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Evaluation of the formation of volatiles and sensory characteristics in the industrial production of white wines using different commercial strains of the genus *Saccharomyces*

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Abstract

The composition of white wines produced industrially from natural grape musts using pure or mixed cultures of various commercially available yeast strains of the genus *Saccharomyces*, inoculated directly or by the *pied de cuve* method was studied. For each wine sample six chemical parameters were analysed by GC and a further six by conventional methods, and Principal Component Analysis was performed. Differences in certain of those parameters appeared to be related to the sensory attributes of the wines.

Keywords: *Saccharomyces*; Wine-making; Alcoholic fermentation; Volatiles; Sensory characteristics

1. Introduction

The use of active dry yeasts of the genus *Saccharomyces* in the manufacture of young white wines is a common practice at wine cellars because of increasing consumer demand for wines with strong fresh, fruity aromas evocative of the grape variety from which they have been made. The Airén grape variety is used in the La Mancha region of Spain in the manufacture of wines. That variety does not have a strong aroma (González Viñas, Perez-Coello, Salvador, Cabezudo & Martin Alvarez, 1996; González Viñas, Perez-Coello, Cabezudo & Martin Alvarez, 1998), making necessary a suitable fermentation process that will strengthen must aromas to the greatest possible extent. To that end, fermentation processes currently employed involve temperature control and the use of commercial yeast strains to produce wines with sensory characteristics acceptable to consumers.

The yeast strain employed is an important factor bearing in mind certain metabolic differences that exist among *S. cerevisiae* strains (Houtman & Du Plessis, 1985; Cavazza, Versini, Dalla Serra & Romano, 1989; Ubeda, Briones & Izquierdo, 1998), and for that reason there is a large range of commercially available yeasts that can be used in the manufacture of different types of white wine.

The object of the present study was to assess the influence of various commercial yeast strains on the physicochemical and sensory characteristics of white wines made on an industrial scale from musts of the Airén grape variety and to compare those results with the results of native, or spontaneous, fermentation.

2. Materials and methods

Must employed and inoculation of the yeasts: Only the highest quality Airén grape musts were used in the different fermentations, and thus the last must fractions obtained by pressing (high pressure) were excluded. The sugar content ranged between 11.1 and 11.7 Baumé, the total acidity in g of tartaric acid per litre was between 3.4 and 4.5, and the pH was between 3.5 and 3.6. Before fermentation the musts were sulphited to a final SO_2 concentration of between 60 and 80 ppm.

The fermentation tanks used were of stainless steel (AISI 304 quality) with cooling jackets and a capacity of 100 000 l; temperature was controlled to between 16°C and 18°C. The tanks used for the spontaneous fermentations was also made of stainless steel of the same

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characteristics and had a capacity of 1 750 000 l. Partial temperature control was employed for one of the spontaneous fermentations. The must used in that spontaneous fermentation was partially cooled by means of the addition of fresh, unclarified must and circulating through a heat exchanger circuit that achieved a temperature drop of 3–4°C, so that the temperature was held to between 25°C and 29°C.

In the direct inoculation method, the yeasts were suspended in warm water at 35°C according to the manufacturer's instructions, and after a few minutes the suspension was added to a fermentation tank containing 3500 l of fresh must. The next day, after fermentation had begun, more fresh must was added to half of the tank's capacity, and the following day the tank was filled to four-fifths of capacity. In the *pied de cuve* method, when the density of the fermenting must (obtained as it has been described above) had attained around 1030–1040 g/l, 4000–5000 l were transferred to another tank and fresh must was then added to four-fifths of capacity. Table 1 summarizes the type of inoculation, namely, direct inoculation, the *pied de cuve* method or spontaneous fermentation, fermentation temperature, and commercial yeast cultures used in the form of either a pure strain or a mixed culture consisting of a combination of strains.

Physicochemical analysis: The official methods of the Office Internationale de Vigne et Vin (OIV, 1990) were employed for the conventional determinations. Volatile acidity and SO_2 were analysed every two days, total acidity and reducing sugars weekly, and alcohol content on completion of fermentation. Temperature and density were measured daily.

Analysis of the major volatile components was carried out by Direct Injection, using 2-pentanol as an internal standard. A Hewlett-Packard model 5830 gas chromatograph equipped with a flame ionisation detector and a Teknokroma S.C. (3 m × 2 mm i.d.) packed column was used. The column operating conditions were the following. Carrier gas: nitrogen; oven temperature: 75°C; injector temperature: 175°C; detector temperature: 225°C; and injection volume: 0.5 μl.

Sensory analysis: On completion of fermentation, the wines were evaluated by a group of 12 expert panelists. All panelists were university staff members experienced in wine sensory analysis and ranging in age between 20 and 40 yr. Wines were presented in random order at 10°C in coded standard wine-tasting glasses according to ISO standard 3591 (ISO, 1977) and covered with a watch-glass to minimize volatile components from escaping. Three wines in each session were evaluated and mineral water was provided for rinsing between wines. All samples were evaluated in a fully equipped tasting room according to ISO standard 8589 (ISO, 1988).

The unfiltered wine samples were taken directly from the cellar and were evaluated by panelists on the same day. For this reason, we asked panelists to rank the wines by consensus into three groups, according to the sensory attributes intensity (low, moderate and high).

Statistical analysis: The results of the physicochemical analysis were processed by Principal Components Analysis (PCA) to investigate the possibility of categorizing separated groups of wines on fermentation characteristics basis. Multivariate Principal Component Analysis (PCA) was performed using the MVSP statistical package (version 2.2.) (Multivariate Statistical Package, 1986).

3. Results

The lag phase, end of fermentation, and density values all proved to be independent of the method of inoculation employed: direct inoculation and by the *pied de cuve* method (not shown).

Table 1
Characteristics of yeasts used, type of inoculation and control of temperature during fermentation

Wines	Specie of *Saccharomyces*[a]	Type of inoculation	Control of temperature (16–18°C)
A	*cerevisiae/bayanus*	Direct seed	Yes
B	*cerevisiae/bayanus*	*Pied du cuve*	Yes
C	*cerevisiae* var *uvarum*	Direct seed	Yes
D	*cerevisiae* var *uvarum*	*Pied du cuve*	Yes
E	*cerevisiae*	Direct seed	Yes
F	*cerevisiae*	Pied du cuve	Yes
G	*cerevisiae* var *uvarum*	Direct seed	Yes
H	*cerevisiae* var *uvarum*	*Pied du cuve*	Yes
I	*cerevisiae*	Direct seed	Yes
J	*cerevisiae* var *bayanus*	*Pied du cuve*	Yes
K	Wild biota	Spontaneous	Yes
L	Wild biota	Spontaneous	Yes[b]

[a] All different yeast were purchased from several suppliers in La Mancha.
[b] Temperature control between 25°C and 28°C.

Table 2
Mean values of the chemical variables analysed in the wines

Wine samples	Density (g/l)	Total acidity (g/l)	SO_2 (mg/)	Volatile acidity (g/l)	Percent alcohol	Reducing sugars (g/l)	Time of fermentation (days)
A	991	4.1	64	0.37	12.0	1.6	20
B	991	4.0	64	0.30	12.6	2.1	27
C	991	3.9	60	0.22	11.5	2.0	20
D	991	3.9	102	0.37	11.9	2.0	17
E	991	4.0	45	0.18	11.6	1.7	21
F	993	3.9	83	0.37	12.1	2.4	40
G	993	3.9	176	0.37	11.8	3.3	41
H	991	4.0	64	0.30	12.0	1.8	20
I	991	4.1	90	0.18	11.6	2.1	17
J	992	4.1	32	0.33	12.3	1.6	20
K	991	4.0	80	0.33	12.3	1.7	17
L	993	4.1	90	0.18	12.4	2.3	14

Table 3
Mean values (mg/l) for the major volatile compounds analysed

Wine sample	Acetaldehyde	Ethyl acetate	Methanol	*n*-Propanol	Isobutanol	Isoamyl alcohols
A	57.035	52.112	52.743	21.122	22.976	124.685
B	79.459	80.385	71.864	25.477	31.351	132.660
C	52.859	60.381	51.378	22.374	33.467	148.149
D	113.864	82.805	50.118	23.481	26.610	110.823
E	42.588	60.023	48.823	26.137	31.579	145.983
F	94.219	49.818	50.505	20.114	23.919	140.789
G	243.386	61.818	79.048	25.046	26.114	151.320
H	64.233	52.636	50.932	24.520	27.391	154.752
I	26.486	49.093	50.328	19.924	30.043	128.728
J	32.124	79.939	81.666	32.015	57.127	185.064
K	68.732	44.711	44.879	21.552	19.685	106.405
L	37.302	19.654	76.997	41.695	34.678	170.530

Table 2 presents the results of the physicochemical analyses. Density, total acidity, and alcohol content were all typical of fermentations using Airén grape musts. The abnormally high values of SO_2 in sample G account for the lengthy duration of fermentation. Still, the resistance of *Saccharomyces* to that antiseptic was evident in the remaining samples analysed, in which a concentration of 90–100 ppm did not influence the duration, which varied from 14 to 20 days in most cases.

For most samples volatile acidity was less than 0.4 g/l, and the values for the reducing sugars in no case exceeded 4 g/l, consistent with the range for dry wines. The values for the major volatile components are given in Table 3. Sample G exhibited an abnormally high acetaldehyde concentration, 243 mg/l, which had an important effect on the sensory results. The concentration of acetaldehyde is closely related to, for instance, the presence of SO_2, which blocks reduction of the acetaldehyde to ethanol. Concentrations of the rest of the components were within the normal ranges for this grape variety.

Statistical analysis of the data: The PCA run using the physicochemical analysis data indicated that the first three components (PC1-3) explained 77% of the observed variance, all the variables being negatively correlated. The variables with the most influence on PC1 (34.7%) were acetaldehyde (−0.447), reducing sugars (−0.404), SO_2 (−0.403), and duration of the fermentation (−0.392); for PC2 (28.7%), methanol (−0.472), 1-propanol (−0.404), and isoamyl alcohol (−0.439); and for PC3, ethyl acetate (−0.674) and volatile acidity (−0.431).

Fig. 1 plots the distribution of the samples on the coordinate grid defined by PC1 and PC2, with an explained variance of 63.4%, showing that wine samples H, C, I, A, and E made up a homogeneous group characterized by intermediate values for the variables most closely correlated with those two principal components. Another group of samples presented maximum (samples F and G) and minimum (sample J) concentration values for the variables most closely correlated with PC1 (acetaldehyde, reducing sugars and SO_2).

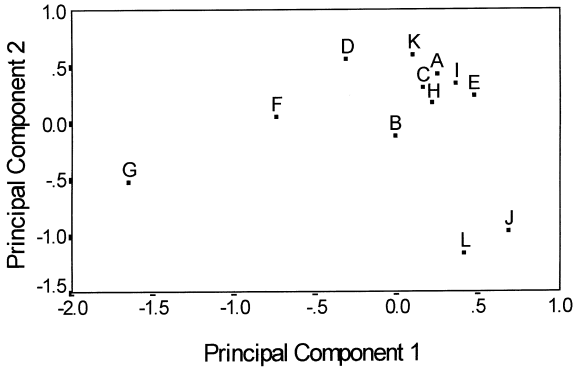

Fig. 1. Distribution of white wine samples in the plane defined by the first two principal components.

Furthermore, samples D, K and, above all, J and L were characterized by minimum and maximum concentration values for the variables most closely correlated with PC2 (methanol, 1-propanol and isoamyl alcohol). The plot of the samples on the coordinate grid defined by PC1 and PC3 (not shown) was similar to the plot in Fig. 1, with samples J, F, G, and L again appearing as outliers. In the literature, certain of the variables selected by the PCA have been considered to be dependent upon the yeast strain employed, and indeed 1-propanol has been used to characterize yeast strains (Rankine, 1967; Soles, Ough & Kunkee, 1982; Marchetti & Guerzoni, 1987; Cavazza, Versini, Dalla Serra & Romano, 1989; Romano & Giudici, 1990; Delteil & Jarry, 1992).

The use of selected yeasts for direct inoculation or *pied de cuve* inoculation, spontaneous fermentation, total or partial temperature control, and yeast species (*Saccharomyces cerevisiae* or *bayanus*) did not affect the placement of the samples on the coordinate grids defined by the different principal components, and indeed the distribution of those variables was observed to be random. However, it must be pointed out that no genetic studies were carried out, hence the yeast species used in the direct inoculation may not have been the same ones that predominated in the *pied de cuve* fermentations.

Sensory analysis was performed after completion of the physicochemical analyses of the wines. The olfactory and gustatory analysis conditions ensured that sensory evaluation of the aroma and flavour characteristics was independent of wine appearance and colour, and an overall evaluation was made based on the attributes deemed most relevant by consensus among the expert assessors. Wine samples F, G, and D were judged to have a strong fresh-citric, green fruit, and apple aroma, and those same aroma characteristics, though less intense, were judged to characterize samples H, I, C, A, K, E, and B. In contrast, wine samples L and J were judged to be quite neutral white wines with an atypical yeasty odour, possibly because sample J had remained in contact with the lees for a certain time and sample L did not benefit from strict temperature control.

The order of preference of samples by the panelists on the basis of their flavour and tactile attributes was the same as in the ranking based on aroma, with three well-differentiated groups. One group consisted of wine samples F, G, and D, which had a perceptibly fruity flavour, moderate acidity, and a pleasantly intense aftertaste. A second group was composed of wine samples H, J, C, A, K, E, and B, which exhibited the same but less pronounced characteristics. A third group comprised wine samples J and L, which, though they were not judged to have any pronounced defects, were awarded the lowest sensory scores, possibly because of the factors just referred to above.

The results of the sensory analysis could be related to the results of the chemical analyses, even though the latter did not take into account minor volatile components. Samples J and L, which earned the lowest sensory scores, had the lowest acetaldehyde content and highest 1-propanol, methanol, and isoamyl alcohol concentrations. Conversely, the wines in the first group (samples F, G, and D) and in the second group (samples H, C, A, K, E, and B) did not present such clear-cut chemical differences.

The results of this study show that simple, fast analysis of a few chemical parameters was useful in predicting the sensory characteristics of the white wines produced, thus demonstrating the feasibility of simple chemical controls before bottling. But, many other physicochemical parameters directly influencing the sensory characteristics of wines exist apart from those analysed in the present study, especially minor volatile components, some of which are dependent upon the yeast strain used. However, the cost, labour-intensiveness, and time constraints involved in analysing such components are impediments to implementation of routine controls at wine cellars where wine production is seasonal in nature.

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