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Use of *Hanseniaspora* spp. in sequential fermentation with *Saccharomyces cerevisiae* to improve the aromatic complexity of Albillo Mayor white wines

Juan Manuel del Fresno[1], Francisco Carrau[2], Carlos Escott[1], Cristian Vaquero[1], Carmen González[1], and Antonio Morata[1]

[1]enotecUPM, Dept. Chemistry and Food Technology, ETSIAAB, Universidad Politécnica de Madrid, 208040 Madrid, Spain
[2]Área Enología y Biotecnología de Fermentaciones, Facultad de Química, Universidad de la Republica, Montevideo 11800, Uruguay

Abstract. *Hanseniaspora* spp apiculate yeasts can be found on ripe grape skins and during the first six days of the alcoholic fermentation. Generally, these yeasts have poor characteristics for its industrial application in winery as they are related with low fermentative power, low resistance to SO_2 and even high volatile acidity production. However, some species have a better fermentative capacity and are producers of certain floral and fruity volatiles. This is the case of the two strains used in this study. *Hanseniaspora vineae* (HV) has a fermentative power around 8-10% v/v, low volatile acidity production and produces high levels of 2-phenylethyl acetate. Similarly, *Hanseniaspora opuntiae* (HO) also produces a low volatile acidity providing sweet and floral aromas, but has a fermentative power around 6% v/v, which means that it must be used in sequential fermentation with *Saccharomyces cerevisiae* (SC). In addition, several studies indicate that both species can increase the mouthfeel and wine body. The aim of this study was to evaluate the use of HV and HO in sequential fermentation with SC to improve the sensory profile of high quality white wines from the neutral grape variety Albillo Mayor. Fermentations were performed in triplicate in 150 L stainless steel barrels with grapes from the 2021 vintage. Pure SC fermentations were used as controls. After the fermentation, the polysaccharide content and the colour was measured, and an intensive study of the aromatic profile was done. The results indicated a higher concentration of polysaccharides in control wines, around 300 mg/L, than in the other fermentations. The aromatic profile varied considerably in the different wines. Up to 1.55 times higher content of 2-phenylethanol was identified in HO wines and up to 3 times higher content of fermentative esters in HV wines, compared to controls. It is noted that only in the sequential fermentations with HV the safranal compound was found. This compound represents the most potent aromatic component of saffron and was only identified in these wines at concentrations of around 9 µg/L.

The use of HO and HV in sequential fermentation with SC could be a powerful technique to increase the aromatic complexity of neutral varietal white wines.

1 Introduction

The apiculate yeasts *Hanseniaspora* spp. are yeasts found on the skins of ripe grapes and in the fermenting must before the sixth day of fermentation. Some of these species show interesting characteristics for the production of quality white wines such as a medium fermentative power, the contribution of floral aromas or the increase of acetate esters and benzenoids levels [1]. The two species used in this study were *Hanseniaspora vineae* (HV) and *Hanseniaspora opuntiae* (HO). HV has a medium fermentative power (8-10% *v/v*), produces low volatile acidity, provides high levels of 2-phenylethyl acetate, with a consequent floral impact on wines [2], and produces de novo terpenes [3]. In addition, this yeast also showed a colour protective effect on rosé wines [4]. HO is the other species used; this strain produces low volatile acidity and the expression of floral and sweet aromas [5]. However, HO must be always used with *Saccharomyces cerevisiae* (SC) in sequential fermentation because it has a fermentative power around 6% *v/v*. Both HV and HO have been shown to increase the mouthfeel and body of wines. The aim of this study was to evaluate the use of HV and HO in sequential fermentation with SC to improve the sensory profile of white wines from the Albillo Mayor grape variety.

2 Materials and methods

The trial was carried out on a semi-industrial scale in Bodegas Comenge cellar (D.O. Ribera del Duero, Spain) using Albillo Mayor grapes from the 2021 vintage. Figure 1 shows schematically the assay performed as well as the analyses carried out after alcoholic fermentation. The yeast strains used were: HV strain T02/5A selected by the team of Professor Francisco Carrau (Universidad de la República, Montevideo, Uruguay); The HO strain A56 selected by enotec-UPM (Universidad Politécnica de Madrid, Spain); and the SC Fermivin 3C (Oenobrands SAS) was used as control and to finish the sequential fermentations. Pneumatic pressing was used to obtain the must from the Albillo Mayor white grape variety. One hundred fifty litre stainless steel barrels were used for fermentations in triplicate. The barrels were inoculated with 5 L (≈3%) of liquid inoculum that was prepared in YPD media. SC fermentations were used as controls in this study and were inoculated at time zero. On day six, these controls were also inoculated with SC to keep the same volume in all triplicates.

The polysaccharides content was measured by the HPLC-refractive index (RI) technique, according to the method described by [6]. Colour parameters were measured by visible spectrophotometry using a 1 mm plastic cuvette. Gas chromatography with flame ionisation detection (GC-FID) was used to analyse fermentative volatiles, the method is described in [7]. Gas chromatography with thermal desorption coupled to tandem mass spectrometry (GC-MS/MS) was used to analyse aroma compounds related to freshness [7].

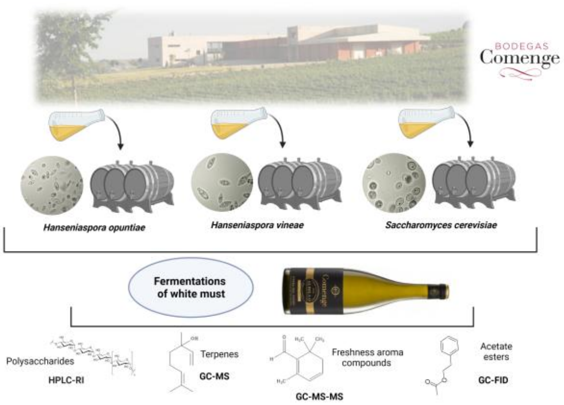

Figure 1. Graphical summary of the trial in Bodegas Comenge.

3 Results

The spectrophotometric parameters measured in the wines were colour intensity, tonality and CIELAB coordinates. The only differences identified among all these parameters were in lightness (L*). The highest values of around 96 were found in the HO wines, which were statistically higher than those of the HV and SC wines. The most significant results were obtained from analyzing the polysaccharide content and aromatic profile of the wines studied.

4 Polysaccharides content

The polysaccharides present in wine come from grapes (pectic polysaccharides [8]) and from the transfer of cell wall polysaccharides from yeasts (mannoproteins). In this study, the total polysaccharide content was measured by HPLC-RI, assuming that the variations obtained are due only to the transfer of cell wall polysaccharides from the yeasts studied or to their interaction with grape polysaccharides. Figure 2 shows the results obtained after alcoholic fermentation. The control wines fermented with SC showed polysaccharide values of around 300 mg/L. These values were statistically higher than that found in the wines fermented with *Hanseniaspora* genus yeasts. No significant differences were identified between HO and HV wines. Since SC was inoculated into the *Hanseniaspora* barrels on the sixth day, it is possible that the transfer of polysaccharides is affected by the interaction between the two yeast species.

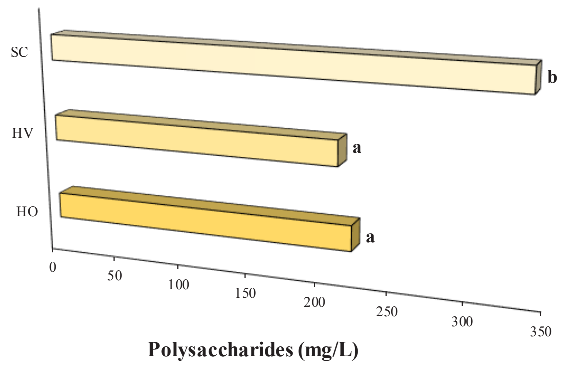

Figure 2. Polysaccharides (mg/L) detected in white wines afther the fermentation process. Different letters indicate the significant differences ($p < 0.05$).

5 Fermentative volatile compounds

Carbonyl compounds, higher alcohols and esters were measured by GC-FID. No significant differences in the content of carbonyl compounds such as acetaldehyde, diacetyl or 2,3-Butanediol were identified in the wines studied. In relation to the higher alcohols, 2-phenylethanol is noted for its significant sensory impact on wines described as 'rose-like' and 'floral' [9], the content of this compound was found in values of around 28 mg/L in HO wines (Fig. 3), these values were significantly higher than the values identified in the rest of the wines. Another of the aromatic groups studied were the volatile esters, these compounds are considered the main source of fruity aroma in wines [10]. HV wines were found to have values that were up to three times higher than SC wines and up to two times higher than HO wines when considering the overall sum of these components. A particularly important ester is 2-phenylethyl acetate, considered a compound that provides floral aromas to wines [11]. The apiculate yeasts produced up to 2.8 times more quantity of this compound than the controls (SC).

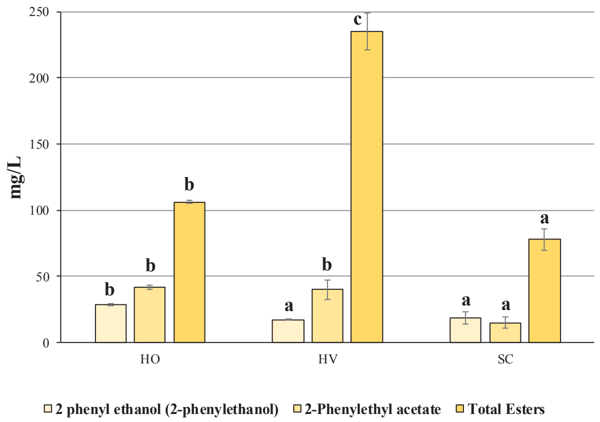

Figure 3. Total esters, 2-phenylethanol and 2-Phenylethyl acetate (mg/L) measured by GC-FID after the fermentation process. Bars with the same letter are not significantly different ($p < 0.05$).

6 Freshness-related aroma compounds

By using GC-MS-MS, seventeen volatile compounds from various groups were analysed. Only five volatile compounds related to wine freshness have been found. Among these compounds, safranal stands out, which is a C10 norisoprenoid ($C_{10}H_{14}O$) with a monoterpene aldehyde chemical structure (Fig. 4). The strongest fragrant component of saffron is safranal [12]. This compound was identified only in wines fermented with HV at concentrations around 9 µg/L. Further research is needed to understand how *H. vineae* metabolism affects the presence of safranal in Albillo Mayor white wines.

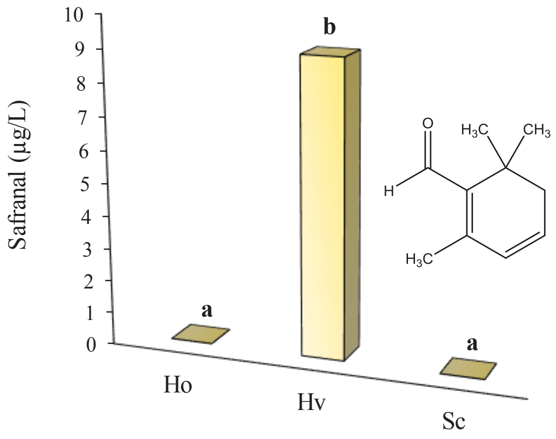

Figure 4. Safranal (µg/L) measured by GC-MS-MS after the fermentation process. Bars with the same letter are not significantly different ($p < 0.05$).

7 Conclusions

Yeasts of the genus *Hanseniaspora* had a lower cell wall polysaccharide release capacity than SC. Some differences were identified in the colour of the white wines obtained; the wines fermented by HO were more transparent. The wines obtained showed clear differences in their aromatic profile. In relation to the volatile compounds from alcoholic fermentation, the HO wines had a higher presence of 2-phenyl ethanol. However, HV produces more fruity esters but with similar quantities of 2-phenylethyl acetate compared to HO. Interestingly, the potent aromatic compound safranal was identified in the HV fermented wines. Selected yeasts of the *Hanseniaspora* genus can be a powerful tool to increase the aromatic complexity of white wines.

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