

Enhancement of flavour properties in wines using sequential inoculations of non-*Saccharomyces* (*Hansenula* and *Torulaspora*) and *Saccharomyces* yeast starter

P. M. IZQUIERDO CAÑAS^{1),2)}, A. T. PALACIOS GARCÍA³⁾ and E. GARCÍA ROMERO¹⁾

¹⁾ Instituto de la Vid y el Vino de Castilla-La Mancha, Tomelloso, Spain

²⁾ Parque Científico y Tecnológico de Albacete, Albacete, Spain

³⁾ Laboratorios Excell Ibérica, Logroño, La Rioja, Spain

Summary

In order to produce wines with greater complexity of aroma and taste, fermentations were carried out by sequential inoculation of non-*Saccharomyces* (*Hansenula anomala* and *Torulaspora delbrueckii*) and *Saccharomyces cerevisiae* yeasts in 'Airén' variety white grape musts. The wines made by sequential inoculation had a higher pH and higher levels of acetates, compounds that supply a fruity note. The wines inoculated with *Hansenula anomala* presented a lower concentration of lineal alcohols and thioalcohols, which are respectively responsible for herbaceous and cooked notes. The fermentations performed by sequential inoculation of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* produced wines with lower levels of volatile acidity, acetaldehyde and carbonyl compounds. In preference tasting, the 'Airén' wines obtained by co-inoculation with the species *Hansenula anomala* scored highest for their sensory qualities and were particularly appreciated for their floral and/or fruity notes.

Key words: Alcoholic fermentation, *Hansenula anomala*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*.

Introduction

The composition of the wine in terms of the volatile products of alcoholic fermentation depends not only on fermentation conditions but also on the yeast strain or species inoculated. Nevertheless, the role of the yeasts in flavour development during vinification is not very well known even now (DUBOURDIEU *et al.* 2006). Spontaneous fermentations of musts generally begin with the growth of non-*Saccharomyces* yeast species of the genera *Candida*, *Debaryomyces*, *Dekkera*, *Hansenula*, *Pichia*, *Torulaspora* and *Zygosaccharomyces*, also known as low-fermentation yeasts (HEARD and FLEET 1985). Growth of these yeast species is limited to the first two or three days of fermentation, after which their population declined from the toxic effect of the ethanol and from the temperature effect (SALVADO *et al.* 2011). At the same time as these yeasts are disappearing, strains of the high-fermentation species *Saccharomyces cerevisiae* begin to multiply until they are the sole lead of fermentation. Although they only grow

during the first few days of fermentation, non-*Saccharomyces* yeasts produce higher concentrations of diverse compounds that can considerably influence the quality of the wine (GIL *et al.* 1996).

In the last few years, studies of non-*Saccharomyces* yeasts have shown that these can make a considerable positive contribution to the vinification process, intensifying and improving the sensory properties of the wines, and they can influence them in original and distinct ways (CIANI, 1997, CIANI *et al.* 2010).

Some researchers have investigated the effect of non-*Saccharomyces* yeasts on the sensory characteristics of wines (EGLI *et al.* 1998, HENICK-KLING *et al.* 1998), using starter cultures which along with *Saccharomyces cerevisiae* contain yeasts of the genera *Kloeckera*, *Cryptococcus*, *Hanseniaspora*, *Candida*, *Pichia* and *Hansenula* (FLEET and HEARD 1993, FLEET 2008), and they have produced wines with significant differences in terms of chemical composition and sensory character (EGLI *et al.* 1998). For instance, FERRARO *et al.* 2000 noted the numerous possibilities offered by the use of non-*Saccharomyces* yeasts in wineries. In particular, the species *Torulaspora delbrueckii* and *Candida stellata* have been studied for their sensory effects during alcoholic fermentation (MARTÍNEZ *et al.* 1990, TORO and VÁZQUEZ 2002, BELY *et al.* 2008).

In this innovative approach to vinification, the key factor in the aromatic complexity of wines is the outcome of the succession of non-*Saccharomyces* and *Saccharomyces* yeast populations during alcoholic fermentation (ROJAS *et al.* 2003, VIANA *et al.* 2008).

The reason for this enhanced aromatic complexity is that, unlike *Saccharomyces* species which do not generally produce significant amounts of extracellular enzymes (MCKAY 1990), non-*Saccharomyces* yeasts secrete a number of enzymes (esterases, glycosidases, β -glucosidases, proteases, cellulases, etc.) (STRAUSS *et al.* 2001) which can interact with the substrate present in the must, improving some stages in the wine-making process and the wine characteristics, especially the aroma (CHAROENCHAI *et al.* 1997, FERNANDEZ *et al.* 2000).

'Airén' is the white grape variety most abundantly grown in Spain, chiefly in the La Mancha region. This is a rather neutral variety, and therefore the aromatic quality of the wines depends essentially on the secondary metabolites produced by the yeasts that carry out the alcoholic fermentation.

In this research, non-*Saccharomyces* and *Saccharomyces* yeasts were sequentially inoculated during two successive harvests to examine the sensory impact that this kind of inoculation can have on wines from a grape of low aromaticity, in this case 'Airén'. *Hansenula anomala* and *Torulaspota delbrueckii* were chosen since they are the yeasts initially present in musts which in principle possess no sensory defect, with a view to reproducing the succession of yeasts which has been observed in numerous studies on vinification ecology (IZQUIERDO *et al.* 1997).

Material and Methods

Fermentation assays: 'Airén' white wines were made in the 2007 and 2008 harvest years. After the grapes had been crushed and pressed, pectolytic enzymes (Lallzyme HC, Lallemand, 2 g·hL⁻¹) were added to the musts, which were decanted statically for 12 h at 15 °C and then distributed evenly in eight 100 L tanks.

In all cases, lysozyme (15 g·hL⁻¹) was added at the start of alcoholic fermentation to prevent the growth of lactic bacteria and thus assure that the end results were only due to the predominant action of the inoculated yeasts. The inoculation scheme for the selected yeasts was as follows:

- Two tanks were inoculated with *Saccharomyces cerevisiae* yeasts following the manufacturer's instructions (Uvaferm VN[®] Lallemand). These tanks were used as controls for fermentation with *Saccharomyces cerevisiae* (VN wines).

- Two tanks were inoculated with *Hansenula anomala* (selected in our laboratory from the la Mancha Region), and when the density fell by approximately 15 units, they were inoculated again with the VN[®] yeast (HAVN wines).

- Two tanks were inoculated with *Torulaspota delbrueckii* (selected in France by Lallemand), and when the density fell by approximately 15 units, they were inoculated again with the VN[®] yeast (TOVN wines).

- Two tanks were inoculated with *Hansenula anomala*, and when the density fell by approximately 15 units, they were inoculated again with *Torulaspota delbrueckii*. When the density once again fell by a further 15 units, they were inoculated anew with the VN[®] yeast (HATOVN wines).

The fermentation temperature was 18 °C; devatting was performed at a density of 1010. The wines were decanted and sulphited, then clarified, stabilised and filtered following standard procedures prior to bottling.

Physicochemical analysis: The wines were analytically characterised. The following parameters were determined: alcoholic degree, glucose and fructose, total acidity, pH, volatile acidity, malic acid and glycerol. Official analytical methods were followed in all cases (OIV 2004).

Analysis of volatile compounds: Samples were analysed by GC/MS using a Thermo Quest mod. Trace GC gas chromatograph and a DSQII mass detector with a single quadrupole analyser. All chromatograms were obtained in electronic impact mode at 70 eV. The selected detector and electron multiplier temperatures were 250 °C and 250 V, respectively.

For the major volatile compounds, 200 mL of wine was steam distilled up to a volume of 200 mL; 1 µL of distilled wine with 4-methyl-2-pentanol as the internal standard was directly injected. The chromatographic conditions were as follows: CP-Wax 57 CB (Varian Inc.) 50 m x 0.32 mm and 0.2 µm phase thick column, with helium as the carrier gas (1.7 mL·min⁻¹, split 1/25); injector temperature, 220 °C; oven temperature, 43 °C (5 min) -4 °C·min⁻¹ -100 °C -20 °C·min⁻¹ -190 °C (1 min).

For analysis of the minor volatile compounds, 500 mL of wine containing 100 µL of 10 g·L⁻¹ 4-nonanol as the internal standard was extracted over 24 h with 250 mL of a 60:40 mixture of pentane and dichloromethane. The extracts were concentrated by distillation in a Vigreux column to 2 mL and then kept at -20 °C until analysis. 2 µL of the extract was injected into a BP21 column (SGE) (50 m x 0.32 mm internal diameter and 0.25 mm thick) of FFAP phase (polyethylene glycol treated with TPA). Chromatographic conditions were: oven temperature, 43 °C (15 min) -2 °C·min⁻¹ -125 °C -1 °C·min⁻¹ -150 °C -4 °C·min⁻¹ -200 °C (45 min) and helium as the carrier gas (1.4 mL·min⁻¹, split 1/15, splitless time 0.5 min.).

Separate compounds were identified by their mass spectra and their chromatographic retention times, using commercial products as standards. Quantification was performed by analysing the characteristic m/z fragment for each compound using the internal standard method. Results for non-available products were shown as the ratio between the area of each compound and that of the internal standard.

Sensory analysis: Descriptive sensory analysis was performed following the Sensory Profile method according to standard Iso 11035, by 14 and 10 trained tasters in 2007 and 2008, respectively. The descriptors were scored on a scale of 0 to 10 (0 absence of the descriptor and 10 maximum intensity).

A hedonic preference test was also performed in which the tasters were asked to list the wines in order of preference, awarding each one points on a scale of 1 to 4. Finally, the average score was calculated and a preference ranking was made.

Statistical analysis: The chemical and sensory data were analysed statistically using the ANOVA test, the Student-Newman-Keuls test of multiple comparison of means and principal component analysis (PCA), using SPSS software.

Results and Discussion

Physicochemical analysis: The results of the physicochemical parameters of the wines are presented in Tab. 1. The wines differed significantly in terms of alcohol, glucose and fructose contents and total acidity, but the trends were not the same in the two different vintages, and therefore no conclusion was reached. No differences between wines were observed in terms of malic acid content. In both vintages, the pH increased in wines subjected to sequential inoculation of either *Hansenula anomala* or *Torulaspota delbrueckii*. It is also important to note that

Table 1

Mean value and standard deviation of the physicochemical parameters studied in wines

Yeasts	VN		HAVN		TOVN		HATOVN	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2007								
Alcohol (% v/v)	13.58 ^a	0.12	14.01 ^c	0.03	13.69 ^{ab}	0.01	13.83 ^b	0.10
Glucose and Fructose	0.29 ^a	0.22	0.68 ^b	0.21	0.23 ^a	0.20	0.14 ^a	0.02
Total acidity (g·L ⁻¹)	4.92 ^c	0.11	4.37 ^b	0.06	4.09 ^a	0.02	4.41 ^b	0.14
pH	3.54 ^a	0.02	3.70 ^b	0.00	3.67 ^b	0.01	3.64 ^b	0.06
Volatile acidity (g·L ⁻¹)	0.31 ^a	0.01	0.49 ^b	0.01	0.28 ^a	0.02	0.53 ^b	0.07
L-Malic acid (g·L ⁻¹)	1.70	0.03	1.60	0.08	1.65	0.18	1.79	0.25
Glycerol	6.14	0.63	5.98	0.10	6.60	0.34	6.13	0.27
2008								
Alcohol (%)	13.80 ^b	0.18	13.68 ^{ab}	0.04	13.46 ^a	0.18	13.61 ^{ab}	0.05
Glucose and Fructose	0.04 ^{ab}	0.02	0.04 ^{ab}	0.01	0.06 ^b	0.01	0.03 ^a	0.00
Total acidity (g·L ⁻¹)	4.45 ^a	0.03	4.76 ^d	0.02	4.57 ^b	0.08	4.66 ^c	0.03
pH	3.48 ^a	0.01	3.51 ^b	0.01	3.54 ^c	0.02	3.52 ^b	0.01
Volatile acidity (g·L ⁻¹)	0.38 ^b	0.02	0.43 ^c	0.04	0.31 ^a	0.04	0.44 ^c	0.01
L-Malic acid (g·L ⁻¹)	1.51	0.07	1.49	0.05	1.48	0.11	1.53	0.08
Glycerol	6.40 ^{ab}	0.01	6.36 ^a	0.12	6.65 ^c	0.07	6.54 ^{bc}	0.04

Different superscripts (^{a, b, c}) indicate significant differences between the yeasts tested for $\alpha = 0.05$ according to the Student-Newman-Keuls test.

in both vintages, the wines inoculated with *Torulaspora delbrueckii* at the start of alcoholic fermentation (TOVN) presented less volatile acidity than the wines in which alcoholic fermentation was carried out only with *Saccharomyces cerevisiae* (VN). The principal feature of *Torulaspora* is its ability to ferment sugars slowly, so that it produces lower levels of volatile acidity (SUÁREZ and IÑIGO 1992) and the osmotolerance (BELY *et al.* 2008, RENAULT *et al.* 2009). On the other hand, in samples inoculated with *Hansenula anomala*, volatile acidity levels were higher than in the VN controls. Nevertheless, these values were still within the normal range for comparable wines. *Hansenula* is, along with *Saccharomyces cerevisiae*, the yeast most commonly found in musts. Its alcohol producing power is very low (4-5°), and so is its alcohol yield (21 to 22 g of sugar/1° of alcohol). It produces more volatile acidity and is easily eliminated by sulphiting owing to its low resistance to SO₂ (ZOECKLEIN *et al.* 1995).

Glycerol is the second largest component of wine after ethanol (in quantities ranging from 6 to 10 g·L⁻¹). Glycerol lends mouthfeel and a silky character to the wine (SUÁREZ 1997). In the 2007 vintage, there were no appreciable differences between wines, but in the 2008 vintage, the glycerol concentration increased slightly in wines fermented with *Torulaspora delbrueckii* and declined in those fermented with *Hansenula anomala*.

Volatile compounds: Tab. 2 shows the concentrations of volatile compounds determined in 'Airen' wines. Since there were more than 150 compounds identified, it was decided to present the data as sums of families of compounds, except in some cases.

The first point of interest was the considerable decline in the acetaldehyde content of wines inoculated with *T. delbrueckii* (TOVN). The same decrease was observed in both vintages, with statistically significant differences between wines. Acetaldehyde is formed during alcoholic fermenta-

tion by decarboxylation of pyruvic acid, although it can also be produced by ethanol oxidation. In excessive amounts, it produces woodiness or an oxidized taste (LAMBRECHTS and PRETORIUS 2000). In a study that reported the aromatic profile of various yeast strains, ROMANO *et al.* 2003 noted that there was little variability in acetaldehyde production among strains of *Saccharomyces cerevisiae*, whereas there was considerable variability among strains of *Hansenula*.

The same tendency was noted in carbonyl compounds (aldehydes and ketones) as in the case of acetaldehyde, namely a considerable decline in the TOVN wines of both vintages, with major and statistically significant differences.

In the case of ester production (Tab. 2), the use of non-*Saccharomyces* yeasts at the start of alcoholic fermentation produced an increase in the acetate contents of the wines (total acetates and ethyl acetate). These results are corroborated by CARRASCOSA *et al.* (2005), who found that wines made with pure non-*Saccharomyces* cultures contained higher ester concentrations than wines fermented with *Saccharomyces cerevisiae* alone.

This point is important since acetates are responsible for the fruity aromas of wines (ROJAS 2002) and such an increase should enhance that sensorial property (LAMBRECHTS and PRETORIUS 2000). Similarly, other authors (HENICK-KLING *et al.* 1998) have concluded that the fruity aroma of wines from 'Riesling' grapes made with non-*Saccharomyces* yeasts was more intense than in wines made using only *Saccharomyces cerevisiae* starters. Other studies have shown that the genera *Candida*, *Hansenula* and *Pichia* possess a greater ethyl acetate production capacity than *Saccharomyces cerevisiae* strains (NYKÄNEN 1986; PLATA *et al.* 2003).

On the other hand, the use of non-*Saccharomyces* strains produced lower contents in ethyl ester in the 2008 vintage, with significant differences between wines; the

Table 2

Mean value and standard deviation of the volatile compounds studied in wines

Yeasts	VN		HAVN		TOVN		HATOVN	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2007								
Acetaldehyde ¹	30.28 ^b	0.03	32.98 ^b	1.47	19.80 ^a	4.43	24.23 ^a	1.15
Aldehydes and ketones ¹	30.77 ^c	0.04	33.43 ^c	1.45	20.14 ^a	4.44	24.67 ^b	1.08
Total Acetates ¹	35.96 ^a	0.90	49.25 ^b	0.03	54.37 ^b	4.73	41.66 ^a	3.76
Ethyl acetate ¹	31.30 ^a	0.73	43.45 ^c	0.16	47.22 ^c	3.59	36.86 ^b	3.38
Ethyl esters ¹	5.78	0.96	5.57	0.12	5.20	0.12	5.29	0.35
Esters of dicarboxylic acids ¹	2.88 ^b	0.32	1.25 ^a	0.12	1.98 ^{ab}	0.29	2.13 ^{ab}	0.74
Linear alcohols ¹	483.34 ^d	2.10	274.86 ^a	2.32	347.51 ^c	18.63	310.25 ^b	19.95
C6 alcohols ¹	0.95 ^b	0.55	0.63 ^a	0.04	0.62 ^a	0.01	0.83 ^{ab}	0.20
Thioalcohols ¹	1.49 ^b	0.85	0.67 ^a	0.04	1.19 ^{ab}	0.22	1.05 ^{ab}	0.45
Benzene derivatives ¹	51.46 ^b	3.69	27.97 ^a	0.10	38.12 ^a	1.13	33.86 ^a	9.61
Furfuryl derivatives ²	27.10 ^a	4.39	30.40 ^a	2.13	31.00 ^a	1.15	45.29 ^b	4.70
Acids ¹	16.50 ^a	4.94	31.17 ^b	1.55	24.58 ^b	2.51	24.00 ^b	3.04
Volatile phenols ¹	1.93	0.53	1.23	0.20	1.20	0.03	1.63	0.31
Lactones ¹	2.61	0.46	2.15	0.12	2.14	0.40	2.34	0.23
Terpenes ²	5.51 ^b	0.30	3.78 ^a	0.06	3.31 ^a	0.27	7.80 ^c	1.77
Norisoprenoids ²	2.83 ^b	0.18	3.28 ^c	0.11	2.14 ^a	0.16	3.44 ^c	0.25
2008								
Acetaldehyde ¹	33.88 ^b	3.97	31.05 ^b	3.25	22.44 ^a	3.29	29.44 ^b	1.45
Aldehydes and ketones ¹	34.28 ^b	4.02	31.42 ^b	3.38	22.68 ^a	3.31	29.78 ^b	1.43
Total Acetates ¹	39.28	1.48	53.99	11.13	40.51	4.87	53.31	0.16
Ethyl acetate ¹	33.75	1.66	47.42	11.30	33.84	4.40	46.86	0.16
Ethyl esters ¹	6.57 ^b	0.45	5.36 ^a	0.22	5.71 ^a	0.24	5.35 ^a	0.20
Esters of dicarboxylic acids ¹	6.14 ^a	0.95	5.76 ^a	0.43	10.63 ^c	0.93	7.93 ^b	1.02
Linear alcohols ¹	256.5 ^{ab}	9.35	246.77 ^a	19.61	287.34 ^b	2.21	282.83 ^b	21.85
C6 alcohols ¹	0.77 ^b	0.06	0.67 ^a	0.02	0.78 ^b	0.03	0.67 ^a	0.07
Thioalcohols ¹	1.07 ^b	0.05	0.54 ^a	0.04	1.02 ^b	0.20	0.39 ^a	0.03
Benzene derivatives ¹	31.73 ^b	7.37	20.34 ^a	2.66	30.44 ^b	3.91	17.31 ^a	0.84
Furfuryl derivatives ²	23.75 ^{ab}	1.13	19.96 ^a	0.89	25.72 ^b	2.76	20.38 ^a	2.20
Acids ¹	17.36 ^a	1.33	18.66 ^{ab}	1.38	20.61 ^b	1.12	19.01 ^{ab}	0.33
Volatile phenols ¹	4.72 ^a	0.50	4.67 ^a	0.33	5.90 ^b	0.37	5.39 ^{ab}	0.18
Lactones ¹	8.14	0.80	8.77	0.78	7.11	0.17	7.96	0.60
Terpenes ²	17.75	4.07	20.10	0.50	22.74	2.22	18.08	1.25
Norisoprenoids ²	7.82	0.76	9.66	1.98	9.88	0.63	10.82	0.73

¹ mg·L⁻¹; ² µg·L⁻¹; Different superscripts (a, b, c) indicate significant differences between the yeasts tested for $\alpha = 0.05$ according to the Student-Newman-Keuls test.

same did not occur in the 2007 vintage, where all the wines presented similar concentrations of these compounds. Moreover, the use of *Hansenula anomala* caused a decline in dicarboxylic acid ester contents in both vintages.

In the case of linear alcohols, which impart herbal notes to the wine (ROJAS 2002), in both vintages there was a decrease in the concentration of these compounds in wines inoculated with *Hansenula anomala*. In wines inoculated with *Torulaspora delbrueckii*, the decline was smaller in the 2007 vintage, whereas in the 2008 vintage there were slight increases.

When non-*Saccharomyces* strains were used, there was a statistically significant decrease in the concentration of C6 alcohols in both vintages. As in the case of linear alcohols, this decline was more pronounced in wines inoculated with *Hansenula anomala* at the start of alcoholic fermentation. Inoculation with *Hansenula* further produced a

considerable decline in thioalcohol concentrations in the wines of both vintages. This development could enhance the aromatic quality of the wines since these compounds are responsible for leafy, cooked, oniony and other aromas. Sequential inoculation with non-*Saccharomyces* yeasts produced a decrease in concentrations of benzene derivatives in both vintages, particularly in those initially inoculated with only *Hansenula anomala*. On the other hand, non-*Saccharomyces* strains appeared to produce a slight increase in concentrations of furfuryl derivatives, although these results were not repeated in the 2008 vintage, where their concentration declined slightly in wines inoculated with *Hansenula anomala* alone.

Tab. 2 shows an increase in the concentration of organic acids in wines of both vintages inoculated with non-*Saccharomyces*, which was much greater in the 2007 vintage. There were significant differences between wines

inoculated with *Saccharomyces cerevisiae* alone (VN) and all the other wines.

Concentrations of norisoprenoids increased with *Hansenula anomala* in the 2007 vintage, a pattern that was confirmed in all wines made with non-*Saccharomyces* strains in the 2008 vintage.

In the case of other volatile components (phenols, terpenes and lactones) there were no major differences between the wines subjected to sequential inoculation and the ones made with *Saccharomyces cerevisiae* only.

Sensory analysis: Fig. 1 shows the projection of aroma and taste descriptors best correlated with principal components 1 and 2. It also presented the centroids of the wines from the 2007 vintage of the Airén variety. These two principal components explain up to 80 % of the variance.

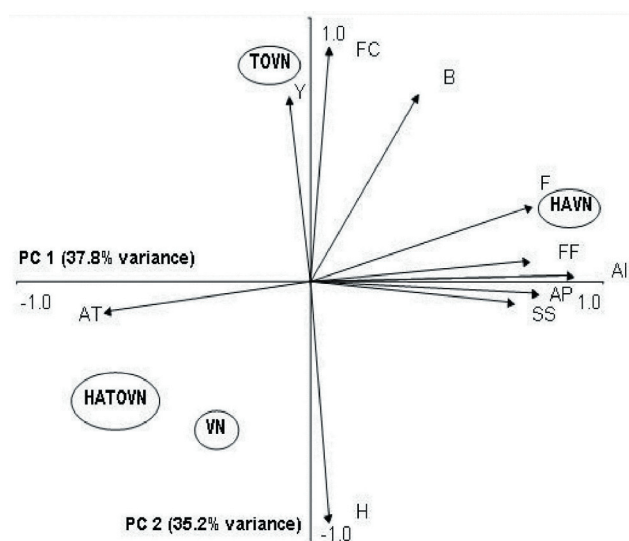


Fig. 1: Sensory analysis of 2007 wines: Projection of aroma and taste descriptors best correlated with principal components 1 and 2. AI: Aromatic intensity; AP: Aftertaste persistence; AT: Alcoholic taste; B: Balsamic; F: Fruit; FF: Fresh fruit; FC: Flavour complexity; SS: Sweet smell; H: Herbaceous; Y: Yeast.

Three groups of wines could be distinguished in 2007. HATOVN and VN wine had higher alcoholic taste and were more herbaceous than TOVN and HAVN.

The TOVN were the most complex wines, presenting strong yeasty and balsamic notes, and were the least herbaceous. The HAVN wines stood clearly apart from the rest, presenting the most intense aroma, fruitiness, confectionery notes (sweet smell) and long aftertaste.

The results were not exactly the same in the 2008 (Fig. 2) as in the 2007 vintage; the TOVN wine could be found along with the group comprising HATOVN and VN wines, which were placed in the zone of greatest balsamic notes. The HAVN wines were again separated from the rest and presented a notably longer-lasting aftertaste, with more intense sweet smell, floral and fruity notes. These results approximately match the findings of similar study (EGLI *et al.* 1998) which reported sensory analysis of 'Riesling' and 'Chardonnay' wines made by spontaneous fermentation in the presence of non-*Saccharomyces* strains with starter cultures of two different strains of *Saccharomyces*

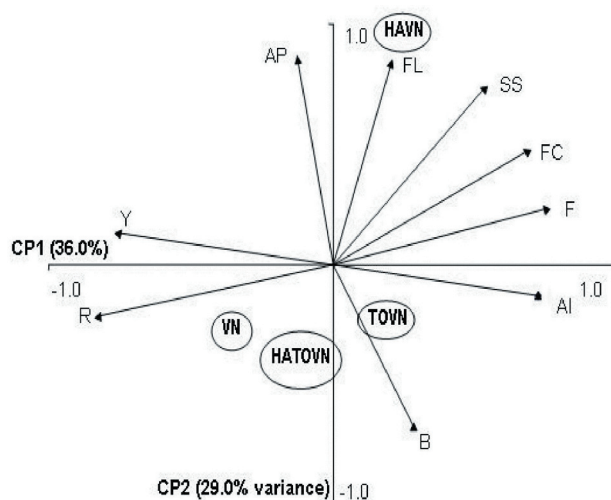


Fig. 2: Sensory analysis of 2008 wines: Projection of aroma and taste descriptors best correlated with principal components 1 and 2. AI: Aromatic intensity; AP: Aftertaste persistence; B: Balsamic; F: Fruit; FC: Flavour complexity; FL: Floral; SS: Sweet smell; Y: Yeast; R: Reduction.

cerevisiae. In the opinion of the tasting panel, the wines of both grape varieties, made by spontaneous fermentation, scored higher because they presented a more intense floral and fruity aroma.

As was to be expected from the results of the descriptive sensory analysis, of the 2007 wines, the tasters preferred the ones sequentially fermented with the *Hansenula anomala* strain (HAVN) (Fig. 3). In second place, they scored the wines made with the strain *Torulaspora delbrueckii* (TOVN) and in last place the ones made with the two non-*Saccharomyces* strains (HATOVN) and the ones inoculated with a pure *Saccharomyces cerevisiae* culture (VN). The results for the 2008 vintage were similar; the tasters again preferred the wines fermented sequentially with the *Hansenula anomala* strain (HAVN) with statistically significant higher scores ($p \leq 0.05$). The other three wines were marked with lower scores and no differences were found among them.

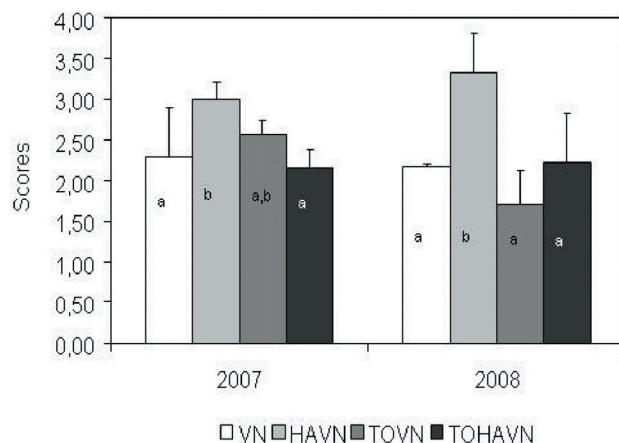


Fig. 3: Sensorial hedonic scores of 'Airen' wines in the two studied harvest (mean value and deviation standard). Different letters indicate significant differences between the yeasts tested for $\alpha = 0.05$ according to the Student-Newman-Keuls test.

Conclusions

Sequential inoculation with non-*Saccharomyces* followed by *Saccharomyces* yeasts strains produces changes in the chemical composition of wines which affect their sensory characteristics. Our results differed depending on which non-*Saccharomyces* strain was used in the starter. In two consecutive vintages, the use of *Hansenula anomala* followed by *Saccharomyces cerevisiae* improved the aroma and sensory quality of Airén white wines, which presented greater aromatic complexity.

Non-*Saccharomyces* yeasts clearly have considerable potential, as yet unexploited, as microorganisms that can influence both the aroma of the wine and the actual vinification process. Therefore, more comprehensive studies are needed to select strains with characteristics that can positively influence the wine-making process while minimising their possible negative effects. The ultimate aim would be to provide wineries with yeast genera other than *Saccharomyces* in the form of LSA for use on an industrial scale with a view to producing wines with particular and distinctive sensory characteristics.

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References

- BELY, M.; STOECKLE, P.; MASNEUF-POMARED, I.; DUBOURDIEU, D.; 2008: Impact of mixed *Torulasporea delbrueckii*-*Saccharomyces cerevisiae* culture on high-sugar fermentation. *Int. J. Food Microbiol.* **122**, 312-320.
- CARRASCOSA, A. V.; MUÑOZ, R.; GONZÁLEZ, R.; 2005: *Microbiología del Vino*. AMV, Spain.
- CHAROENCHAI, C.; FLEET, G. H.; HENSECHKE, P. A.; TODD, B. E. N.; 1997: Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes. *Aust. J. Grape Wine Res.* **3**, 2-8.
- CIANI, M.; 1997: Role, enological properties and potential use of non-*Saccharomyces* wine yeasts. *Recent Res. Dev. Microbiol.* **1**, 317-331.
- CIANI, M.; COMITINI, F.; MANNAZZU, I.; DOMIZIO, P.; 2010: Controlled mixed culture fermentation: a new perspective on the use of non-*Saccharomyces* yeasts in winemaking. *FEMS Yeast Res* **10**, 123-33.
- DUBOURDIEU, D.; TOMINAGA, T.; MANSNEUF, I.; PEYROT DES GACHONS, C.; MURAT, M. L.; 2006: The role of yeast in grape flavour development during fermentation: the example of Sauvignon Blanc. *Am. J. Enol. Vitic.* **57**, 81-88.
- EGLI, C. M.; EDINGER, W. D.; MITRAKUL, C. M.; HENICK-KLING, T.; 1998: Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. *J. Appl. Microbiol.* **85**, 779-789.
- FERNÁNDEZ, M.; UBEDA, J. F.; BRIONES, A. I.; 2000: Typing of non-*Saccharomyces* yeasts with enzymes activities. *Int. J. Food Microbiol.* **59**, 29-36.
- FERRARO, L.; FATICHENTI, F.; CIANI, M.; 2000: Pilot scale vinification process using immobilized *Candida stellata* cells and *Saccharomyces cerevisiae*. *Process Biochem.* **35**, 1125-1129.
- FLEET, G. H.; 2008: Wine yeasts for the future. *FEMS Yeast Res.* **8**, 979-995.
- FLEET, G. H.; HEARD, G. M.; 1993: *Microbiology and Biotechnology, Yeast-growth during winemaking*. G. H. Fleet Wine Harwood Academic Publishers, Chur.
- GIL, J. V.; MATEO, J. J.; JIMÉNEZ, M.; PASTOR, A.; HUERTA, T.; 1996: Aroma compounds in wine as influenced by apiculate yeasts. *J. Food Sci.* **61**, 1247-1249.
- HEARD, G. M.; FLEET, G. H.; 1985: Growth of natural yeast flora during the fermentation of inoculated wines. *Appl. Environ. Microbiol.* **50**, 727-728.
- HENICK-KLING, T.; EDINGER, W.; DANIEL, P.; MONK, P.; 1998: Selective effects of sulphur dioxide and yeast starter addition on indigenous yeast populations and sensory characteristics of wine. *J. Appl. Microbiol.* **84**, 865-876.
- IZQUIERDO, P. M.; UBEDA, J.; BRIONES, A.; 1997: Study of the karyotype of wine yeasts isolated in the region of Valdepeñas in two consecutive vintages. *Food Microbiol.* **14**, 221-225.
- LAMBRECHTS, M. G.; PRETORIUS, I. S.; 2000: Yeast and its importance to wine aroma. A Review. *S. Afr. J. Enol. Vitic.* **21**, 97-129.
- MARTÍNEZ, J.; TOLEDANO, F.; MILLÁN, C.; ORTEGA, J. M.; 1990: Development of alcoholic fermentation in non-sterile musts from "Pedro Ximénez" grapes inoculated with pure cultures of selected yeasts. *Food Microbiol.* **7**, 217-225.
- McKAY, A. M.; 1990: Degradation of polygalacturonic acid by *Saccharomyces cerevisiae*. *Lett. Microbiol.* **11**, 41-44.
- NYKÄNEN, L.; 1986: Formation and occurrences of flavour compounds in wine and distilled alcohol beverages. *Am. J. Enol. Vitic.* **37**, 84-96.
- OIV 2004: *Reweil des Méthodes Internationales d'Analyse de Vins et des Mousts*. Ed. OIV (Office International de la Vigne et du Vin). Paris.
- PLATA, C.; MILLÁN, C.; MAURICIO, J. C.; ORTEGA, J. M.; 2003: Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. *Food Microbiol.* **20**, 217-224.
- RENAULT, P.; MIOT-SERTIER, C.; MARULLO, P.; HERNÁNDEZ-ORTE, P.; LAGARRIGUE, L.; LONVAUD-FUNEL, A.; BELY, M.; 2009: Genetic characterization and phenotypic variability in *Torulasporea delbrueckii* species: potential applications in the wine industry. *Int. J. Food Microbiol.* **134**, 201-210.
- ROJAS, V.; 2002: *Actividades esterasicas en levaduras vinicas*. Doctoral thesis University of Valencia.
- ROJAS, V.; GIL, J. V.; PINAGA, F.; MANZANARES, P.; 2003: Acetate ester formation in wine by mixed cultures in laboratory fermentations. *Int. J. Food Microbiol.* **86**, 181-188.
- ROMANO, P.; FIORE, C.; PARAGGIO, M.; CARUSO, M.; CAPECE, A.; 2003: Function of yeast species and strains in wine flavour. *Int. J. Food Microbiol.* **86**, 169-180.
- SALVADÓ, Z.; ARROYO-LÓPEZ, F. N.; GUILLAMÓN, J. M.; SALAZAR, G.; QUEROL, A.; BARRIO, E.; 2011: Temperature Adaptation Markedly Determines Evolution within the Genus *Saccharomyces*. *Appl. Environ. Microbiol.* **77**, 2292-2302.
- STANDARD ISO 11035; 1994: Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach. *Sensory Analysis*.
- STRAUSS, M. L. A.; JOLLY, N. P.; LAMBRECHTS, M. G.; VAN RENSBURG, P.; 2001: Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. *J. Appl. Microbiol.* **91**, 182-190.
- SUÁREZ, J. A.; IÑIGO, B.; 1992: *Microbiología Enológica*. Fundamentos de Vinificación. Mundi-Prensa (2nd ed), Madrid.
- SUÁREZ, J. A.; 1997: *Levaduras Vínicas*. Funcionalidad y Uso en Bodega. Mundi-Prensa, Madrid.
- TORO, M. E.; VÁZQUEZ, F.; 2002: Fermentation behaviour of controlled mixed and sequential cultures of *Candida cantarelli* and *Saccharomyces cerevisiae* wine yeast. *World J. Microbiol. Biotechnol.* **18**, 347-354.
- VIANA, F.; GIL, J. V.; GENOVÉS, S.; VALLÉS, S.; MANZANARES, P.; 2008: Rational selection of non-*Saccharomyces* wine yeasts for mixed starters based on ester formation and enological traits. *Food Microbiol.* **25**, 778-785.
- ZOECKLEIN, B. W.; FUGELSANG, K. C.; GUMP, B. H.; NURY, F. S.; 1995: *Wine Analysis and Production*. Chapman & Hall, New York.