Frontiers in Bioscience Elite

Front. Biosci. (Elite Ed) **2023**; 15(3): 18
https://doi.org/10.31083/j.fbe1503018

Original Research

Influence of Native *Saccharomyces cerevisiae* Strains on Malvasia *aromatica* Wines

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Academic Editor: Baohong Zhang

Submitted: 28 February 2023 Revised: 10 April 2023 Accepted: 10 May 2023 Published: 17 July 2023

Abstract

Background: In the search of tools to deal with climate change-related effects along with the aim of avoiding the loss of aromatic typicity in wine, two native yeasts strains of *Saccharomyces cerevisiae* (CLI 271 and CLI 889) were evaluated to determine their influence on white Malvasia *aromatica* wines aroma composition and sensory characteristics. **Methods**: The strains were tested versus a commercial yeast strain (LSA). The fermentations were performed on grape must of the Malvasia *aromatica* variety previously macerated. Wine quality was studied by analysis of oenological parameters together with volatile aroma components using gas chromatography coupled to flame ionization detector (GC-FID) to quantify major volatiles compounds and headspace-solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS) to determine terpenoids and C13-norisoprenoids. Sensorial analysis was also realized by an experienced taster panel. **Results**: Wines from locally-selected yeasts strains used had lower volatile acidity levels and higher concentration of aromatic compounds compared to the commercial strain ones. The yeast strain *S. cerevisiae* CLI 271 provided wines with a higher concentration of esters related to fruity attributes, especially isoamyl acetate. The tasting panel highlighted the strong floral character of wines from *S. cerevisiae* CLI 889 fermentation. **Conclusions**: The use of microorganisms well adapted to climatic conditions can be used to produce quality wines of the Malvasia *aromatica* variety.

Keywords: native yeast; aroma; climate change; white wines; Malvasia *aromatica*

1. Introduction

The use of native yeast strains in a particular region is an important instrument with the aim of improving the sensory quality of wines. Native yeast strains are the microorganisms best adapted to the characteristics of terroir, climate and cultivar condition from which they have been isolated and therefore may be able to enhance the aromas, structure and color of the wine [1,2].

Fermentation using autochthonous yeasts have aroused great interest to try to extract the typical organoleptic characteristics of a region. However, the quality of the product can be highly variable between consecutive seasons and it is also difficult to know exactly which yeasts are acting. On the other hand, the yeast activity during a spontaneous fermentation could contribute to fewer desirable attributes to the wine. In addition, natural fermentations can lead to slow or stopped fermentations and the spread of contaminating yeasts. To avoid this variability, commercial active dry wine yeasts (LSA from *Saccharomyces* strains) are used. A successful implantation of inoculated LSA encourages a rapid start of fermentation and a total consumption of fermentable sugars [3], however it has been shown that a product of uniform quality can be obtained using commercial yeast throughout the different vintages [4,5]. Nevertheless, some winemakers consider that in this way the differential character of the harvests, aromatic variability and varietal sensory nature could be lost [6,7]. To keep away from this loss of typicality, today there is a tendency to select autochthonous strains that are adapted to each wine-growing area and, therefore, to the climatic conditions, to the grape varieties of each territory and to the practices and techniques of winemaking used [8]. These strains will be responsible, partially, on the sensory characteristics of the wines obtained in each region.

The vineyard is a crop whose correct development is influenced by the climate. The suitability of wine-growing areas to reach optimum levels of sugar, pH, color and aromatic components, which are necessary for the production of quality wines, depends on weather conditions throughout the growing period [9,10]. In order to manage climate change-related effects, the adaptation tools on winemaking can be implemented at the winery level or at the vineyard level [11]. In oenology, innovations can be considered an important strategy to protect against climate variations related effects by focusing on specific hazards towards the improvement of the production. These techniques include changes in winemaking practices by using better adapted microorganisms.

Yeasts contribute to wine aroma by several mechanisms: the compounds formed during alcoholic fermentation have a decisive influence on the volatile composi-

tion of wine, the novo biosynthesis of volatile compounds and transformation of neutral grape compounds into flavor-active components. The main groups of compounds that form the fermentation aroma are esters, higher alcohols, and volatile acids, as well as varietal compounds. Aroma is one of the most influential factors on wine quality and consumers preferences [12]. Climatic conditions can affect the correct development of the varietal aroma compounds of the grape [13] and could contribute to the synthesis of non-desirable aromas through alcoholic fermentation by altering the aroma quality of the wine. The mitigation techniques developed before, during or after fermentation will have influence on the wine final aroma composition. Several studies have been carried out to determine this influence in order to improve the sensory quality of wines. Skin-contact treatment has been proposed as a first measure of adaptation to climate change related effects in our laboratories [14]. As a second measure, we use native yeast strains as strategy to protect against climate variations effects.

Madrid is located in the center of Spain with specific climatic conditions with a predominance of hot summers, cold winters and low levels of rainfall. Climate forecasts indicate a progressive increase in temperatures, a decrease in rainfall, and a greater frequency of extreme events such as frosts, storms and heat waves with greater incidence in the center of the Iberian Peninsula [15]. These events could compromise the correct development of the technological and aromatic ripening of the grapes, preventing the production of intense aromatic white wines.

The aim of this work was the use of autochthonous and better adapted microorganisms in order to intensify the aroma potential of winemaking white wines in the Protected Designation of Origin (PDO) "Vinos de Madrid" preserving the sensory and distinctive characteristics of the region. We choose cv. Malvasia *aromatica*, a white grape variety of Italian origin with great aromatic potential [16–18] and physical-chemical characteristics that give rise to musts with high acidity and low pH, for its interest and potential application as a suitable varietal to improve the organoleptic quality of the Madrid wines.

2. Materials and Methods

2.1 Vintage, Yeast Strains and Vinification Procedure

Malvasia *aromatica* white grapes cultivated in Madrid winegrowing region were hand-harvested and elaborated in the Experimental Winery from IMIDRA Institute at the "Finca El Encín", in Alcalá de Henares, Spain.

The *S. cerevisiae* yeast strains (CLI 271 and CLI 889) were isolated from wineries of the PDO "Vinos de Madrid" and selected based on its good oenological properties by the Oenological Microbiology Laboratory of the Department of Agrifood Research at Madrid Institute for Rural, Agriculture and Food Research and Development (IMIDRA) Institute. These two native strains are well-known and widely tested at IMIDRA laboratories. Specifically, *S. cerevisiae* CLI 889 presented a high fermentative capacity and optimal implantation rate, resistance to ethanol, fruity and fresh character and it is low-producer of acetic acid, hydrogen sulfide and sulfur dioxide [19,20]. Likewise, the use of CLI 271 strain was interesting for its capacity of consumption of amino acids precursor of oxidation notes, reducing oxidation notes after sensorial analysis [21]. These strains were tested versus a *S. cerevisiae* active dry commercial yeast (LSA Vario from Agrovin), considered as control. The fermentations were performed on must previously macerated at 10 °C for 18 h. After cold maceration, Malvasia *aromatica* must showed 21.4 °Brix and 164 mg/L of yeast assimilable nitrogen (YAN), the pH value was 3.32 and the titratable acidity was 5.4 (expressed as g/L of tartaric acid). The must was divided into seven tanks. Moreover, the addition of nutrients (Actimax plus 30 g/hL) was done at the beginning and half of fermentation to encourage the growth of yeasts.

Three were inoculated with the CLI 271 yeast, three others with the CLI 889 strain and the last one with the commercial yeast (LSA, was used as a control). Each *S. cerevisiae* strain was inoculated in grape must at a concentration of 10^6 cells/mL, from a pre-culture grown for 48 h in Yeast extract Peptone Dextrose (YPD) liquid medium at 28 °C. Fermentation took place under controlled temperature of 16 °C and was followed daily by measuring density (Proton 20969 and 28271 densimeters, Spain).

2.2 Oenological Parameters

Pre- and post-fermentation parameters were analyzed from each treatment replication. International Organisation of Vine and Wine (OIV) official methods [22] were used for the analysis of °Brix, free and total sulphur dioxide, pH, titratable acidity, residual sugars and volatile acidity. Fermentation kinetic was controlled by daily monitoring of the density. The fermentative capacity was valuated as the difference between the initial and the final sugar content.

2.3 Aromatic Analysis of Wines

Twenty-six major volatiles were determined by gas chromatography coupled to flame ionization detector (GC-FID; Agilent Technologies, Santa Clara, CA, USA) with the method described by Ortega [23] and 9 minor volatiles by gas chromatography coupled to mass spectrometry (GC-MS; Agilent, Santa Clara, CA, USA) following the method proposed by Yuan and Qian [24].

The extraction of major aroma compounds was performed in dichloromethane using DB-WAX - high-polarity, polyethylene glycol (PEG) column (60 m × 0.32 mm × 0.5 µm film thickness) from J&W Scientific (Folsom, CA, USA). For sample preparation, conical bottom glass tubes were used and the following were added: 3.9 g of ammonium sulfate, 6.3 mL of milli-Q grade deionized water 2.7 mL of wine, 20 µL of an internal standard solution (2-Butanol, 4-Methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone and 2-Octanol) and 250 µL of dichloromethane.

The oven temperature was initially programmed at 40 °C for 5 min, and then ramped to 200 °C. A constant helium flow of 2 mL/min was used. Two mL of aroma extract were injected at 250 °C in splitless mode. Total run time was 75 min per sample. All samples were analyzed in duplicate.

Terpenoids and C13-norisoprenoids (minor volatiles) were determined using headspace-solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS) following the method proposed by Yuan and Qian [24] using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent). Two mL of the wine sample were diluted with 8 mL of a citric acid solution (0.5 g/L citric acid, pH 3 saturated with sodium chloride) and 20 µL of 4-octanol (100 µg/L) used as internal standard; all were mixed with a small magnetic stir bar. For volatile extraction a 50/30 µm Divinylbenzene (DVB)/Carboxen (CAR)/Polydimethylsiloxane (PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used. The vials for chromatography (20 mL of volume, Agilent Technologies, Santa Clara, CA, USA) were tightly capped and equilibrated at 50 °C in a thermostatic bath for 10 min and extracted by SPME fiber for 50 min at the same temperature with stirring (1000 rpm). To desorb the analytes, the fiber was manually inserted into the injection port of the GC at 230 °C. A DB-Wax column from J&W Scientific (Folsom, CA, USA) (60 m × 0.32 mm × 0.5 µm film thickness, Phenomenex, Torrance, CA, USA) was employed to separate the analytes. Carrier gas (helium) was set at a constant flow rate of 1 mL/min. The oven temperature was initially set at 40 °C for 2 min, raised to 230 °C at 5 °C/min for 15 min.

2.4 Sensory Analysis

Descriptive sensory analyses were performed by a trained panel of 8 people (4 expert tasters and 4 habitual consumers) from the IMIDRA Institute. This panel had been previously trained in a normalized tasting room in the recognition of wine flavor. Wines were compared by triangle tests (International Organization for Standardization (ISO) 4120:2004) to assess whether aroma differences existed between the different *S. cerevisiae* fermentations. Sensory descriptive analysis was performed in used to describe and quantify attributes of the wines on the basis of a scale from 1 (low intensity) to 10 (high intensity). The chart included a visual phase (color, color intensity, vivacity/brightness), an olfactory phase (aroma intensity and quality, fruity, vegetal, floral, alcoholic and alteration aroma (off flavour) such as oxidation or microbiologic) and a gustatory phase (alcoholic character, acidity, fruity, vegetal/herbaceous, bitter, body/structure, salty, global taste quality). A hedonic classification was also carried out establishing the order of preference of the samples presented. The final score was obtained as the mean of the wine evaluations with their respective standard deviation and interpreted by graphical representation.

2.5 Statistical Analysis

The statistical processing of the data was carried out with software SPSS ver. 20.0 (SPSS, Inc, Chicago, IL, USA). Analysis of variance (ANOVA) was applied on oenological parameters, volatile compounds and sensory attributes of the wines. Tukey honestly significant difference (HSD) post-hoc tests were used to establish the significance of differences between means to assess significance ($p < 0.05$). Principal component analyses (PCA) to evaluate the influence of yeast strains on the volatile composition of wines.

3. Results and Discussion

3.1 General Wine Composition

The three studied *S. cerevisiae* strains could complete the vinifications (residual sugars below 4 g/L) and the time required to carry out the fermentation was the same.

Physico-chemical parameters of wines remained within the legal limits established by European Regulation for table wines elaboration: titratable acidity ≥4.5 g/L (Commission Regulation (EC) No 557/94 of 14 March 1994 laying down a transitional measure regarding the total acidity content of the table wine produced in Spain and Portugal and released to the markets in those Member States for 1994), volatile acidity ≤1.08 g/L (EC 1493/99), pH 2.8–3.4, alcohol degree ≥9–<15 (Commission Regulation (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines), total SO_2 ≤210 mg/L (EC 1493/99). All wines obtained presented similar analytical characteristics (**Supplementary Table 1**) only volatile acidity marked the highest differences. The *S. cerevisiae* CLI 271 (0.33 g/L) and CLI 889 (0.37 g/L) strains had lower volatile acidity values than commercial control LSA (0.50 g/L). This parameter can play a relevant role in wine aroma and its excessive content is highly detrimental to wine quality. During alcoholic fermentation, the usual quantity of volatile acidity produced by *S. cerevisiae* is between 0.25 g/L and 0.50 g/L [25], so all Malvasia wines are among this normal range.

3.2 Influence of Yeast Strain on Aroma Profile of Malvasia Wines

The concentration of varietal and major aroma compounds in the wines produced by the tested yeasts was evaluated. Table 1 (Ref. [26–31]) shows the average and standard deviations of the volatile detected in the different fermentations together with their odor threshold (OTH) [26–31]. The compounds were classified into chemical families to define their impact on wine and check if there were differences between the yeast strains used. From all the volatile compounds identified, those whose concentrations were higher than their OTH are considered as aroma-contributing compounds. Comparing the total volatiles obtained in the three elaborations, CLI 889 strain wines showed a higher

Table 1. Concentration of volatile compounds analyzed in wines fermented with CLI 271 (n = 3), CLI 889 (n = 3) and LSA (n = 1) strain. (average ± sd).

Compounds	OTH*	CLI 271	CLI 889	LSA	Ref.
Terpenols (µg/L)					
β-Myrcene	-	1.13 ± 0.17	1.48 ± 0.17	1.51	-
α-Terpinene	-	0.17 ± 0.02	0.17 ± 0.02	0.21	-
Limonene	15	0.39 ± 0.07	0.42 ± 0.06	0.49	[26]
γ-Terpinene	-	1.15 ± 0.15	1.46 ± 0.14	1.63	-
Linalool	25	67.53 ± 11.35	74.29 ± 7.60	77.01	[27]
α-Terpineol	250	20.24 ± 4.42	17.53 ± 2.62	18.01	[27]
β-Citronellol	100	12.19 ± 2.04	18.76 ± 1.83	16.11	[28]
Geraniol	30	17.71 ± 1.66	22.85 ± 1.87	22.80	[26]
Total		120.51± 17.24	136.96 ± 15.08	137.77	
C_{13}-norisoprenoids (µg/L)					
β-Damascenone	0.05	1.18 ± 0.19	1.22 ± 0.12	1.39	[28]
Total		1.22 ± 0.12	1.18 ± 0.19	1.39	
Alcohols (mg/L)					
Isobutanol	40	33.74 ± 7.68	40.04 ± 4.21	35.35	[27]
1-Butanol	150	0.96 ± 0.11	0.35 ± 0.02	0.32	[26]
Isoamyl alcohol	30	285.22 ± 73.93	315.86 ± 9.03	224.05	[27]
1-Hexanol	8	1.46 ± 0.23	1.65 ± 0.16	1.53	[27]
Cis-3-hexen-1-ol	0.4	0.02 ± 0.00	0.02 ± 0.00	0.02	[27]
Methionol	1	1.36 ± 0.28	0.57 ± 0.03	0.57	[27]
β-Phenylethanol	14	52.42 ± 5.91	34.59 ± 0.98	38.15	[27]
Total		375.19 ± 86.57	393.08 ± 12.12	299.99	
Lactones (mg/L)					
γ-Butyrolactone	35	4.85 ± 0.88	7.15 ± 0.51	8.95	[29]
Total		4.85 ± 0.88	7.15 ± 0.51	8.95	
Fatty acids (mg/L)					
Isobutyric acid	2.30	1.47 ± 0.44	1.73 ± 0.26	1.85	[30]
Butyric acid	0.17	0.55 ± 0.09	0.67 ± 0.09	0.87	[27]
Isovaleric acid	0.03	2.77 ± 0.56	2.41 ± 0.25	2.11	[27]
Hexanoic acid	0.42	3.10 ± 0.29	2.58 ± 0.46	2.51	[27]
Octanoic acid	0.50	3.34 ± 0.42	2.60 ± 0.41	2.63	[27]
Decanoic acid	1	0.22 ± 0.05	0.20 ± 0.04	0.33	[27]
Total		11.44 ± 0.74	10.19 ± 1.35	10.3	
Esters (mg/L)					
Ethyl butyrate	0.02	0.30 ± 0.04	0.33 ± 0.05	0.40	[27]
Ethyl isovalerate	0.003	0.21 ± 0.06	0.15 ± 0.01	0.22	[27]
Isoamyl acetate	0.03	2.83 ± 0.53	1.29 ± 0.15	1.81	[27]
Ethyl hexanoate	0.01	0.66 ± 0.00	0.51 ± 0.09	0.62	[27]
Hexyl acetate	1	0.15 ± 0.02	0.11 ± 0.02	0.14	[31]
Ethyl lactate	154	3.35 ± 0.62	3.82 ± 0.73	2.73	[26]
Ethyl octanoate	0.58	0.72 ± 0.03	0.53 ± 0.09	0.64	[26]
Ethyl 3-Hydroxy-butyrate	20	0.12 ± 0.02	0.20 ± 0.06	0.13	[29]
Diethyl succinate	1.20	0.00 ± 0.00	0.17 ± 0.02	0.10	[27]
2-Phenylethyl acetate	0.25	0.52 ± 0.12	0.18 ± 0.03	0.27	[27]
Total		8.85 ± 0.83	7.29 ± 1.16	7.05	
Carbonyl compounds (mg/L)					
Diacetyl	0.10	0.15 ± 0.03	0.26 ± 0.11	0.37	[28]
Benzaldehyde	5	0.18 ± 0.04	0.07 ± 0.02	0.15	[27]
Total		0.33 ± 0.05	0.33 ± 0.06	0.51	
Total (mg/L)		400.78 ± 87.41	418.19 ± 10.45	326.94	

*OTH: Odour threshold values gives in mg/L except terpenols and β-damascenone which are in µg/L.

LSA: Active dry yeast.

concentration of total volatiles (418.41 mg/L) than those elaborated with CLI 271 and LSA strains (400.78 mg/L and 326.94 mg/L respectively).

The total concentrations of higher alcohols range from 299 mg/L in the wine fermented with LSA to 393 mg/L in the wine fermented with CLI 889 strain. Isoamyl alcohol involved more than 70% of the total alcohols and together with β-phenylethanol exceeded their OTH in all wines. This compound is related to floral aromas with attributes of roses and is considered to contribute positively to wine aroma [32]. Isobutanol and methionol also contributed to the aroma of wines fermented with CLI 271 and CLI 889 respectively.

The total ester content was higher in wines fermented with CLI 271 strain. Esters are very important for the aroma of wine; they are related to fruity aromas [33]. Isoamyl acetate and ethyl lactate were the dominant esters founded in higher concentrations in wines elaborated with CLI 271 strain. Ethyl isovalerate, ethyl butyrate, ethyl hexanoate, ethyl octanoate and 2-phenylethyl acetate exceeded or were very close to the perception threshold in all wines. These results are in agreement with Balboa-Lagunero [21] in white wines of cv. Palomino fino fermented with different yeast strains where resulting wines from CLI 271 fermentation stood out for their ester content.

As regards fatty acids, butyric, isovaleric, hexanoic and octanoic acids were the main ones found in the wines. The concentration was higher in wines fermented with CLI 271 strain, moreover all of them exceeded the olfactory threshold, therefore they will have a significant aromatic impact on the resulting wines. Although the presence of fatty acids is often associated with off-flavours, they will play an important role in the aromatic balance considering their synthesis antagonistic to the hydrolysis of the ester analogues [20,32].

The lactone family is characterized by typical fruity aromas. Only γ-butyrolactone was detected and it resulted in different concentrations for the three preparations. The wine made with LSA showed almost twice the concentration compared to the wine made with strain CLI 271, however, the wine made with strain CLI 889 obtained very similar concentrations to that of LSA. As expected, in none of the cases it was found in concentrations above its perception threshold, this family is mainly found in wood-aged wines to which it contributes important sensory characteristics [34]. Wine elaborated with the LSA strain showed the highest levels of aldehydes and ketones compared to wines fermented with 889 and 271 strains mainly due to the high diacetyl content. This group of compounds is related to oxidation aromas in wine [35]. In previous studies, *Palomino* must fermented with CLI 271 had the lowest concentration values of some aldehydes that are negatively correlated with wine's quality; also, these samples obtained the lowest scores in the sensory test for oxidation related descriptors [21].

Regarding the terpene family, the main monoterpenes found were linalool, α-terpineol, geraniol and β-citronellol whose concentrations were variable depending on the yeast strain used, thus, the wines from CLI 889 and LSA strains showed the highest levels of monoterpenes. Linalool, with concentrations above 60 µg/L, was the most abundant and was found above its olfactory perception threshold in all wines. This terpene provides the wine with floral and citrus notes characteristic of Muscat [36]. The compounds analyzed in this family are characterized by their floral and fresh notes. Their presence will be very important because of their influence on wine quality. Traditionally, these compounds have been related to the grapes and not to the fermentation processes, but it has been demonstrated that they are synthesized "de novo" by *S. cerevisiae*, depending on the redox situation and the level of nitrogen available in the fermentation medium [1]. Therefore, the concentration of free terpenes released into the wine will not only depend on the content of aromatic precursors and the grape variety.

It is important to highlight the significance of β-damascenone as the only aroma representative of the C13-norisoprenoid family. Its perception threshold is very low and in all three wines exceeds it, providing floral aromas with violet attributes. Similar results were found in Chardonnay wines [37].

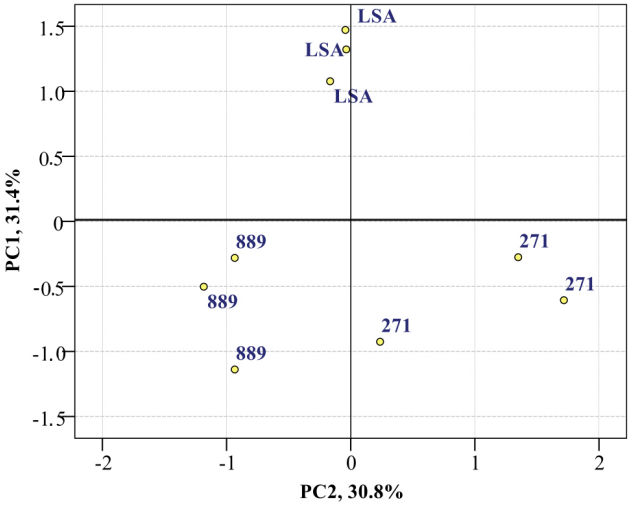

Fig. 1. Principal component analysis (PCA) of volatile composition data. Malvasia *aromatica* wines fermented with *S. cerevisiae* CLI 271, CLI 889 and LSA strains in the squares formed by Principal component 1 (PC1) (31.4%) and Principal component 2 (PC2) (30.8%).

In addition, PCA of aroma composition using analytical triplicates of control LSA wines (Fig. 1) was performed in order to reveal the compounds that best differentiated between the Malvasia wines elaborated different yeast strains. According to PCA results, two main components explaining 62.2% of the total variance were retained (with a factor

loading >0.5000). The first component with 31.4% of the variance explained mainly groups the variables related to wines fermented with LSA strain in the positive plane and those related to wines elaborated with the CLI 889 strain in the negative plane. The main compounds related to factor 1 in LSA wines are α-terpinene, diacetyl, ethyl butyrate and ethyl isovalerate. With the exception of α-terpinene (whose OTH is not known), all of them were found above the olfactory perception threshold and were therefore relevant in the aroma of these wines. The most significant variables of component 1 related to CLI 889 wines were isoamyl alcohol and cis-3-hexen-1-ol. As for the second component which reveals 30.8% of the variance, it is represented in the positive plane by pleasant aroma compounds (β-phenylethanol, ethyl hexanoate and isoamyl acetate) but also unpleasant aroma compounds (methionol, benzaldehyde, 1-butanol) where wines from yeast strain CLI 271 are classified, and diethyl succinate and β-citronellol compounds in the negative plane where wines from yeast strain CLI 889 are located. A clear orderly disposition respect on the aromatic composition of wines have been obtained depending on the *S. cerevisiae* employed in the fermentation process.

3.3 Influence of S. cerevisiae Strains on Sensory Profile of Malvasia Wines

A descriptive tasting of wines was realized at visual, olfactory and gustative levels which results were statistically treated in order to establish the main differences found among the wines fermented with the three yeast strains in study. Differences between wine samples with a significance level of 0.1% ($p < 0.001$) were considered very significant, of 1% ($p < 0.01$) considered significant and of 5% ($p < 0.05$) considered low significant. Visual descriptors were scored very similar between wines, only wine elaborated with LSA strain obtained a score significantly higher than others in terms of vivacity/brightness character (Fig. 2A). In turn, the color of all wines was described as straw yellow with greenish tones.

Regarding aroma descriptors (Fig. 2B), the wines elaborated with CLI 889 and LSA obtained very similar general quality notes (5.6 and 5.8 respectively), very low defects and non-significant differences on olfactory level. Also, these wines reached the best scores in terms of aroma intensity, aroma quality and flowery descriptor. However, CLI 889 wines were considered more alcoholic than others but without significant differences, and together with LSA wines noticeably more floral than wines from CLI 271 fermentations ($p < 0.01$). Tasters highlighted the high floral character of CLI 889 wines with anise notes.

About gustative analysis (Fig. 2C), wines from CLI 889 and LSA fermentations obtained again similar profiles. Both gained the highest outcome for global quality descriptor, with significant differences between CLI 271 and LSA wines ($p < 0.05$). Fruity character was highlighted in CLI

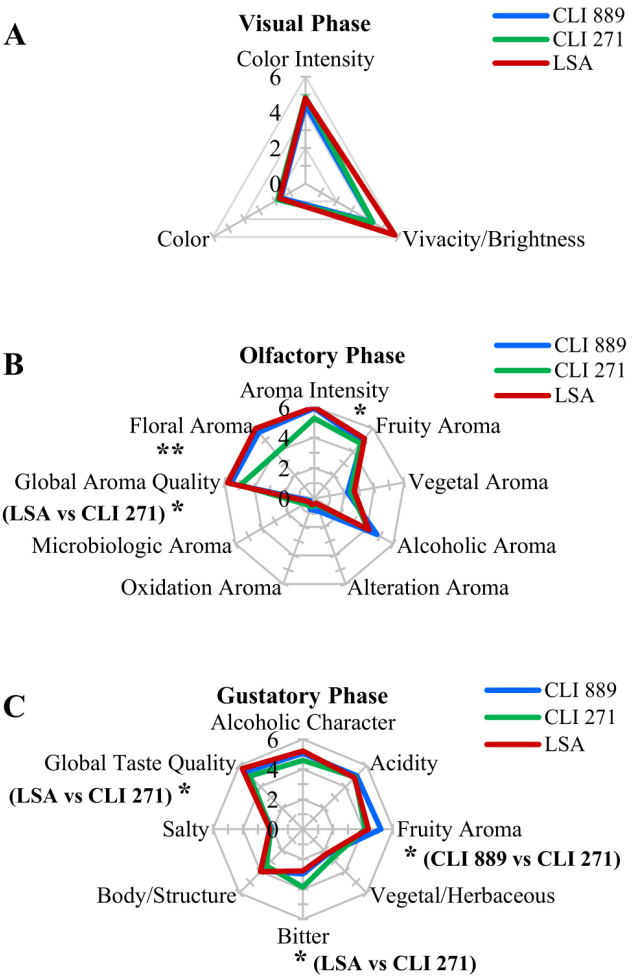

Fig. 2. Descriptive analysis of Malvasia wines elaborated with *S. cerevisiae* CLI 271, CLI 889 and LSA strains. (A)Visual phase. (B) Olfactory phase. (C) Gustative phase. The asterisks * and ** correspond to significant differences between wines from different yeast strains at $p < 0.05$ and $p < 0.01$, respectively.

Table 2. Results of triangular tests.

Test	Series	Success rate (%)	Preference (%)		
			CLI 889	CLI 271	LSA
T1	S1	5	70	30	
	S2	Ns			
	S3	5			
	S4	5			
T2	S1	Ns	50		50
	S2	5			
	S3	1			
	S4	1			
T3	S1	1		53	47
	S2	5			
	S3	Ns			
	S4	1			

Ns, Not significant.

Table 3. Volatile compounds with OAV ≥1 in Malvasia *aromatica* wines.

			Concentration			OAV[2]		
			CLI 271	CLI 889	LSA	CLI 271	CLI 889	LSA
Compounds	OTH[1]							
Varietal volatiles (µg/L)								
Linalool	25	Floral, citric	67.53	74.29	77.01	2.70	2.97	3.08
β-Damascenone	0.05	Floral, lilac	1.28	1.22	1.39	23.67	24.40	27.80
Major volatiles (mg/L)								
Isobutanol	40	Alcohol	33.74	40.04	35.35	0.84	1.00	0.88
Isoamyl alcohol	30	Vegetal/Herbaceous	9.51	10.53	7.47	9.51	10.53	7.47
Methionol	1	Cooked vegetable Bitterness	1.36	0.57	0.57	1.36	0.57	0.57
β-Phenylethanol	14	Roses	3.74	2.47	2.73	3.74	2.47	2.73
Ethyl butyrate	0.02	Acid fruit, apple	0.30	0.33	0.40	14.96	16.41	20.10
Ethyl isovalerate	0.003	Sweet fruit, orange, blackberry	0.21	0.15	0.22	69.20	51.50	72.77
Isoamyl acetate	0.03	Banana	2.83	1.29	1.81	94.37	43.01	60.19
Ethyl hexanoate	0.01	Acid fruit, apple	0.66	0.54	0.62	65.72	53.85	62.25
Ethyl octanoate	0.58	Acid fruit, apple	0.72	0.55	0.64	1.24	0.95	1.10
2-phenylethyl acetate	0.25	Green apple	0.52	0.18	0.27	2.10	0.73	1.06
Diacetyl	0.10	Butter	0.15	0.26	0.37	1.50	2.60	3.67
Butyric acid	0.17	Cheese	0.55	0.57	0.87	3.21	3.33	5.11
Isovaleric acid	0.03	Blue cheese	2.77	2.41	2.11	92.18	80.41	70.47
Hexanoic acid	0.42	Cheese	3.10	2.58	2.51	7.37	6.15	5.97
Octanoic acid	0.50	Butter, rancid	3.34	2.60	2.63	6.68	5.21	5.26

[1]OTH: Odor Threshold Value. Sensory descriptor and OTH values were found in the references included in Table 1.

[2]OAV: Odor Activity Value calculated by dividing concentration by odor threshold value of the compound.

889 wines found significant differences with CLI 271 elaborations ($p < 0.05$). This fruity character of CLI 889 has previously been emphasized in young white wines elaboration [19,20,38,39]. The vegetal/herbaceous and bitter character was superior in CLI 271 wines.

On the other hand, triangular tests were done to determine whether descriptive analysis was determinant to distinguish the samples. Discriminant triangular tastings were carried out using dark glasses and four series of tastings were performed by each type of wine: CLI 889 *vs.* CLI 271, CLI 889 *vs*. LSA, CLI 271 *vs*. LSA. Moreover, the panelists realized a hedonic classification according to their preference. Preferences were taken into account only when tasters answered correctly on each test. After first test (Table 2), the panelists were able to differentiate between wines from CLI 889 and CLI 271 in three of the four series with a 5% of significance level by triangle tests. The tasting panel showed preference by CLI 889 wines in 70% of cases versus 30% of them selected CLI 271 wines. The second test exhibited an elevated percentage of correct responses when CLI 889 and control LSA were compared. In this case, three of four series were distinguished with significance level of 5% (series 2) and of 1% (series 3 and 4), but was not nevertheless manifested preference between the wines (Table 2). Finally, CLI 271 and LSA wines were compared. The level of correct responses was also very significant in this test, the tasters identified clearly the wines from two yeast strains in three of the four series (significance levels of 1% and 5%), and they expressed preference for CLI 271 wine (Table 2).

To estimate the sensory contribution of aromatic compounds to overall aroma of wine, the odor activity value (OAV) was calculated for all aroma compounds in study (Table 3). The OAV is obtained from the ratio between the concentration of certain compound and its perception threshold. Thus, a volatile compound contributes to overall aroma when its concentration is beyond its odor threshold value (OTH); so, odorants with OAV ≥1 are considered direct contributors to wine aroma [28,32]. The wine elaborated with *S. cerevisiae* CLI 271 strain obtained higher concentrations of β-phenylethanol (roses), isoamyl acetate, ethyl octanoate and 2-phenylethyl acetate (fruity, fresh). Although other compounds with OAV >1 related to unpleasant flavors were found in this wine such as isovalerianic, hexanoic and octanoic acids (cheesy, rancid) (Table 3). This may have contributed to the scores of intensity and global quality aroma were not very high in this CLI 271 wine. On the other hand, there are interesting correlations between volatile compounds and sensory analysis results in wines from CLI 889 and LSA yeast strains. It is worth noting that CLI 889 wine with the highest scores of alcoholic notes (Fig. 2B) also presented the largest concentration of higher alcohols as isobutanol and isoamyl alcohol compounds (OAV ≥1) (Table 3). In turn, CLI 889 and LSA strains received high scores in floral character (Fig. 2B),

also had more elevated concentration of varietal aromas such as linalool and β-damascenone. This could mean that both elaborations obtained high intensity and global quality valuations than wines fermented with CLI 271 strain.

4. Conclusions

Based on these results, we can affirm that the selected indigenous strains, *S. cerevisiae* CLI 271 and CLI 889, showed a higher synthesis or release of aroma compounds than the commercial strain. Furthermore, no notable differences were found in the release of varietal compounds between strains CLI 889 and LSA, though the tasting panel highlighted the intense floral character of CLI 889 wines, and the strain CLI 271 was able to generate more esters related to fruity aromas. Therefore, the employment of locally-selected yeast strains from Mediterranean vineyard of Madrid better adapted to their climatic conditions can be used for the elaboration of quality wines from Malvasia *aromatica* variety, which in turn can be introduced as an alternative grape variety for improving the organoleptic quality of the regional wines.

Availability of Data and Materials

All the generated data and the analysis developed in this study are included in this article.

Author Contributions

JC and JMC designed and performed the research. MG and TA selected and inoculated the yeasts. JC, VR and JMC developed the chemical analysis and gas chromatography. JC analyzed the data. JC and MG wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

JMC acknowledges the INIA for pre-doctoral fellowships FPI-INIA 2012. Authors acknowledge the IMIDRA, Finca El Socorro for providing material of Malvasia *aromatica* used in this work and Microbiology Laboratory from Agrifood Research Department for yeast strain used for the winemaking.

Funding

This research was funded by the Spanish National Institute for Agricultural and Food Research and Technology (INIA), grant number RTA2011-00095-C02-01 funded. JMC was recipient of a PhD grant funded by INIA. M.G. was recipient of a PhD grant funded by IMIDRA.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbe1503018.

References

[1] Carrau FM. Native yeasts for low input winemaking. Biodiversity, selection and characterization. Agrociencia. 2005; 9: 387–399. (In Spanish)

[2] García M, Esteve-Zarzoso B, Crespo J, Cabellos JM, Arroyo T. Influence of native *Saccharomyces cerevisiae* strains from D.O. "Vinos de Madrid" in the volatile profile of white wines. Fermentation. 2019; 5: 94.

[3] Longo E, Cansado J, Agrelo D, Villa TG. Effect of Climatic Conditions on Yeast Diversity in Grape Musts from Northwest Spain. American Journal of Enology and Viticulture. 1991; 42: 141–144.

[4] Ribéreau-Gayon P. New Developments in Wine Microbiology. American Journal of Enology and Viticulture. 1985; 36: 1–10.

[5] Heard GM, Fleet GH. Growth of Natural Yeast Flora during the Fermentation of Inoculated Wines. Applied and Environmental Microbiology. 1985; 50: 727–728.

[6] Carrau FM, Gioia O, Neirotti E. Importance of the "killer" phenomenon in the population of dominant yeasts in winemaking at the industrial level. III Simp. Latin Vitic. Enol., Mendoza 19-23 September. National Institute of Viticulture (INV): Mendoza, Argentina. 1988. (In Spanish).

[7] Ramey D. Low input in winemaking-let nature do the work. In Stockley CS, Sas A, Johnstone RS, Lee TH (eds.) Proceedings of the Ninth Australian Wine Industry Technical Conference (pp. 26–29). Winetitles: Adelaide, Australia. 1995.

[8] Heard GM. Novel yeasts in winemaking-Looking to the future. Food Australia. 1999; 51: 347–352.

[9] Adams DO. Phenolics and Ripening in Grape Berries. American Journal of Enology and Viticulture. 2006; 57: 249–256.

[10] Mira de Orduña R. Climate change associated effects on grape and wine quality and production. Food Research International. 2010; 43: 1844–1855.

[11] Nicholas KA, Durham WH. Farm-scale adaptation and vulnerability to environmental stresses: Insights from winegrowing in Northern California. Global Environmental Change. 2012; 22: 483–494.

[12] Polášková P, Herszage J, Ebeler SE. Wine flavor: chemistry in a glass. Chemical Society Reviews. 2008; 37: 2478–2489.

[13] Crespo J, Rigou P, Romero V, García M, Arroyo T, Cabellos JM. Impact of climate change on bound fraction of aroma compounds of cv. Muscat a petits grains blancs berries. In Escribano Bailón M, García Estevez I, González Paramás A, Dueñas Patón M (eds.). 10th In Vino Analytical Sciences Symposium IVAS 2017 (pp. 118–119). University of Salamanca: Salamanca, Spain. 2017.

[14] Crespo J, Romero V, García M, Arroyo T, Cabellos JM. Influence of skin-contact treatment on aroma profile of Malvasia *aromatica* wines in D.O. "Vinos de Madrid." In Morata A (ed.). Grapes and Wine (pp. 137–144). IntechOpen: Rijeka, Croatia. 2021.

[15] Compés R, Sotés V. Compés R, Sotés V. The wine sector facing the challenge of climate change. In Public and private mitigation and adaptation strategies in the Mediterranean.1st edn. Cajamar Caja Rural: Almería, Spain. 2018. (In Spanish)

[16] Cabello F, Ortiz JM, Muñoz Organero G, Rodríguez Torres I, Benito A, Rubio C, *et al*. Grapevine varieties in Spain. Editorial Agrícola Española: Madrid, Spain. 2011. (In Spanish)
[17] Amerine MA, Roessler EB. Composition of wines. In Freeman WH (ed.). Wines- their sensory evalution (pp. 72–77). W.H. Freeman: San Francisco, USA. 1976.
[18] Flanzy C. Oenology: scientific and technological fundamentals. 1st edn. Mundi Prensa: Madrid, Spain. 2000. (In Spanish)
[19] Arroyo T. Study of the influence of different oenological treatments on the evolution of the microbiota and on the quality of wines elaborated with "Airén" variety, In "Vinos de Madrid" DO. University of Alcalá: Madrid, Spain. 2000. (In Spanish)
[20] Cordero-Bueso G, Esteve-Zarzoso B, Gil-Díaz M, García M, Cabellos J, Arroyo T. Improvement of Malvar wine quality by use of locally-selected *Saccharomyces cerevisiae* strains. Fermentation. 2016; 2: 7.
[21] Balboa-Lagunero T, Arroyo T, Cabellos JM, Aznar M. Yeast selection as a tool for reducing key oxidation notes in organic wines. Food Research International. 2013; 53: 252–259.
[22] International Organisation of Vine and Wine (OIV). Methods of analysis of wines and musts. Official Methods of Analysis of Musts and Wines of the International Organisation of Vine and Wine. 2012. Available at: http://www.oiv.int/oiv/infoen methodesinternationalesvinenmethodesinternationalesvin (Accessed: 15 September 2014).
[23] Ortega C, López R, Cacho J, Ferreira V. Fast analysis of important wine volatile compounds development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. Journal of Chromatography A. 2001; 923: 205–214.
[24] Yuan F, Qian MC. Development of C13-norisoprenoids, carotenoids and other volatile compounds in *Vitis vinifera* L. Cv. Pinot noir grapes. Food Chemistry. 2016; 192: 633–641.
[25] Bely M, Rinaldi A, Dubourdieu D. Influence of assimilable nitrogen on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. Journal of Bioscience and Bioengineering. 2003; 96: 507–512.
[26] Etiévant PX. Wine. In Maarse H (ed.) Volatile Compounds in Foods and Beverages (pp. 483–546). 1st edn. Routledge: New York. 2017.
[27] Ferreira V, López R, Cacho JF. Quantitative determination of the odorants of young red wines from different grape varieties. Journal of the Science of Food and Agriculture. 2000; 80: 1659–1667.
[28] Guth H. Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. Journal of Agricultural and Food Chemistry. 1997; 45: 3027–3032.
[29] Aznar M, López R, Cacho J, Ferreira V. Prediction of Aged Red Wine Aroma Properties from Aroma Chemical Composition. Partial least Squares Regression Models. Journal of Agricultural and Food Chemistry. 2003; 51: 2700–2707.
[30] Van Gemert LJ, Nettenbreijer AH. Compilation of odour threshold values in air and water. National Institute for Water Supply, Voorburg, and Central Institute for Nutrition and Food Research: Zeist, The Netherlands. 1977.
[31] Chaves M, Zea L, Moyano L, Medina M. Changes in Color and Odorant Compounds during Oxidative Aging of Pedro Ximenez Sweet Wines. Journal of Agricultural and Food Chemistry. 2007; 55: 3592–3598.
[32] Gil M, Cabellos JM, Arroyo T, Prodanov M. Characterization of the volatile fraction of young wines from the Denomination of Origin "Vinos de Madrid" (Spain). Analytica Chimica Acta. 2006; 563: 145–153.
[33] Ferreira B, Hory C, Bard MH, Taisant C, Olsson A, Le Fur Y. Effects of skin contact and settling on the level of the C18:2, C18:3 fatty acids and C6 compounds in burgundy Chardonnay musts and wines. Food Quality and Preference. 1995; 6: 35–41.
[34] Perestrelo R, Fernandes A, Albuquerque FF, Marques JC, Câmara JS. Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorant compounds. Analytical Chimica Acta. 2006; 563: 154–164.
[35] Escudero A, Cacho J, Ferreira V. Isolation and identification of odorants generated in wine during its oxidation: a gas chromatography-olfactometric study. European Food Research and Technology. 2000; 211: 105–110.
[36] Marais J. Terpenes in the Aroma of Grapes and Wines: A Review. South African Journal of Enology and Viticulture. 1983; 4: 49–58.
[37] Li H, Tao YS, Wang H, Zhang L. Impact odorants of Chardonnay dry white wine from Changli County (China). European Food Research and Technology. 2008; 227: 287–292.
[38] García M, Greetham D, Wimalasena TT, Phister TG, Cabellos JM, Arroyo T. The phenotypic characterization of yeast strains to stresses inherent to wine fermentation in warm climates. Journal of Applied Microbiology. 2016; 121: 215–233.
[39] García M, Esteve-Zarzoso B, Crespo J, Cabellos JM, Arroyo T. Yeast monitoring of wine mixed or sequential fermentations made by native strains from D.O. "Vinos de Madrid" using real-time quantitative PCR. Frontiers in Microbiology. 2017; 8: 2520.