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Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine

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Abstract

Musts from Airen grapes to which ammonium (100, 300 mg/l) and amino acids had been added (doubling the level of amino acids in that must) were fermented with three different yeast strains. Statistical treatments showed that the strain of yeast is the major factor affecting wine volatile composition, but must nitrogen supplementation also has an influence. The addition of any source of nitrogen to the must reduces the contents in the wine of β-phenylethanol (ca. 65% reduction), methionol (ca. 70%) and isoamyl alcohol (40–65%) and increases wine content of propanoic acid by 30–130%. Wines from musts supplemented with ammonium are richer in ethyl lactate and c-3-hexenol and wines supplemented with amino acids are richer in γ-butyrolactone and isobutanol. From the sensory point of view, must supplementation with ammonium brings about a decrease in sulphury notes and an increase in citric flavour. The effect of amino acid supplementation depends on the yeast strain. In one case, the effect is similar to that of ammonium supplementation; in the others an increase in fruity and fusel notes was obtained.

Keywords: Must; Wine; Ammonium; Amino acids; Aroma; Yeast

1. Introduction

The aromatic complexity of wines varies, depending on the variety of grape used, the aromas produced during the fermentation and those developed during the aging process. The production of both, aromas and spoilage compounds, by the yeasts can be significantly affected by winery practices such as clarification, airation, yeast strains, nutrient addition and temperature of fermentation.

It is a common practice in vineyards to supplement the must with diammonium phosphate (DAP), urea or yeast nutrients to prevent problems related to nitrogen deficiency: slow and stuck fermentations and SH_2 production. This addition must follow some criterion, since the addition of large amounts of ammonium in the must can result in later problems. Wines with higher amounts of residual nitrogen run greater risks of microbiological instability and production of ethylcarbamate.

The nitrogen composition of the must affects fermentation kinetics, the production of aromatic and spoilage compounds, of ethanol and glycerol (Albers, Larsson, Liden, Niklasson, & Gustafsson, 1996) and also urea, the main precursor of ethylcarbamate, a carcinogen present in wine. The two main sources of yeast-assimilable nitrogen are primary amino acids and ammonium (Butzke, 1998). Several studies on the effect of ammonium addition on most of these parameters have been carried out, but little is known about its influence on the formation of most aromatic compounds.

In particular, SH_2 production is usually controlled in vineyards by the addition of DAP to must. Jiranek, Langridge, and Henschke (1995) have demonstrated that, when there is little ammonium in the exponential phase, more SH_2 is produced than when there is ammonium present. Recently, Marks, van der Merwe, and van Vuuren Hennie (2003), using genetic studies,

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demonstrated that assimilable nitrogen is a key factor in the regulation of SH_2 during alcoholic fermentation.

On the other hand, the addition of ammonium to musts with a low nitrogen level produces an increase in the fermentation speed during the exponential phase, with a significant increase of biomass, but it does not prolong the time during which the cells can multiply (Monteiro & Bisson, 1992; Ough, 1964). If there were high nitrogen concentrations in the must, Jiranek, Langridge, and Henschke (1991) observed that the differences in the fermentation speed of different yeasts were smaller than when the musts were poor in nitrogen.

Increase of assimilable nitrogen in must grape, by means of vinegrape fertilization, or by addition of ammonium, produces a diminution in the formation of fusel alcohols (Vos & Gray, 1979; Rapp & Versini, 1991; Ough & Bell, 1980; Castor, 1954).

Webster, Edwards, Spayd, Peterson, and Seymur (1993) have demonstrated that the addition of a good source of nitrogen to a must affects yeast metabolism and the fermentation bouquet, yielding a "cleaner" but less complex wine. They also found that wines from fertilized vines contain more esters and were different by sensory analysis.

The yeast strain is another factor to consider in the formation of the fermentation aroma. Some authors find that, the yeast used affects aromatic compounds, esters, and alcohols, which modify the fruity notes of the wines obtained (Perez-Coello, Briones Perez, Ubeda Iranzo, & Martin Alvarez, 1999). They mainly find differences related to the distinctive capacity of yeasts to assimilate nitrogen. The yeasts with greater demand for nitrogen produce higher concentrations of esters during the fermentation, and those with lesser demands produce greater concentrations of higher alcohols (Perez-Coello et al., 1999; Torrea, Fraile, Garde, & Ancin, 2003). Nevertheless, Ubeda and Briones (2000) find that, although the amounts of volatiles formed are different, there are no clear sensory differences between the wines obtained.

On the other hand, the studies conducted by Marchetti and Guerzoni (1987), with 16 different yeast strains, clearly indicate that the composition of the initial must influences the aroma of the wine obtained to a greater extent that the yeast strain used.

Considering the widespread use of ammonium addition and active dry yeasts in vineyards, it is necessary to know the effect that such an addition may have on the aroma of the wine and the influence of different yeasts commonly used in wine production. This article describes the study of 15 wines made with Airen, the largest *vitis vinifera* cultivar in Spain. The must was supplemented with different amounts of ammonium and/or amino acids, and three different yeast strains were used in the study.

2. Materials and methods

2.1. Reagents and standards

The pure reference compounds used in the quantitative analysis of volatile compounds were purchased from Aldrich (Gillingham, UK), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), Poly Sciences (Niles, IL), Lancaster (Strasbourg, France), and Chemservice (West Chester, PA). Individual amino acids were from Sigma. Diammonium phosphate and formaldehyde were obtained from Panreac S.A. (Barcelona, Spain). Water was obtained from a Milli-Q purification system (Millipore, Beldford, MA).

2.2. Samples and vinification

2.2.1. Grapes

Must from *vitis vinifera* grapes of Airen variety was used. Two types of experiments were performed:

2.2.2. Experiment 1

Must Airen (Airen 1) was divided into three batches of 600 ml. Each batch consisted of three samples, each inoculated with active dry yeasts *Saccharomyces cerevisiae*: Fermicru AR2 (L1), Stellevin NT116 (L2) and LW LVCB CT1+ (L3) obtained from DSM Food Specialties Oenology S.A.S. (France). The first batch was the control (no ammonium added), samples from the second batch were supplemented with 100 ppm of ammonium, and samples from the third with 300 ppm. The experiment was replicated.

2.2.3. Experiment 2

The concentrations of individual amino acids in the Airen 1 must were measured; then, solutions of each one of the amino acids were prepared and added to the must in order to double the original concentration of each amino acid. This batch was identified as Airen 2. A second batch was prepared by further addition of 100 ppm of ammonium to this amino-acid enriched must. Both batches were divided into three samples and each sample was inoculated with one of the three aforementioned yeasts. The experiment was replicated. All the samples used in the study are presented in Table 1.

Table 1
FAN in all the musts used in the present study

Musts	FAN (mg N/l)
Airen 1 (A1)	175
Airen 1 + 100 ppm ammonium	240
Airen 1 + 300 ppm ammonium	375
Airen 2 (A2 = A1 + amino acids)	274
Airen 2 + 100 ppm ammonium	342

Fermentations were carried out in 500 ml Erlenmeyer flasks locked by Muller valves. Fermentation flasks were kept at 20 °C in a Climas incubator (Barcelona, Spain). Yeasts were inoculated in the must in a proportion of 0.3 g/l. To do this, 0.5 g of dry yeast were rehydrated in a sterile flask in 6 ml of sterile deionised water with 0.5 g of glucose for 15 min at 35 °C.

The experiments were done in duplicate, and the progress of the fermentations was monitored by weighing. At the end of the fermentation, the solutions were centrifuged at 5000 rpm for 10 min and the supernatant was taken for analysis.

2.3. Determination of free α-amino nitrogen (FAN) and free amino acids

Free α-amino nitrogen (FAN) was calculated by formol titration (Zoecklein, Fugelsang, Gump, & Nury, 2001). The free amino acids were quantified by HPLC, following the method described in Hernandez Orte, Ibarz, Cacho, and Ferreira (2003).

2.4. Analysis of volatiles

The volatile compounds were analyzed by the procedure proposed by Ortega, Lopez, Cacho, and Ferreira (2001).

2.5. Chemometric study

Cluster analysis was performed with NTSYS from Exeter Software (Setauket, NY, USA). ANOVA was carried out with Statview (SAS Institute, Cary, NC, USA). Principal component analysis was carried out with Unscramble from CAMO ASA (Oslo, Norway).

2.6. Sensory evaluation

Wines were evaluated on the same day as the fermentation finished. The tasting panel was composed of 10 trained members (seven women, three men, aged 24–45), all of them part of the laboratory staff, with a long experience in wine tasting and in aroma evaluation. Panel members were invited to first describe aroma notes defining the samples. Descriptors were recoded and classified into five categories (sulphur, fruity, citric, toasted and fusel). Each category had a predefined standard in the laboratory (a local Pilsen beer for sulphur, ethyl hexanoate for fruity, cider for citric, a mixture of isoamylalcohol and β-phenylethanol for fusel, and acetylpyrazine for toasted). The wine samples were coded and presented randomly to the panel, and the aroma intensity on each sensory descriptor was measured using an unstructured scale.

3. Results and discussion

3.1. Effect of the nitrogen addition on the fermentation speed

In the present study, the level of assimilable nitrogen of a must of the Airen variety has been modified by addition either of ammonium or amino acids, as indicated in Table 1. In all cases the FAN is above 140 mg N/l, a level considered by several authors (Butzke, 1998; Ingledew & Kunkee, 1985) to be the minimum amount necessary to complete the fermentation in samples within a normal range of sugar content. Therefore, we cannot expect to see a large effect on fermentation kinetics as a result of the nitrogen addition. In fact, the statistical study performed on the curves of weight loss did not show significant differences related to the addition of ammonium or amino acids. Nevertheless, differences were found in the speed associated with the yeast ($p = 0.0315$, $F = 5.202$). Yeast strain 3 showed significantly superior kinetics to that of strain 1.

3.2. Hierarchy of effects in the formation of aromatic compounds

The matrix of data formed by the 30 samples and the data of 30 volatile components were studied, using different data treatment strategies: cluster analysis, analysis of variance (ANOVA) and principal component analysis. Cluster techniques, whatever the similarity (or dissimilarity) parameter used, showed that samples first group according to the strain of yeast used in the fermentation (data not shown). Similar conclusions are reached by ANOVA. Table 2 shows the results of a 2-factor ANOVA (yeast, 3 categories and ammonium addition, 3 levels) performed on the subset of data corresponding to the first three musts of Table 1, and Table 3 shows the results of the 3-factor ANOVA (yeast, 3 categories, ammonium addition, 2 levels, and amino acid addition, 2 levels) carried out on the first, fourth and fifth samples of Table 1. As can be seen from both Tables, the yeast shows an effect on 21 of the compounds studied, whereas the addition of ammonium affects 15 and amino acids affect only eight components.

The subset of data composed of those 21 volatile compounds identified by ANOVA (Tables 2 and 3) as significantly affected by any of the factors considered in the experiment, was further processed by principal component analysis. Results of this study are shown in Figs. 1 and 2. As can be seen, the set of data has a strong latent structure. The first and second principal components (retaining 25% of total variance each) classify samples according to the strain of yeast, while the third and fourth principal components (retaining 17% and 9% of the total variance, respectively) classify samples according to the amount of FAN present in the must

Table 2
Significant differences in the concentration of volatiles found in a two-way ANOVA carried out on wines made with Airen must supplemented with different amounts of ammonium (0 control, 100 ppm and 300 ppm) and fermented with three different yeast strains

Compound	Yeast strain		Ammonium addition		Interaction (strain × ammonium)	
	p	*F*	*p*	*F*	*p*	*F*
Propanoic acid	0.0179	6.501	0.0031	11.71		
Isobutyric acid	<0.0001	105.8	0.008	8.644		
Butyric acid	0.0007	18.19				
Hexanoic acid	<0.0001	36.04				
Octanoic acid	0.0002	26.00				
Decanoic acid	0.0111	8.323				
Isobutanol	<0.0001	105.6	0.0039	10.96	0.0483	3.685
1-butanol	<0.0001	30.83	0.0038	11.04		
Isoamyl alcohol	0.0043	10.63	0.0002	24.49		
1-hexanol	<0.0001	175.9	0.0042	10.72		
c-3-hexenol	0.022	6.005				
Methionol	0.0001	29.98	<0.0001	93.05		
β-phenylethanol	0.0014	14.97	<0.0001	154.7		
Ethyl isobutyrate	<0.0001	159.3				
Isoamyl acetate	0.107	7.844				
Ethyl hexanoate					0.0496	3.645
Ethyl lactate	0.0445	4.488	0.0043	10.59		
Ethyl 3-hydroxybutyrate	0.0001	28.87	0.0018	13.91		
Phenylethyl acetate	<0.0001	67.37			0.0287	4.489
Diacetyl	0.0068	9.143	0.0156	6.847		
γ-butyrolactone	0.016	6.779	0.0155	6.858		

(component 3) and to the type of nitrogen supplementation given to the must (component 4).

3.3. Effect of yeast

Fig. 1(b) shows the loadings of the different volatile compounds in the first and second components. Musts fermented with the yeast strain 2 are richest in fusel alcohol acetates, in diacetyl and in ethyl-3-hydroxybutyrate, while they are poorest in isobutyric acid, ethyl isobutyrate, isobutanol and hexanol. The reverse composition is found in those musts fermented with yeast strain 1. Finally, musts fermented with yeast 3 are particularly rich in volatile compounds related to the synthesis of fatty acids (butyric, hexanoic, octanoic and decanoic acids, γ-butyrolactone and ethyl hexanoate), and particularly poor in fusel alcohol acetates, methionol, β-phenylethanol and ethyl isobutyrate.

A closer look at Fig. 1(a) also reveals that the addition of amino acids to the must seems to emphasize the differential characteristics of yeast strain 2, but not those of yeast strains 1 and 3. In a similar way, the addition of ammonium to the must accentuates those differential characteristics of yeast 3. These observations explain some of the interactions (yeast × ammonium and yeast × amino acid) shown in Tables 2 and 3.

3.4. Effect of nitrogen supplementation on volatile composition

The effect of nitrogen supplementation can be seen in Fig. 2(a) and (b). As a first approximation, it can be said that samples are ordered along principal component 3 according to their content of nitrogen. The variable loadings on this component (Fig. 2(b)) show that the addition of any kind of nitrogen to the must brings about a reduction in the levels of β-phenylethanol, methionol and isoamyl alcohol and an increase in the levels of propanoic acid. The cases of β-phenylethanol and methionol are remarkable and are presented in Fig. 3. In this figure, the concentration of these alcohols has been plotted against the level of FAN in the must, independent of its type (ammoniacal or aminic). It can be observed that similar trends are observed for the three yeasts and that the form in which the nitrogen has been added seems not to play any role. Two different responses are observed. At low levels of FAN, the addition of any nitrogen source to the medium has an intense effect on the level of the component, which diminishes similarly for all yeasts. From a certain level of FAN, which seems to be related to the tendency of formation of these components by the yeast, and which is found to be between 250 and 300 mg/l, the effect of the addition of more nitrogen is attenuated, and the amount of these components formed converges, reducing the differences between yeasts. The levels of these components are reduced by factors higher than 4 with the addition of nitrogen. A similar plot can also be drawn for isoamyl alcohol, although the relationship is less clear, particularly for yeast strain 2. These observations seems to be in agreement with reports from other authors, about the effect of nitrogen fertilizers and the addition of nitrogen to must, on the level of certain components (Cantagrel, Symonds, & Carles, 1982; Margheri, Versini, Gianotti,

Table 3
Significant differences in the concentration of volatiles found in a three-way ANOVA carried out on wines made with must from Airen supplemented with ammonium (0 and 100 ppm), and/or with amino acids (0 and ×2) and fermented with three different yeast strains

Compound	Yeast		Ammonium addition		Amino acid addition		Interaction (amino acid × ammonium)		Interaction (amino acid × yeast)		Interaction (yeast × ammonium)		Interaction (amino acid × ammonium × yeast)	
	p	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*
Propanoic acid			0.0068	10.64										
Isobutyric acid	<0.0001	28.10			0.0061	11.04	0.0297	6.082						
Butyric acid			0.036	5.551										
Hexanoic acid	<0.0001	31.28			0.0223	6.869			0.0488	3.924				
Octanoic acid	0.0002	19.44							0.0156	5.999				
Decanoic acid	0.0002	18.86			0.0005	21.80			0.0036	9.336			0.0285	4.853
Isobutanol	<0.0001	97.47												
1-butanol	0.0002	18.58												
Isoamyl alcohol	0.0408	4.228	0.0037	12.94	0.0002	27.50			0.0194	5.572				
1-hexanol	0.0009	13.18												
Methionol	<0.0001	37.33	<0.000-1	42.91	<0.0001	109.4	0.0019	15.68	0.0201	5.506				
β-phenylethanol	<0.0001	43.92	<0.000-1	77.67	<0.0001	521.1	<0.0001	45.43					0.0107	6.788
Ethyl butyrate	0.0075	7.569	0.032	5.886							0.0064	7.939	0.0107	6.787
Isoamyl acetate	<0.0001	24.48							0.0074	7.578			0.0260	5.021
Ethyl hexanoate	0.0158	5.973	0.0356	5.599							0.0233	5.228	0.0014	11.88
Ethyl lactate	0.0017	11.43	0.0021	15.29	<0.0001	45.19								
Ethyl 3-hydroxy-butyrate	<0.0001	32.94			0.0015	16.82								
Phenylethyl acetate	<0.0001	97.78							0.0469	3.990			0.0117	6.601
Diacetyl	0.0166	5.884												
γ-butyrolactone	0.0003	17.62												

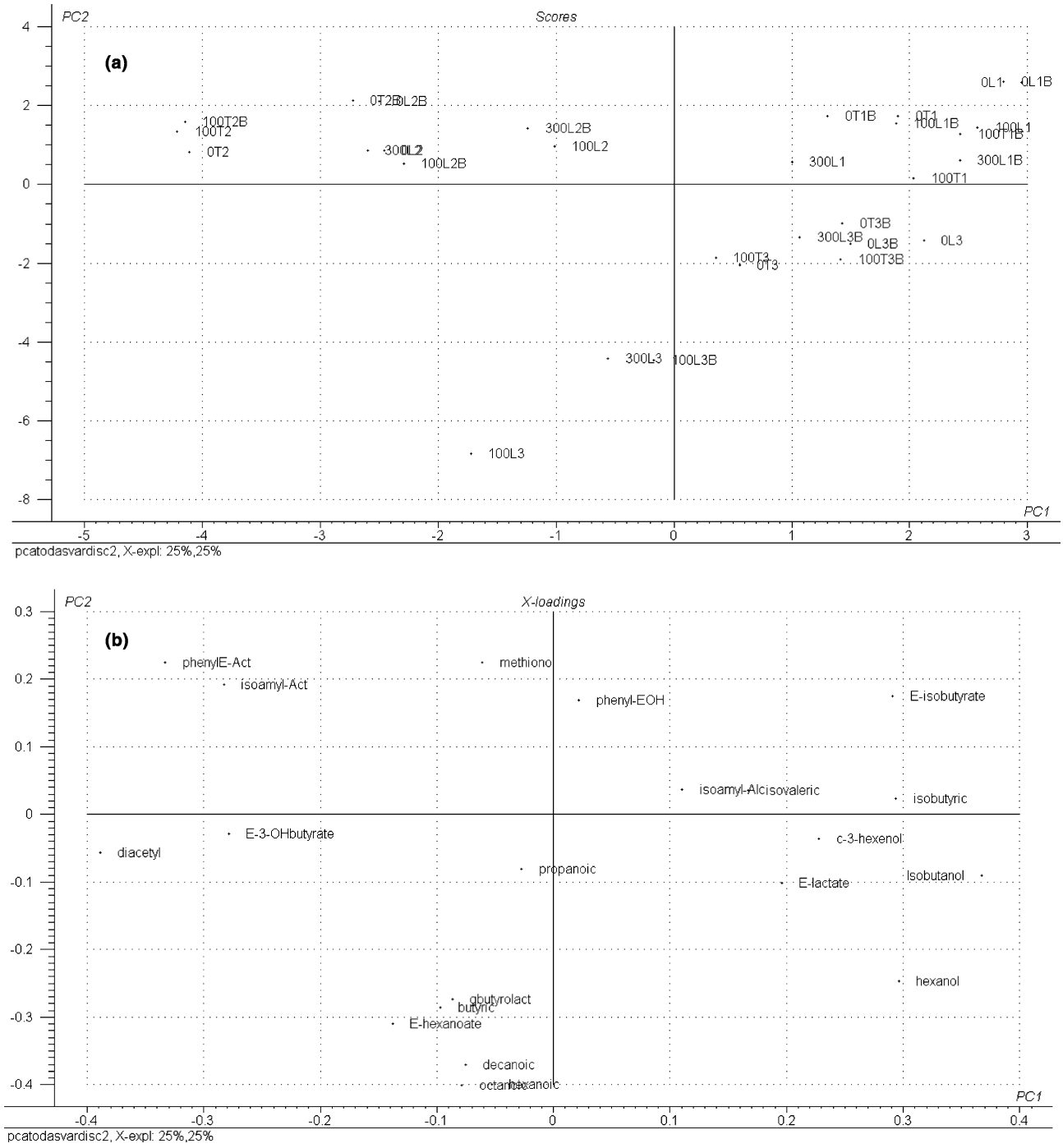

Fig. 1. Results of the principal component analysis carried out on the volatile compound data matrix: (a) scores of the 30 wine samples in the plane formed by the two first principal components; (b) loadings of the variables on the first two principal components. Key: first number, 0, 100 or 300 refers to the amount of ammonium added; the letter that follows refers to the addition of amino acids, L = no addition, T = addition; the second number refers to the yeast strain; the last B is the replicate sample.

& Pellegrini, 1984; Ough & Bell, 1980; Seeber, Sferlazzo, Leardi, Serra, & Versini, 1991).

The effect of the ammonium addition can be explained by the increased capacity of the yeast to transform the synthesized α-ketoacids, avoiding their accumulation and later expulsion to the medium after their reduction to higher alcohols. The addition of amino acids should produce, a priori, a different effect, since one would expect an increase of products derived from their catabolism, which would be in agreement with results presented in a recent study (Hernandez Orte, Cacho, & Ferreira, 2002). The fact that no increase is observed seems to confirm that, if the amino acidic profile of the must is maintained, as is the case, there is

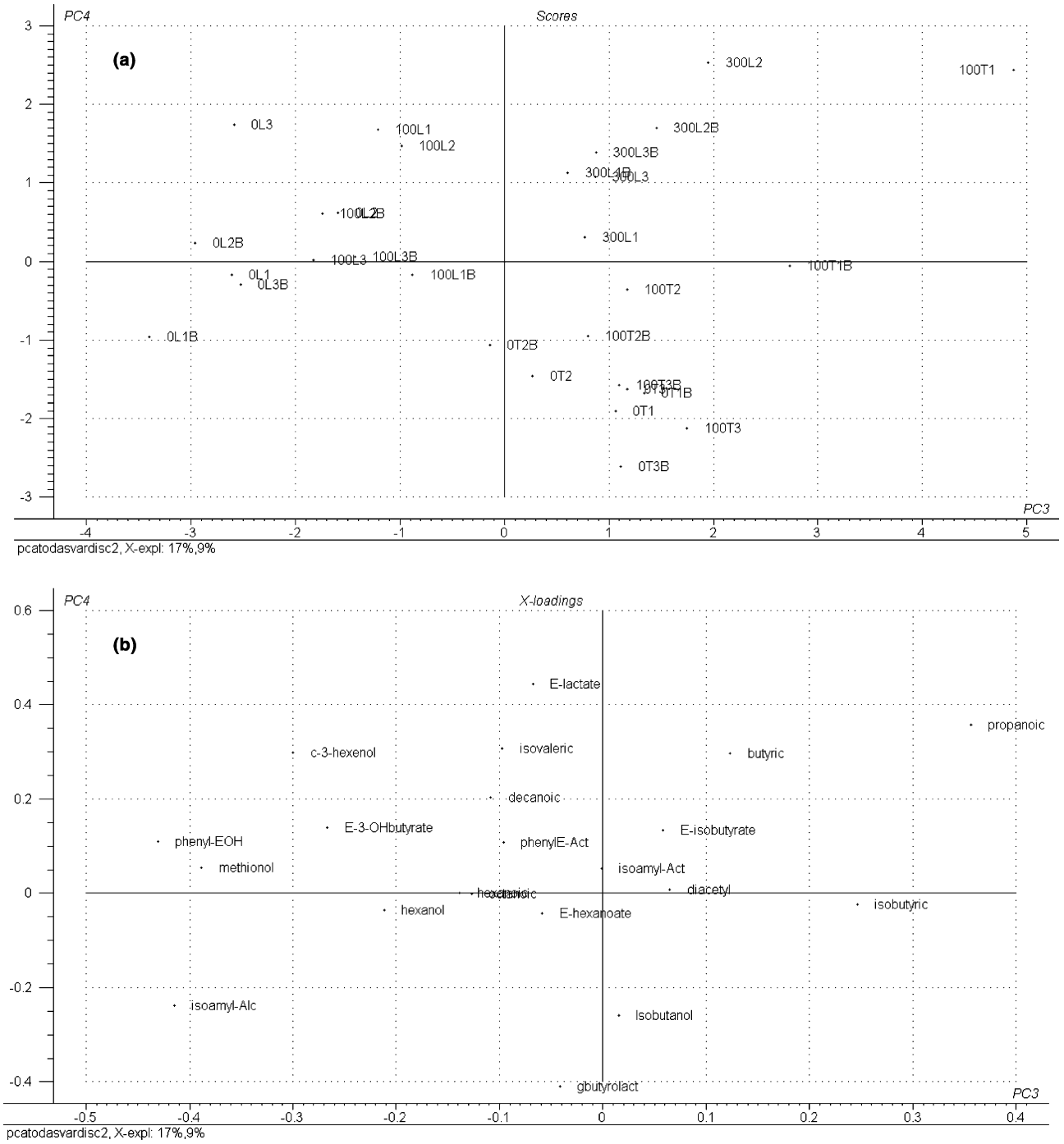

Fig. 2. Results of the principal component analysis carried out on the volatile compound data matrix: (a) scores of the 30 wine samples in the plane formed by the third and fourth principal components; (b) loadings of the variables on the third and fourth principal components. The key as explained in Fig. 1.

no increase of catabolism that can lead to alteration of the volatile profile. On the other hand, it should be emphasized that the proportion between the fusel alcohols formed at low and high levels of FAN roughly corresponds to the ratio, total fusel alcohols/fusel alcohols formed by catabolism (Bidan, 1975). This might indicate that the anabolic route in the formation of higher alcohols is only important at low levels of FAN, and that from a certain value (for this must between 250 and 300 mg/l), there is only formation via a catabolic route.

Fig. 2(a) also reveals the existence of differences between samples fermented with different types of nitrogen. Nearly all samples supplemented with ammonium have positive loadings in component 4, while nearly all samples supplemented with amino acids can be found in

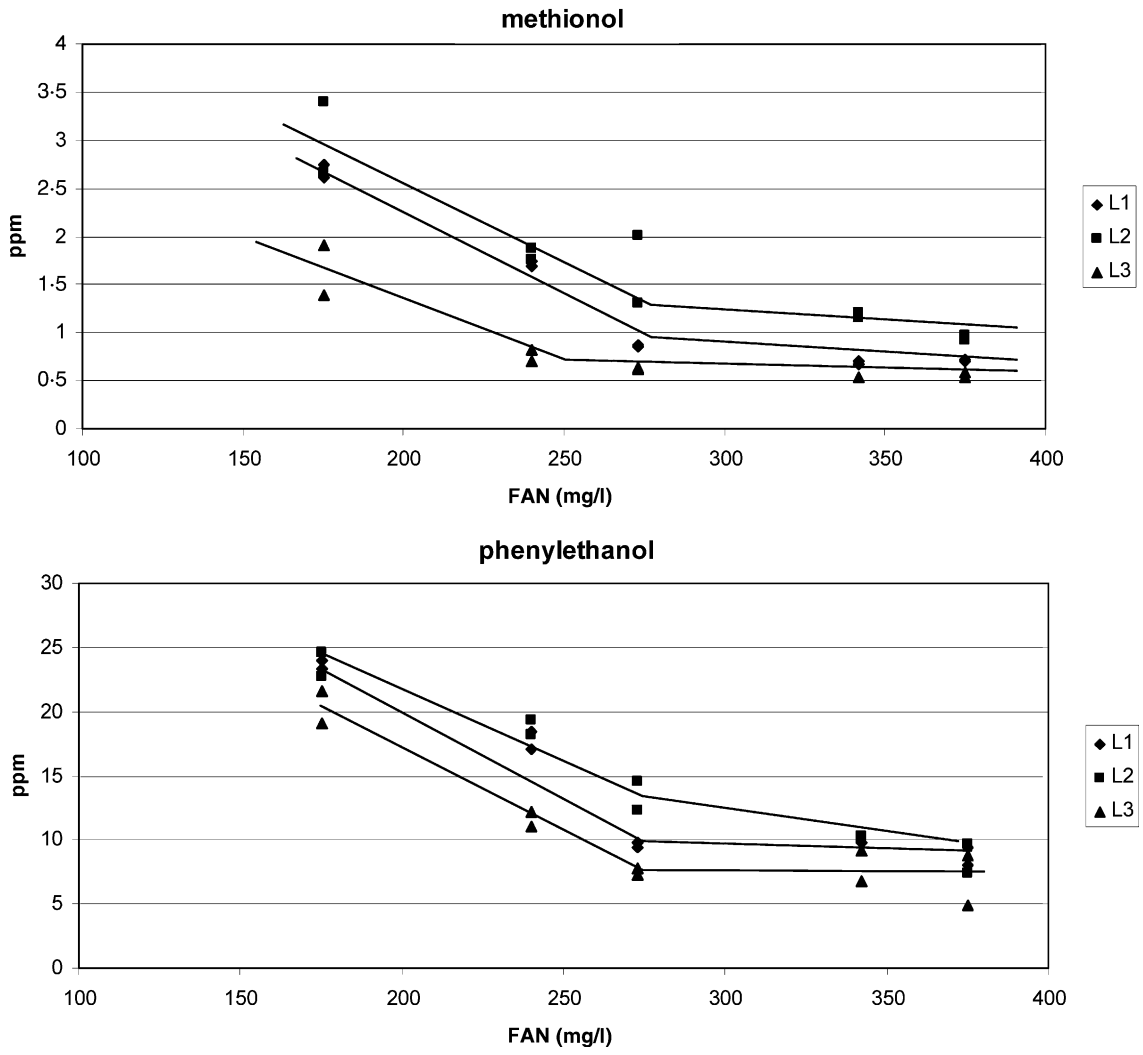

Fig. 3. Relationship between the wine content in methionol and β-phenylethanol and the FAN contained in the must. Points at 175 mg N/l FAN correspond to the original must, at 240 to the 100 ppm addition of ammonium, at 273 to the addition of amino acids, at 342 to the combined addition of amino acids and 100 ppm ammonium, and at 342 to the addition of 300 ppm of ammonium.

the area defined by negative loadings in this component and positive loadings in component 3. As can be seen from Fig. 2(b), the most important differences between both set of samples are due to the levels of isobutyric acid (not for yeast strain 2), isobutanol and γ-butyrolactone, all of which are higher in samples supplemented with amino acids, and in ethyl lactate and c-3-hexenol, which are higher in samples supplemented with ammonium. It also can be appreciated that this effect is less important for samples fermented with yeast strain 2.

3.5. Sensory analysis

The 30 wines obtained in the experiment were analysed by sensory descriptive analysis. Data on the four most important descriptors (sulphury, citric, fusel and fruity) were processed by principal component analysis. Fig. 4(a) shows the sample scores in the plane formed by the two first principal components (retaining 51% and 26% of the total variance, respectively), while the variable loadings on these components can be seen in Fig. 4(b). As before, there is a clear classification of samples but, in this case, the nitrogen supplementation seems to be the factor with highest effect. Samples not supplemented with nitrogen appear in the left bottom corner of the PC plane. Samples supplemented with 100 ppm of ammonium occupy the centre right of the plane, while those supplemented with 300 ppm of ammonium lie in the upper central part of the plane. In terms of sensory descriptors, this implies that un-supplemented samples are the richest in sulphury notes, and that the addition of ammonium has, as a consequence, reduction of the sulphury character and an increment in the citric notes. It is remarkable that this result is independent of the yeast used. The effect of supplementing the must with amino acids is quite different and depends to a

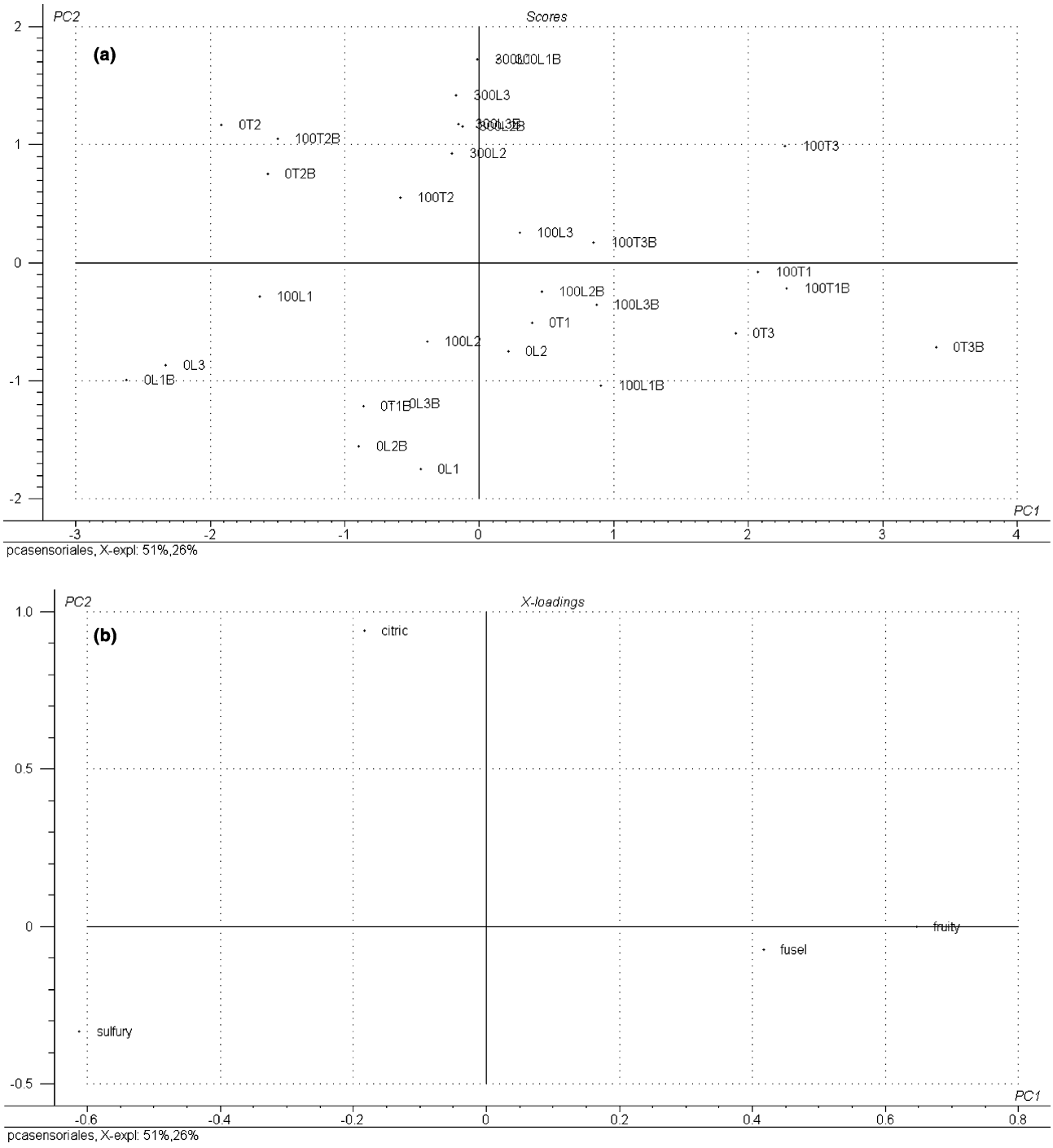

Fig. 4. Results of the principal component analysis carried out on the sensory scores data matrix: (a) scores of the 30 wine samples in the plane formed by the two first principal components; (b) loadings of the variables on the first two principal components; key as in Fig. 1.

great extent on the yeast strain. Amino-acid supplemented musts, fermented with yeast strain 2, scored high in citric and sulphury and become quite similar to wines made from musts supplemented with high amounts of ammonium. Musts fermented with strain 3 were less sulphury and more fruity and fusel. Finally, those amino-acid supplemented musts fermented with strain 1 were less sulphury and, in this case, the combined supplementation of amino acids and ammonium produced wines high in fruity and fusel notes and low in sulphury character (see Table 4).

Unfortunately, the available analytical data are insufficient to completely interpret sensory properties. Most of the changes shown in Fig. 4 are due to changes in sulphur-containing volatiles, which were not analysed. These compounds are not only responsible for

Table 4
Volatile composition of the 15 wines (averages of replicate values) obtained in the present experiment

	Yeast Strain 1					Yeast Strain 2					Yeast Strain 3				
	Control	100 ppm	300 ppm	aa	aa + 100 ppm	Control	100 ppm	300 ppm	aa	aa + 100 ppm	Control	100 ppm	300 ppm	aa	aa + 100 ppm
Propanoic acid	1.12	1.55	1.42	1.23	2.51	1.53	1.39	2.04	1.41	1.69	1.38	1.63	1.88	1.49	1.71
Isobutyric acid	2.79	2.31	2.08	2.82	5.21	1.06	0.94	1.14	1.06	1.06	1.86	1.61	1.39	2.45	2.66
Butyric acid	1.05	1.15	1.24	1.13	1.67	1.40	1.44	1.34	1.29	1.33	1.45	1.71	1.49	1.37	1.30
Isovaleric acid	1.16	1.26	0.76	0.97	1.02	0.82	0.71	1.43	0.74	0.66	1.89	0.75	0.98	0.85	0.81
Hexanoic acid	2.09	2.39	2.40	2.29	2.17	2.80	3.11	2.27	2.72	2.67	4.12	5.40	4.06	3.26	3.67
Octanoic acid	2.88	3.37	3.74	3.46	3.03	4.06	3.92	3.13	3.95	3.87	5.46	7.80	5.69	4.21	4.73
Decanoic acid	0.42	0.56	0.70	0.27	0.50	0.88	0.71	1.05	0.49	0.45	1.25	2.41	1.76	0.70	0.61
Isobutanol	109	90.2	69.0	100	90.2	25.7	24.4	25.1	28.3	23.8	79.6	81.9	57.4	103	93.4
Isoamyl alcohol	263	194	128	170	98.6	160	142	107	160	146	201	195	124	150	134
1-hexanol	1.26	1.19	1.20	1.00	0.97	0.98	0.98	0.88	0.84	0.78	1.36	1.33	1.25	1.21	1.23
c-3-hexenol	0.24	0.23	0.23	0.20	0.21	0.22	0.22	0.20	0.19	0.19	0.23	0.23	0.21	0.20	0.21
Methionol	2.67	1.71	0.72	0.86	0.68	3.03	1.81	0.95	1.66	1.18	1.65	0.77	0.56	0.63	0.54
β-phenylethanol	23.6	17.7	8.71	9.65	9.75	23.7	18.8	8.53	13.4	10.2	20.4	11.6	6.82	7.51	6.97
Ethyl isobutyrate	0.11	0.11	0.11	0.10	0.12	0.05	0.05	0.05	0.03	0.05	0.06	0.05	0.06	0.04	0.03
Isoamyl acetate	1.65	1.66	1.44	1.38	1.09	1.83	1.38	2.08	2.00	2.96	0.49	1.32	0.73	0.63	0.76
Ethyl hexanoate	0.11	0.13	0.14	0.11	0.10	0.16	0.09	0.13	0.12	0.18	0.09	0.38	0.20	0.16	0.13
Ethyl 3-hydroxybutyrate	0.11	0.10	0.09	0.07	0.09	0.22	0.24	0.13	0.21	0.18	0.19	0.19	0.13	0.11	0.09
Ethyl lactate	16.1	18.7	17.6	11.6	13.8	12.1	16.3	17.4	10.6	11.0	15.3	16.0	18.1	13.4	14.7
Phenylethyl acetate	0.08	0.08	0.07	0.07	0.05	0.15	0.10	0.11	0.12	0.15	0.04	0.06	0.04	0.04	0.04
Acetaldehyde	37.7	41.6	49.8	29.5	47.3	44.5	64.6	58.2	49.5	26.0	83.4	60.8	73.1	45.6	70.7
Diacetyl	0.25	0.37	0.50	0.35	0.21	0.66	0.91	1.40	1.33	1.74	0.33	1.04	0.93	0.53	0.74
γ-butyrolactone	2.24	1.99	1.44	1.90	1.98	3.21	2.72	1.12	3.44	3.41	3.18	5.63	2.26	5.24	6.08

All concentrations are in mg/l. Abbreviations: 100 or 300 ppm, wine from must supplemented with 100 or 300 ppm of ammonium, respectively. aa: wine from must supplemented with amino acids.

sulphury notes, but also for masking other aroma nuances. Fig. 4 suggests that the concentration of sulphur-containing volatiles decreases when must is supplemented with ammonium in all cases. However, this effect seems to be dependent on the yeast strain in the cases in which musts were supplemented with amino acids.

4. Conclusions

The volatile composition of wine is strongly influenced by the different factors considered in this study. The strain of yeast is the predominant factor and mainly affects fusel alcohols and their acetates, isoacids and compounds related to the synthesis of fatty acids. The supplementation of must with nitrogen also plays an important role. In general, the higher the FAN level of the must, the lower the levels of methionol, β-phenylethanol and isoamyl alcohol and the higher the level of propanoic acid. The type of nitrogen supplementation (ammonium or amino acids) also has some importance. Samples from musts supplemented with ammonium have higher levels of ethyl lactate and c-3-hexenol, while samples from must supplemented with amino acids have higher levels of γ-butyrolactone, isobutanol and isobutyric acid. From a sensory point of view, nitrogen supplementation is the main factor introducing differences. Wines from musts supplemented with ammonium become more citric and less sulphury, independent of the yeast strain. However, sensory properties of wines from musts supplemented with amino acids depend on the yeast strain. In one case, the sensory effect is similar to that observed when adding high amounts of ammonium. For the two other strains, wines were richer in fruity and fusel notes.

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