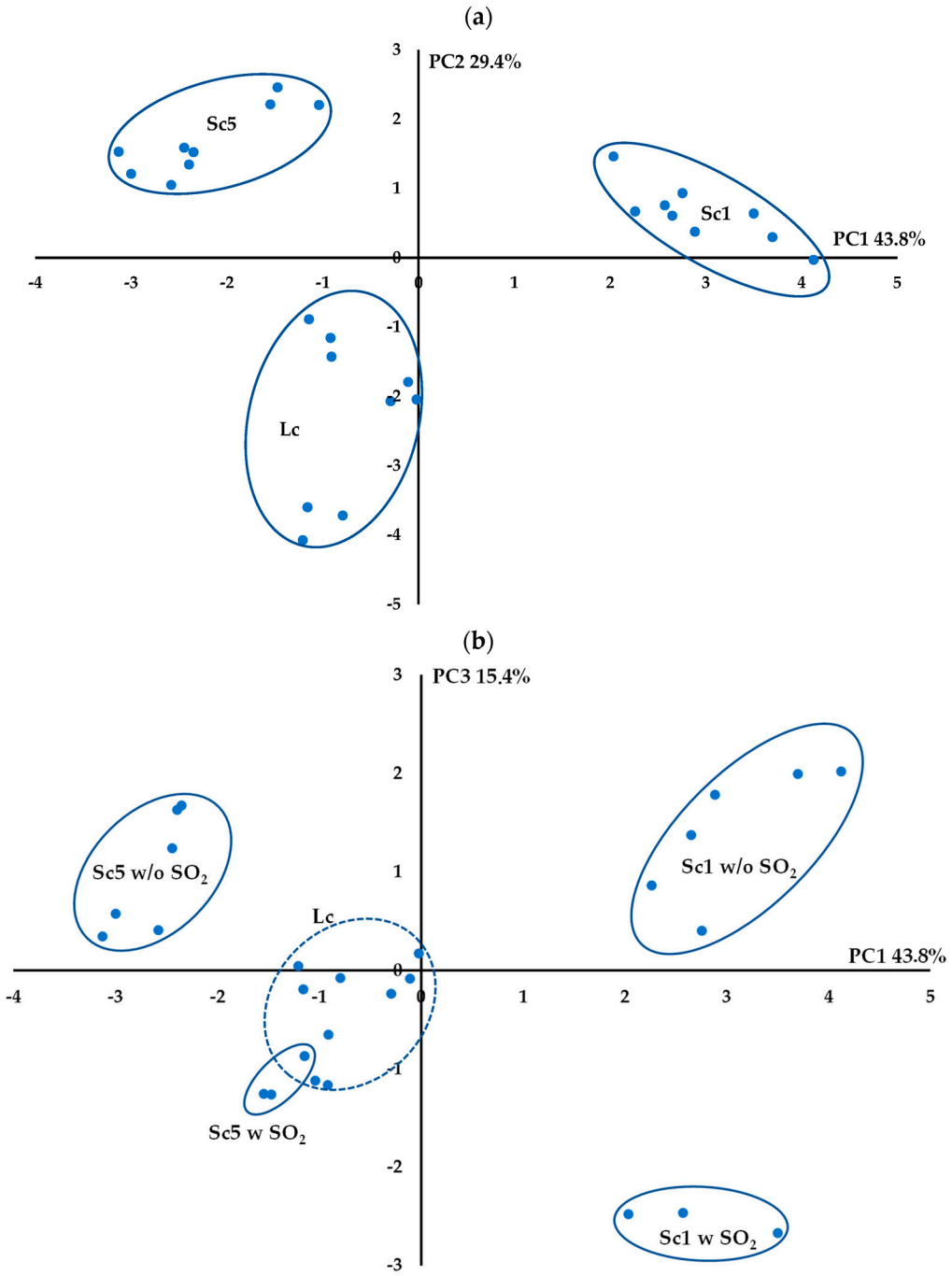

Figure 3. Principal component analysis (PCA) of the wines obtained. (**a**) represents the interaction between PC1 and PC2, and (**b**) shows the interaction between PC1 and PC3.

4. Conclusions

The selected yeast strains are capable of fully completing the fermentation of musts with high sugar concentrations, and the presence of sulfur dioxide does not affect its completion. However, there is a delay in the start of fermentation compared to commercial yeast.

The yeast strain is the factor that most influences the volatile composition of wines. In fact, out of the 45 volatile compounds analyzed, 39 of them depend on the yeast strain. Among the different strains tested, the Sc1 yeast strain stands out in terms of aroma production, followed by the commercial yeast strain.

When considering the individual aroma compounds, it is observed that twelve compounds exhibit odor activity values exceeding unity, with most of them being ethyl esters.

These compounds contribute significantly to the fruity aroma series, while others such as β-damascenone and β-ionone contribute to the floral and herbal aromatic series.

The results of multivariate analysis reveal that the Sc1 strain stands out for green fruit, citrus, and spice aromas, while the Lc strain exhibits prominent smoky and herbal notes. Overall, the Sc5 strain demonstrates values above the median. This information can be valuable for winemakers aiming to achieve specific organoleptic characteristics in their wines.

Furthermore, cluster and principal component analyses highlight that the aromatic composition of wines produced with wild yeast strains is more influenced by sulfur dioxide than by the initial sugar content of the must. In contrast, the commercial strain shows the opposite pattern. The fruity, green fruit, and citrus aroma series play a significant role in differentiating between yeast strains, particularly for Sc1, whereas the herbal, floral, and smoky series are more prominent for the Lc strain.

In conclusion, the Sc1 wild yeast strain exhibited a fermentation behavior comparable to that of the commercial yeast, with an increasing content in aroma compounds. However, as each strain contributes a distinct aromatic profile, winemakers can leverage this diversity to craft wines that showcase specific aromas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9060541/s1, Table S1. Major and minor aroma compounds identified in the wines; Table S2. Fermentation kinetic rates of the *Saccharomyces cerevisiae* strains obtained after the fermentation period; Table S3. Odor perception thresholds and aromatic series assigned to the volatile compounds; Table S4. Weight of the aromatic series to the component selected in the principal component analysis; Figure S1. Fermentation kinetics of the assayed yeast in the studied conditions.

Author Contributions: Conceptualization, R.A.P. and N.L.d.L.; methodology, R.A.P., J.C.M., T.G.-M. and N.L.d.L.; formal analysis, S.P.-F. and F.J.M.-G.; investigation, S.P.-F. and F.J.M.-G.; data curation, R.A.P. and F.J.M.-G.; writing—original draft preparation, S.P.-F. and F.J.M.-G.; writing—review and editing, R.A.P., J.C.M., T.G.-M. and N.L.d.L.; funding acquisition, R.A.P., J.C.M., T.G.-M. and N.L.d.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Innovation of Spain, grant number TED2021-129208B-100.

Data Availability Statement: The data are available upon request to the authors. Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics (accessed on 1 June 2023).

Conflicts of Interest: All the authors have agreed on and authorized the publication of this manuscript upon the final version. The authors declare that they have no known competing financial interest or personal relationship that could influence the work reported in this paper. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. This work has not been published previously and it is not under consideration for publication elsewhere. Furthermore, if accepted, it will not be published elsewhere either, in English or in any other language, including electronically, without the written consent of the copyright-holder.

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