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EFFECT OF THREE *SACCHAROMYCES CEREVISIAE* STRAINS ON THE VOLATILE COMPOSITION OF ALBARIÑO WINES

INFLUENZA DI TRE CEPPI DI *SACCHAROMYCES CEREVISIAE*
SULLA COMPONENTE VOLATILE DI VINI ALBARIÑO

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ABSTRACT

Must obtained from *Vitis vinifera* L. cv Albariño grapes was inoculated with three strains of *Saccharomyces cerevisiae* (Asln1, Asln2 and Asln20) and fermented under identical conditions. Standard chemical analyses were carried out on the final wines and volatile compounds were analysed by gas chromatography. Statistical analysis of the data obtained by the latter technique showed that the wines produced differed depending on the yeast strain used. The formation of alcohols,

RIASSUNTO

Tre ceppi di *Saccharomyces cerevisiae* (Asln1, Asln2 and Asln20) sono stati inoculati su mosto ottenuto da uve di *Vitis vinifera* L., cv. Albariño, e successivamente fatti fermentare nelle medesime condizioni. Sui vini finiti sono state condotte analisi chimiche routinarie e la componente volatile è stata determinata tramite gas cromatografia. Le analisi statistiche dei dati, ottenuti seguendo le tecniche sopradescritte, hanno messo in evidenza che la differenza riscontrata nei vini prodotti è dovuta al

- Key words: Albariño wine, alcoholic fermentation, *Saccharomyces cerevisiae*, volatile composition -

esters and isoamyl acetate by Asln1, Asln2 and Asln20 differed significantly ($P < 0.05$). The probability associated with the differences for isobutanol and isoamyl alcohol was 99.9%.

ceppo di lievito usato. La formazione di alcoli, esteri e isoamil acetato differisce in modo statisticamente significativo ($P < 0,05$) a seconda dei lieviti impiegati (Asln1, Asln2 and Asln20). La probabilità statistica associata alle differenze riscontrate nei diversi vini per quanto riguarda l'isobutanolo e l'alcool isoamilico è risultata del 99,9%.

INTRODUCTION

Yeasts, key microorganisms in wine-making, conduct alcoholic fermentation. The selection of the yeast strain and fermentation conditions are claimed to be the most important factors influencing the volatile compounds in wine. The aroma profile of wine is derived from the grape through changes in grape flavor precursors caused by yeast. The composition and quality of wine are closely related to the yeast (FLEET and HEARD, 1993; GIL *et al.*, 1996) which produces volatile compounds as secondary products during fermentation. While the volatile metabolites contribute to the fermentation bouquet ubiquitous to all young wines, the amounts of these by-products vary and are yeast-strain specific (LAMBRECHTS and PRETORIUS, 2000).

The main groups of compounds that form the fermentation bouquet are higher alcohols and esters (RAPP and VERSINI, 1991). When present in excess, some fermentation bouquet compounds, such as acetaldehyde, acetic acid, ethyl acetate, higher alcohols and diacetyl, may also be regarded as undesirable (LAMBRECHTS and PRETORIUS, 2000). It is known that volatile compounds of wines vary little during alcoholic fermentation (DUBOURDIEU *et al.*, 1988; DARRIET *et al.*, 1995; MURAT *et al.*, 2001) since the glycosidases of *Saccharomyces cerevisiae* have little effect on terpenic glycosides at the pH of the must.

Studies in which starter cultures and indigenous yeasts have been investigated have shown that there are significant differences in the chemical composition of the resulting wines (EGLI *et al.*, 1998; HENICK-KLING *et al.*, 1998; ESTÉVEZ *et al.*, 2004). PATEL and SHIBAMOTO (2002, 2003) showed that the formation and concentration of the volatile compounds found in Symphony, Napa Gamay and Petite Sirah wines were dependent on the strain of *S. cerevisiae* used.

Furthermore, it has been discovered that during alcoholic fermentation Sauvignon blanc varietal precursors are transformed into aroma varietal compounds by *S. cerevisiae* (DARRIET *et al.*, 1995)

The effect of *S. cerevisiae* yeast strains on the liberation of the volatile compounds was studied in Palomino (ESTÉVEZ *et al.*, 2004), Emir (NURGEL *et al.*, 2002) and Sauvignon blanc (MURAT *et al.*, 2001) wines.

Albariño, a grape variety of *Vitis vinifera* L., is a typical white variety from Galicia in northwestern Spain, mostly on the Atlantic coast (Appellation Controlée Rías Baixas) and in northern Portugal (VQPRD Vinhos verdes). It is characterised by a high intensity of floral aromas. Free monoterpenes are responsible for these floral notes (CARBALLEIRA *et al.*, 2001). Young white wines prepared from Albariño grapes are dominated by fruity and floral aromas (CARBALLEIRA *et al.*, 2001; FALQUÉ *et al.*, 2001). The high

amount of monoterpenes could explain the floral aroma in Albariño wines.

The objective of this study was to determine the role of the selected *S. cerevisiae* strains on the volatile composition of single varietal wines produced from Albariño grapes. Multivariate data analysis techniques were used for these comparisons.

MATERIALS AND METHODS

Yeast

The *S. cerevisiae* yeast strains used in this study (ASIn1, ASIn2 and ASIn20) were isolated from musts obtained from grapes harvested in the Rías Baixas region (Spain) from a winery which had never used commercial yeast starters. The karyotypes of the three yeasts used (PFGE) are shown in Fig. 1. Chromosomal DNA was prepared in agarose plugs following the method of [BELLIS *et al.* \(1987\)](#). Electrophoresis was performed with a Pharmacia-LKB (Pulsaphor) apparatus based on the contour homogeneous electric field (CHEF) principle (DUBOURDIEU and FREZIER, 1990) under the following conditions: 0.8% agarose gel, migration at 10°C, TBE 1X (Tris Sigma 7-9 90 mM, boric acid 90mM, EDTA 2mM pH 8) as the migration buffer, 165 v, pulsed time program: 90 s-20h, 100 s-12 h, 120 s-12 h, 30 s-4 h.

Fermentations

Must from Albariño grapes was collected from a winery in the Rias Baixas region before the addition of sulfur dioxide. The must was transported to the laboratory at 8°C and then stored at -20°C. Prior to fermentation, it was centrifuged (10,000 x g for 20 min) and sterilized by membrane filtration through a Millipore system (0.45 µm membrane); sulfur dioxide was then added (50 mg/L).

The initial sugar concentration was 190 g/L and the pH was 3.1.

Fermentations were performed in 16 L flasks containing 14 L of Albariño must. Yeast cells were cultured in liquid YEPD media, washed twice with sterile water and suspended in the must at a density of 10⁶ cells/mL. Fermentations were conducted at 18°C for 15 days. Samples of 10 mL were periodically collected from the flasks and used to test strain dominance by Pulsed Field Gel Electrophoresis (PFGE).

All fermentations were performed in triplicate. The data obtained on cell growth, as well as the analytical results, are means of three replicates. At the end of fermentation, the wines were centrifuged and sulfur dioxide was added (50 mg/L). The wines were bottled and 1L of each wine was analyzed after 30 days.

Chemical analysis

The following measurements were made to compare the effect of each yeast according to Office International de la Vigne et du vin (OIV, 1990) official methods: pH (measured with a pH meter), ethanol content (by distillation of wine made

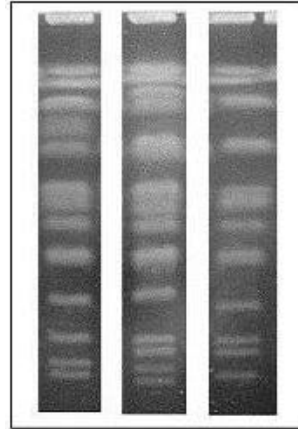


Fig. 1 - Pulsed Field Gel Electrophoresis (PFGE) of *S. cerevisiae* yeast strains (ASIn1, ASIn2 and ASIn20).

with a suspension of calcium hydroxide), total acidity (by titration with bromothymol blue as an indicator), volatile acidity (by titration of the volatile acids separated from the wine by steam distillation and titration of the distillate), total dry extract (by measurement with a densitometer), tartaric acid (gravimetric method), malic acid (enzymatic method) and reducing sugar content (by determination of glucose and fructose using an enzymatic method. Determinations were made in triplicate.

Volatile compounds

The contents of the most relevant aromatic fermentation compounds (higher alcohols, esters and acetates) of the final wines were determined by gas chromatography (GC).

The analyses were carried out using a Hewlett Packard 5890 Series II Gas-Chromatograph equipped with a Flame Ionisation Detector (Hewlett Packard, EEUU) The compounds were separated on a Chrompack CP-Wax 57CB (polyethylene glycol stationary phase; 50 mx0.25 mm id with 0.25 µm film thickness) fused-silica capillary column (Varian, The Netherlands). Instrumental conditions were: injector temperature, 250°C; detector temperature, 260°C; carrier gas, helium at 1.07 mL/min; make-up gas, nitrogen 30 mL/min. The detector gas flow rates were: hydrogen, 40 mL/min and air, 400 mL/min.

Due to the high concentrations of methanol and higher alcohols in the wines, 1 mL of an internal standard solution (1 g of 4-methyl-2-pentanol per 1 L of ethanol) was added to 10 mL of the sample prior to analysis. A 1-µL aliquot of this sample was injected directly and split 1:1. The temperature program was: held at 60°C for 15 min and then increased at 3°C/min to 200°C.

Extraction of esters and acetates was carried out according to the method described by BERTRAND (1981): 2 mL of

3-octanol (50 mg/L) as internal standard and 1 mL of sulphuric acid (1/3) were added to 50 mL of wine. Each sample was extracted three times with 4, 2 and 2 mL of diethyl ether-hexane (1:1, v/v). 1 µL of the organic extract was injected into the chromatograph in splitless mode (30 s). The temperature program was: held at 55°C 15 min and then increased at 3°C/min to 200°C.

Aromatic compounds were identified by comparing the retention times with those of pure compounds and confirmed by GC-MS using a HP5890 Series II coupled to a HP 5989 A mass spectrometer in the EI mode (ionization energy, 70 eV, source temperature 250°C). The acquisition was made in the scanning mode from 10 to 1,000 m/z at 5 scan/s.

Internal standards were used to quantify concentrations of individual compounds.

Statistical analyses

Significant differences among wines for each of the compounds were assessed with one-way analysis of variance (ANOVA) using the procedure of the SAS statistical package (SAS Institute Inc., Cary, N.C., USA).

RESULTS AND DISCUSSION

Chemical composition

The basic wine composition is shown in Table 1. The results were within the normal range of values expected. The wine made with *S. cerevisiae* ASIn20 had the highest ethanol content (11.15% v/v) and extract (26.4 g/L). All wines were fermented to dryness; the concentration of reducing sugar (which determines the capacity of different strains to complete fermentation) was less than 1 g/L. Total acidity ranged from 9.65 to 10.15 g/L; Albariño wines from Galicia are always acidic. Malic and tartaric acid were

Table 1 - Chemical analyses of cv. Albariño wines.

Samples			
Parameter	ASIn1	ASIn2	ASIn20
Ethanol (%v/v)	10.15±0.212	10.35±0.212	11.15±0.221
Total acidity (g/L)	10.15±0.017	9.65±0.919	10.1±1.131
Volatile acidity (g/L)	0.4±0.141	0.375±0.035	0.48±0.028
Reducing sugar (g/L)	<1±0.000	<1±0.000	<1±0.000
Density (20°C)	0.998±0.000	0.998±0.000	0.996±0.001
pH	3.2±0.106	3.25±0.071	3.35±0.163
Extract (g/L)	25±0.000	25.15±0.212	26.4±0.424
Tartaric acid (g/L)	2.5±0.000	2.55±0.071	2.35±0.071
Malic acid (g/L)	7.4±0.707	7.4±0.566	7.05±0.919

Means ± SD. No significant differences were found among the three wines.

present in similar concentrations in all wines produced. No significant differences were found between the three wines.

Volatile composition

The volatile composition of a wine depends on the grape variety, fermentation conditions and the yeast involved. Higher alcohols and esters are the main aroma constituents of wine that are measurable by GC. These compounds are produced mainly by yeast metabolism during fermentation (NURGEL *et al.*, 2002).

The production of major volatiles differed among the three strains (Table 2). A total of 13 free volatile compounds were identified in Albariño wines fermented with the three strains. Strain ASIn2 produced the highest quantities of volatile compounds.

Alcohol

Higher alcohol formation seems to depend on the yeast strain used, the use of amino acids and total nitrogen demand (JINAREK *et al.*, 1991). Small amounts of higher alcohols contribute positively to wine quality, but excessive amounts may impair it (LAMBRECHTS and PRETORIUS, 2000).

The amount of total alcohols in the wines ranged from 109.07 to 227.8 mg/L; the highest amounts were in wines fermented with ASIn2, which also had the highest concentration of methanol (Table 2). The highest concentrations of isoamyl alcohol and isobutanol were obtained with ASIn2, with significant differences among all the wines produced. However, neither of these alcohols were present at levels above the threshold of perception (40 and 60 mg/L respectively) (SIMPSON, 1979; LÓPEZ *et al.*, 1999). The wine with the lowest isoamyl alcohol (14.31 mg/L) and methanol concentrations (52.50 mg/L) was obtained with ASIn20.

Esters

Esters are a group of volatile compounds that impart a pleasant smell and contribute fruity and flowery notes to the overall aroma (LAMBRECHTS and PRETORIUS, 2000). Total ester concentrations in the present wines ranged from 3.51 to 5.93 mg/L (Table 2). Differences were mainly due to ethyl octanoate; ASIn1 and ASIn20 produced the highest amounts of ethyl octanoate, ethyl hexanoate and ethyl decanoate, that are responsible for floral and fruity odours.

Table 2 - Volatile compounds produced by three *S. cerevisiae* strains during fermentation of Albariño musts.

Compound	Descriptors for chemical compounds (CLIFF et al., 2002)	Samples			Sig.
		ASIn1	ASIn2	ASIn20	
Alcohol					
1-Propanol	Floral, fruity, sweet	51.75±6.02	34.47±2.35	27.05±0.70	**
Isobutanol	Fusel	39.75±3.51	68.85±4.60	15.22±1.65	***
Isoamyl alcohol	Marzipan	27.21±3.02	53.93±1.05	14.31±0.25	***
Methanol	Alcohol	52.93±1.02	70.50±2.68	52.50±1.14	*
Total alcohols		171.64	227.8	109.07	
Esters					
Ethyl butyrate	Fruity	0.70±0.18	0.31±0.08	0.45±0.01	*
Ethyl hexanoate	Apple, banana, violets	1.23±0.21	0.86±0.04	1.11±0.03	*
Ethyl octanoate	Pineapple, pear, floral	2.32±0.22	1.12±0.14	1.74±0.07	**
Ethyl decanoate	Floral, sweet	0.94±0.06	0.33±0.01	0.60±0.03	*
Diethyl succinate	Fruity	0.45±0.05	0.80±0.05	0.63±0.03	*
Ethyl laurate	Fruity	0.29±0.10	0.09±0.01	0.50±0.04	*
Total esters		5.93	3.51	5.03	
Acetate					
Isoamyl acetate	Fruity, banana, pear	6.41±0.78	3.82±0.52	1.73±0.05	**
Hexyl acetate	Fruity	0.49±0.02	0.30±0.02	0.29±0.018	ns
Ethyl acetate	Varnish, fruity	87.23±4.27	66.32±3.98	65.33±3.24	ns
Total acetates		94.13	70.44	67.35	

Concentrations are reported in mg/L. The data are mean values of triplicates; Sig.: significance at which means differ as shown by analysis of variance: *, **, *** denote significance at p<0.05, p<0.01, p<0.001, respectively. ns: not significant.

Acetates

The isoamyl acetate concentrations in the wines differed, but were always well above the perception threshold (1 mg/L) (ETIEVANT, 1991). Strains ASIn1 and ASIn2 produced the greatest amounts of total acetates (94.13 and 70.44 mg/L, respectively). ASIn20 produced the least (67.35 mg/L).

Ethyl acetate, the most abundant ester in the volatile fraction of wines, contributes significantly to "acetic nose" defect (LAMBRECHTS and PRETORIUS, 2000). The wine fermented with ASIn20 had the lowest ethyl acetate content, while that fermented with ASIn1 had the highest. The ethyl acetate concentrations

in all the wines were above the threshold of perception (50 mg/L).

Significant differences were found among the three wines for all the variables (95% probability) except for hexyl acetate and ethyl acetate. The probability associated with the differences between isobutanol and isoamyl alcohol was 99.9%.

CONCLUSIONS

Musts from Albariño grapes were fermented to dryness with three *S. cerevisiae* strains isolated in the Rías Baixas region. ASIn20 produced the highest amounts of alcohol. Marked differences in the volatile composition of the fer-

mented musts, determined by GC were dependent upon the yeast strain used. *S. cerevisiae* ASIn1 and ASIn20 produced the greatest quantities of esters, (ethyl butyrate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) which can give a wine a fruity aroma. These results suggest that the production level of these compounds is characteristic of the individual yeast strains, which highlights the importance of characterising yeast strains for industrial use.

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