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ORIGINAL PAPER

Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. Albariño

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Abstract A comparative study was made of the fermentation products of Spanish Albariño wines produced with spontaneous yeast flora and an indigenous selected *Saccharomyces cerevisiae* strain (Alb16). The content of fermentative volatile compounds was determined by gas-chromatography-FID. Fifteen compounds (5 alcohols, 7 esters and 3 acetates) were identified in the two Albariño wines studied. Higher alcohols, ethyl esters (except ethyl hexanoate and ethyl octanoate) and acetates were in greater concentration in the spontaneous fermentation wine than in that with selected Alb16 strain. Principal components analysis showed good separation between the different wines.

Keywords Albariño variety · Indigenous selected yeast · Spontaneous fermentation · Volatile composition

Introduction

The yeast responsible for alcoholic fermentation in winemaking is usually introduced into the must from the surface of the grapes, the surface of winery equipment, or from specifically prepared cultures [8]. The fermentation process can occur either naturally, without inoculation, or by inoculating the must with selected starters [3, 10].

Spontaneous alcoholic fermentation of grape must is a complex process performed by the sequential action of different yeast genera and species, found on the grapes, in the must and in the wine, each contributing to the flavour of wines [11]

Starters are used in many countries and are giving excellent results. Wines produced in this way are usually of a constant quality [8].

The use of locally selected yeast strains (usually belonging to the species *Saccharomyces cerevisiae*), with strain-specific metabolic characteristics can positively affect the final quality of the wine [16, 17].

Several studies have clearly shown the effects of indigenous and inoculated yeast populations on the volatile composition of *Emir* and *Kalecik karasi* wines from Turkey [13, 14], *Monastrell* and *Albariño* from Spain [12, 20].

The aroma of a wine is one of the most important determinants of its quality. A wine may contain over 800 volatile compounds including alcohols, esters, organic acids, phenols, thiols, monoterpenes and norisoprenoids [18]. Among the volatiles compounds derived from yeast metabolism are the esters, alcohols and acetates.

The essence of a wine's flavour is formed during alcoholic fermentation. Ethanol and glycerol are the most abundant alcohols, followed by higher alcohols and esters [12], the combinations of which affect the final aroma of a wine.

Albariño, an aromatic variety of *Vitis vinifera* L. grown in Galicia (NW Spain), is used in the production of quality white wines. Young white wines prepared

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from Albariño grapes are dominated by fruity and floral aromas [4, 7].

Albariño grapes and Albariño-based wines have been the object of several studies [4, 5, 20]. The present study analysed the volatile composition of Albariño wine produced with an indigenous selected *S. cerevisiae* strain (Alb16) and that produced by spontaneous fermentation. Multivariate techniques of data analysis were employed for these comparisons.

Materials and methods

Wine samples and yeast

The must used in this study was that of *Vitis vinifera* cv. Albariño grapes; all the must used was supplied for the winery "Agrupación de Cosecheros de Albariño del Salnés", Galicia, Spain.

The selected yeast used in this study was *S. cerevisiae* Alb16. This strain was isolated from a winery in the *Rias Baixas* region and selected for its fermentative behaviour and desirable oenological characteristics. Sensorial analysis indicates that the aroma of wine elaborated with Alb16 is typical of Albariño wines [20].

The strain was grown on YEPD, (1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) glucose)

Fermentations

All fermentation experiments were performed in triplicate, using 2 l of must in sterile 3 l Erlenmeyer flasks at 18°C.

The must for the Alb16 fermentation was centrifuged (10,000 rpm for 30 min) and sterilised by membrane filtration using a Millipore system (0.45 μm). Yeast cells grown in liquid YEPD media were washed twice with sterile water and added to the must at a concentration of 10^6 cells/ml. Three other replicate samples were allowed to undergo spontaneous fermentation.

At the end of fermentation (20 days), samples were cleared by centrifugation at 10,000 rpm, 15 min. Sulphur dioxide (50 mg/l) was then added. A 1 l centrifuged sample of each wine was used in analytical assays.

Standard chemical analysis of the wines

The standard chemical parameters of the wines were determined according to the method of Ough and Amerine [15]: ethanol content by distillation of wine made alkaline by a suspension of calcium hydroxide, total acidity by titration with bromothymol blue as an indicator and volatile acidity by titration of the volatile acids separated from the wine by steam distillation. pH was measured with a pH meter. All determinations were made in triplicate.

Volatile compounds

The contents of the most relevant aromatic compounds produced during fermentation (higher alcohols, esters and acetates) were determined by gas chromatography (GC) using a Hewlett Packard 5890 Series II gas chromatograph equipped with a flame ionisation detector. The compounds were separated on a CHROMPACK CP-WAX 57CB (polyethylene glycol stationary phase; 50 m × 0.25 mm id with 0.25 μm film thickness) fused-silica capillary column. The instrumental conditions were as follows: 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; air, 400 ml/min.

Due to the high concentrations in of methanol in wines, the determination of these compounds required a 1 ml internal standard solution (1 g of 4-methyl-2-pentanol per 1 l of ethanol) be added to 10 ml of the sample prior to analysis. A 2 μl aliquot of this sample was injected directly and split 1:1. The operating temperature was set at 60°C for 15 min before being raised to 200°C at a rate of 3°C/min.

The extraction of esters and acetates was performed according to the method of Bertrand [2]: 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 as follows: 15 min at 55°C and then a rise of 3°C/min until 200°C.

Identification and quantification

The wine components were identified by comparing their retention times with those of pure standards. An internal standard was used for quantitative purposes.

Statistical analyses

The concentrations of the different aromatic compounds in the two wines were analysed by ANOVA. Principal components analysis (PCA) was performed to identify any factors differentiating the wines. All calculations were performed using the Enterprise Guide 3 System Software (SAS Institute, Cary, NC, USA).

Results and discussion

The general composition of the two wines is shown in Table 1. A small difference is observed in their ethanol and total acidity contents.

In general, spontaneous fermentation produced higher levels of total volatile compounds than did fermentation by inoculation of Alb16 strain (Fig. 1). The greatest differences between wines were observed for total higher alcohols and acetates.

The results for the aromatic compounds were subjected to ANOVA to identify any significant differences between the wines. Table 2 shows the means (of three fermentations per experiment) and standard deviations for 15 variables.

The total higher alcohols content varied from 127.23 to 366.62 mg/l, the highest levels being observed with spontaneous fermentation. When the total higher alcohol concentration of a wine is below 300 mg/l, these compounds contribute towards a desirable complexity. However, when above 400 mg/l, they have a negative influence on quality [19]. Viticultural conditions and the use of different yeast strains during fermentation contribute considerably to variations in higher alcohol profiles and concentrations [9]

Table 1 General composition of Albariño wines

Compound	SF	Alb16
Density (20°C)	0.997	0.995
Ethanol (% v/v)	12	12.2
Volatile acidity (g/l)	0.3	0.3
Total acidity (g/l)	6.5	6.9
pH	3.2	3.2

SF spontaneous fermentation, *Alb16* inoculated with selected yeast)

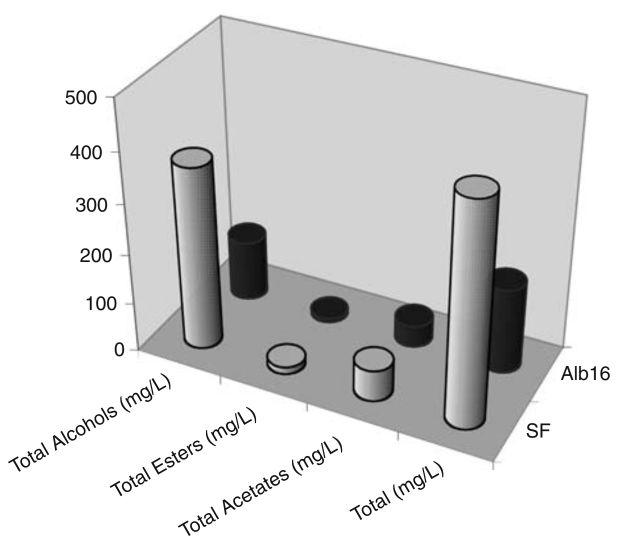

Fig. 1 Total volatiles compounds in Albariño wine fermented with spontaneous flora (*SF*) and Alb16 strain

The highest alcohol concentration detected in the studied wines was that of isoamyl alcohol. The flavour threshold in wine for this compound is between 60 and 180 mg/l [13]; this compound contributes to the sensorial quality of wine, providing it a fruity aroma [18]. Significant differences ($P < 0.001$) between the fermentations were seen only in 1-propanol content.

To a great extent, the fresh, fruity aromas of wines derive from mixture of esters produced by the yeast during fermentation [19]. Esters are particularly important compounds in the aroma of young wines [3, 6]. In the present study, the highest concentration of total esters was found in the wine obtained by spontaneous fermentation. Significant differences ($P < 0.05$) were seen between the wines in terms of their ethyl lactate, ethyl decanoate and ethyl hexanoate contents. Ethyl lactate is formed mainly during malolactic fermentation [13]. However, in this work, little ethyl lactate was produced in the wines fermented with the selected yeast. In the spontaneous fermentation, this compound was probably formed during the fermentation process itself [1].

The wine produced with Alb16 was characterised by lower acetate content than that of the spontaneous fermentation product (Table 2). The most common acetate was ethyl acetate. Significant differences were found only in terms of the isoamyl acetate ($P < 0.01$) and hexyl acetate ($P < 0.05$) concentrations.

Table 2 Mean concentration (mg/l) and standard deviation (SD) of the volatile compounds in Albariño wines fermented with spontaneous flora (SF) and Alb16

Compound	SF		Alb16		Sig.
	Mean	SD	Mean	SD	
Acetal	16.64	10.01	8.03	1.78	NS
Methanol	65.74	9.25	66.03	1.01	NS
1-Propanol	39.75	1.14	27.47	0.48	***
Isobutanol	43.47	26.17	18.24	0.96	NS
Isoamyl alcohol	201.02	88.76	110.46	2.56	NS
Ethyl butyrate	0.72	0.018	0.67	0.02	NS
Ethyl hexanoate	1.44	0.17	1.88	0.02	*
Ethyl octanoate	2.44	0.01	2.48	0.00	NS
Ethyl decanoate	1.2	0.21	0.55	0.04	*
Ethyl succinate	1.19	0.17	0.55	0.05	NS
Ethyl laurate	0.14	0.07	0.06	0.02	NS
Ethyl lactate	7.13	2.30	2.09	0.40	*
Ethyl acetate	56.27	19.52	40.96	1.00	NS
Isoamyl acetate	7.15	0.85	3.00	0.02	**
Hexyl acetate	0.09	0.16	0.30	0.03	*

The data are mean values of triplicates

NS not significant

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$

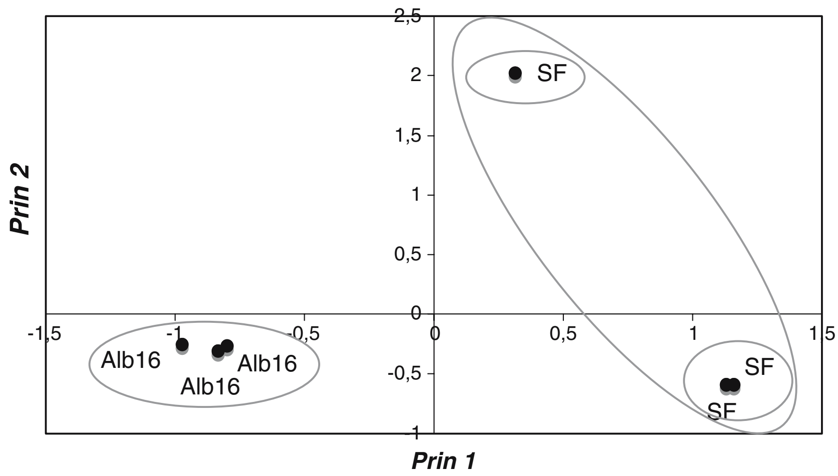

Fig. 2 Principal components analysis (*PCA*) of volatile compound composition. *SF* spontaneous fermentation, *Alb16* fermentation with the Alb16 strain

Of the compounds studied, the spontaneous fermentation samples showed greater contents of higher alcohols, ethyl esters (except ethyl hexanoate and ethyl octanoate) and acetates. These wines were considered the most aromatic.

Figure 2 shows the results of the PCA. The first two principal components (Prin 1 and Prin 2) accounted for 97.98% of the variance (54.91 and 43.07%, respectively). Prin 1 was characterised by 1-propanol, ethyl decanoate, isoamyl acetate and ethyl laurate, all with positive loading, and by ethyl hexanoate and hexyl acetate, with negative loading. For Prin 2 the compounds methanol, isobutanol, isoamyl alcohol, ethyl butyrate, ethyl lactate and ethyl succinate showed positive loading.

The PCA showed good separation of the differently fermented wines; two groups were clearly defined. The spontaneous fermentation group was divided into two subgroups. The effect of Alb16 (increased ethyl hexanoate and hexyl acetate concentrations) was reflected on the negative side of Prin1. The greatest variation between the replicates of the same wine was shown by those that underwent spontaneous fermentation (results reflected on the positive side of Prin 1 and the positive and negative sides of Prin 2).

The wines obtained with the Alb16 strain were more similar to one another (Fig. 2)

Conclusions

A comparative study was conducted on Albariño wine fermented by spontaneous flora and an indigenous selected *S. cerevisiae* strain (Alb16). The gas chromatography data showed that the wines differed in their volatiles contents. The wine produced by spontaneous fermentation showed higher contents in higher alcohols, ethyl esters (except ethyl hexanoate and ethyl octanoate) and acetates. Alb16 yeast strain led to the production of extra ethyl hexanoate and hexyl acetate. The wines obtained by the spontaneous fermentation were more aromatic than those obtained with the Alb16 yeast strain, although the PCA showed the former to be more heterogeneous.

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