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Influence of prefermentation clarification on the higher alcohol contents of wines

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Nitrogenous compounds and the amount of solids have an effect on both the metabolic pathway and on the concentration of higher alcohols (HA) in wines. In this study, the influence of two prefermentation clarification treatments (static sedimentation and vacuum filtration) on the concentration of nitrogen which can be assimilated and glucose consumption have been studied with respect to HA concentration of wines. The results show that total HA decreased with increasing must clarification level, and the clarification is more of a determining factor than amino nitrogen. No relation has been found in these complex mediums between Leu concentration and the formation of isoamyl alcohol nor between Val and isobutyl alcohol. The concentration of *n*-propyl alcohol was unrelated to concentration and consumption of Thr and Gaba. There seems to be a relationship between slower glucose consumption in filtered musts and lower HA concentration in wines.

INTRODUCTION

The main volatile compounds in wines are secondary products arising from alcoholic or malolactic fermentation; their presence in young wines, whites, rosés and reds, is a quality-defining factor (Ferrarese, 1987; Ribéreau-Gayon *et al.*, 1982). Among volatile compounds, higher alcohols (HA) make up the largest group from a quantitative point of view (Rapp & Mandery, 1986).

The mechanism involved in the formation of HA requires a decarboxylation process of the corresponding α-keto acid, followed by a reduction which produces the final alcohol (Sentheshanmuganathan, 1960; Rapp & Versini, 1991). When amino acids are catabolized through Ehrlich's pathway, α-keto acids are mainly produced; for this to occur, glucose is necessary as a source of the α-keto-glutarate which behaves as an acceptor of the amino groups of the amino acids. The alcohols, *n*-propyl, isobutyl, active amyl and isoamyl, are formed from α-keto-butyrate, α-keto-valerate, α-keto-isocaproate and α-keto-β-methylvalerate which are derived from threonine, valine, leucine and isoleucine, respectively (Ouchi *et al.*, 1980). However, in the absence of appropriate amino acids, such as at the beginning of fermentation, α-keto acids and the corresponding HA formed from these are obtained from glucose through a pyruvate pathway (Chen, 1978; Cooper, 1982; MacDonald *et al.*, 1984; Nykänen, 1986; Henschke & Jiranek, 1993). Using radioactive isotope markers, it has been found that 35% of higher alcohols come from the sugars while the remaining 65% have their origin in amino acids (Zoecklein *et al.*, 1990).

The relative amount formed by each pathway for every HA depends on various factors, such as yeast strain (Zeeman *et al.*, 1982; Cabrera *et al.*, 1988), yeast growth (Quain, 1988), fermentation temperature (Ciolfi *et al.*, 1985), must pH (Rankine, 1967), degree of aeration (Crowell & Guymon, 1963) and solids level (Klingshirn *et al.*, 1987).

In addition, grape variety and ripeness affect the concentration of higher alcohols (Cabrera *et al.*, 1988), probably because of the existence of qualitative and quantitative differences in must amino acid composition (Ough & Bell, 1980; Vos, 1981; Herráiz *et al.*, 1989; Rapp & Versini, 1991). Also, the presence of large amounts of insoluble solids in musts during fermentation produces wines with high levels of higher alcohols and esters when compared to wines made with clarified musts (Groat & Ough, 1978). Therefore, the must clarification method (static sedimentation or with a rotary vacuum filter) has an influence on the concentration of different alcohols during the wine stabilization process (Aleixandre & Vélez, 1992). The size of the solid particles also affects HA concentration; it has been found that musts with an average particle size greater than 53 μm result in wines with isobutyl and isoamyl alcohol concentrations which are significantly

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higher than for musts where solid particles with average size under 38 μm predominate (Klingshirn *et al.*, 1987). Nitrogenous compounds also play a fundamental role in HA concentration. It has been found that low concentrations of higher alcohols are obtained, through anabolic pathways, in simple media containing ammonium as the only nitrogenous source (Herráiz *et al.*, 1989; Henschke & Jiranek, 1993). When the medium is supplemented with amino acids or when it is complex, a substantial increment in HA concentration is produced; an exception is *n*-propyl alcohol (Herráiz *et al.*, 1989). Ough and Bell (1980) showed that increasing must nitrogen content over the range 287–766 mg N/litre decreased the total concentration of the higher alcohols, isobutyl and active amyl.

The aim of this study was to observe the influence of two prefermentation clarification treatments, static sedimentation and vacuum filtration, on HA concentration of stabilized wines. The relationships between HA concentration and nitrogenous compounds, which can be assimilated, and with glucose consumption rates have also been studied. Two different kinds of grapes were selected and subjected to prefermentation treatments. Original musts were taken as controls. The musts were fermented to produce the corresponding wines (rosé and white). Prefermentation treatments were done in a wine cellar, and the fermentation was done in a pilot plant to simulate industrial conditions. The musts and wines obtained were analyzed and compared.

MATERIALS AND METHODS

Samples and vinification

Vitis vinifera var. garnacha and *Vitis vinifera* var. viura musts of Navarra Denomination of Origin (NDO) were collected; rosé and white wines were produced in a pilot plant.

Vitis vinifera var. garnacha grapes were crushed and destemmed. The skins were not removed for 17 h. Must was later divided into three fractions. The first was treated with SO_2 (50 mg/litre) but was not subjected to any prefermentative technique. The other two, following refrigeration to 10°C and the addition of SO_2 (50 mg/litre), were clarified by two different prefermentation treatments: decantation and filtration by a rotary vacuum filter. In decantation, the must remained in stainless steel tanks for 24 h before racking. Then, 400 litres of the three musts were subjected to fermentation using *S. cerevisiae* (0.5 g/litre) at 17.7 ± 2°C. In all cases, the fermentation was continued until the concentration of reducing sugars fell below 2.5 g/litre. Finally, wines were stabilized by refrigeration at −5°C for 1 week and then filtered through a cellulose plate filter.

Freshly cropped *Vitis vinifera* var. viura grapes were crushed and destemmed; the skins obtained were not removed for 5–8 h, and the same process as described above for rosé vinification was followed. Average fermentation temperature was 18 ± 2°C. In all cases, the fermentation was continued until the concentration of reducing sugars fell below 2.5 g/litre.

A stainless crusher–stemmer Marzola Marzinox (Marrodán and Rezola, S.A., Logroño, Spain), equipped with a rubber roller was used to press the grapes. The must was filtered through an Espal V-20 rotary vacuum filter (Temavinsa, Logroño, Spain), with a 6500 litre measuring barrel, equipped with a 4 hp shaking motor, a 40 hp vacuum pump and a 7.5 hp feed pump. The diatomaceous earth filter, with a maximum particle size of 52 μm, had a surface area of 30 m^2/g and a filtration volume of 8000–10 000 litre/h.

Vinification was carried out in stainless steel (AISI 316-18/8/2) vertical tanks. Tank dimensions were 0.76 m diameter, 1.1 m height, and the capacity was 400 litres.

Must was inoculated with 0.5 g/litre of Fermivin active dry *Saccharomyces cerevisiae* from Gist brocades. (F. Lafford and Cía, S.A., Pasajes, Spain).

Preparation and HPLC analysis of free amino acids

Analysis was performed with a Waters high-pressure liquid chromatograph (Waters Chromatography Div., Milford, Massachusetts, USA) equipped with two 510 pumps, a U6K injector and a 486 UV–vis detector used at 254 nm. Maxima 820 software was employed for chromatographic control. A PICO.TAG reverse-phase column (300 mm and 3.9 mm i.d.) was used, with a stationary phase of dimethyloctadecylsilyl bonded to amorphous silica (Ref. Waters 10950). Derivatization was performed using a Waters PICO.TAG work station.

Standard solutions, for the analysis of the free amino acids by HPLC, were prepared of 2.5 mmol/ml concentration (Sigma Chemical Co., St. Louis Missouri, USA), except cystine (1.25 mmol/ml). Internal standards were L-2-aminohexanoic acid (L-norleucine) and L-2-amino-4-[methylsulphonyl]butanoic acid (L-methionine sulphone), both from Sigma. In derivatization, phenylisothiocyanate (Pierce, Rockford, Illinois, USA), methanol (Scharlau, S.A., Barcelona, Spain), triethylamine (Aldrich, Milwaukee, Wisconsin, USA) and double-distilled water were used. Mobile phases were prepared with sodium acetate, acetonitrile and methanol (Scharlau) and acetic acid (E. Merck, Darmstadt, Germany). The mobile phases were purified through an HA 0.45 μm Millipore filter. Solvents were of HPLC quality, and reagents of analytical quality.

The PICO.TAG method developed by Waters (Cohen *et al.*, 1989) was followed for amino acids analysis. Samples were cleaned up by ultrafiltration with a Millipore ultrafree MC cartridge, and then L-norleucine and L-methionine sulphone were added as internal standards. After that, precolumn derivatization with phenylisothiocyanate was carried out. The amount of sample injected was 5 μl.

Preparation and GC analysis of higher alcohols

The determination was done with a Varian (Palo Alto, California, USA) model 3700 gas chromatograph

equipped with a flame ionization detector and connected to a Shimadzu C-R1B (Columbia, Maryland, USA) integrator. The chromatographic column used was 2 m long packed with 5% Carbowax 20M on 80-120 Carbopack B.

Standard solutions, for the analysis of higher alcohols by gas chromatography, were prepared of *n*-propyl alcohol (4.45 mmol/l) (Merck), isobutyl alcohol (2-methyl-1-propanol) (4.11 mmol/l) (Panreac, Barcelona, Spain) and isoamyl alcohol (3-methyl-1-butanol) (3.65 mmol/litre) (Panreac). As internal standard, 4-methyl-2-pentanol (49.0 mmol/litre in 40% ethyl alcohol) was used (both from Janssen Pharmaceutica, Geel, Belgium). All reagents were of analytical quality.

Volatile compounds were concentrated by simple distillation, collecting a distillate volume 90–95% of the original sample volume; distilled water was then added to the distillate restoring the original sample volume (Amerine & Ough, 1976). Higher alcohols were identified and quantified through gas chromatography using nitrogen as carrier gas (20 ml/min). Injector and detector temperatures were 150°C. Oven temperature was 40°C during the first 10 min. Oven temperature was then increased at 10°C/min until a temperature of 110°C was reached. This temperature was maintained for 30 min.

Nitrogen contents

Must proteins were precipitated with trichloroacetic acid at 55%, using 1 ml for every 10 ml of must. Precipitation was performed at 0°C, and the must was then centrifuged at 4000 rpm. The supernatant was decanted and the nitrogen content in the residue analysed using the method described by the Office International de la Vigne et du Vin (1990), but modified by the addition of $CuSO_4$ and K_2SO_4 as catalysts instead of Se and $HgSO_4$. Protein analysis of the wines was performed by Bradford's modified method (Waters *et al.*, 1991). The results were then expressed as protein nitrogen.

Total nitrogen and ammonium nitrogen were obtained according to the methods described by the Office International de la Vigne et du Vin (1990).

Distillation of total and ammoniacal nitrogen was performed with Tecator automatic steam equipment (Tecator AB, S-26321 Höganäs, Sweden).

Turbidity and enological parameters

Enological parameters were obtained according to the methods described by the Office International de la Vigne et du Vin (1990).

The must's turbidity was determined using a 18900 Hach turbidimeter (Hach Co., Loveland, Colorado, USA) prepared for coloured samples.

All determinations were performed in quadruplicate on representative samples of musts and wines. The results given in the tables and figures include standard errors (SE).

RESULTS AND DISCUSSION

General characteristics of musts and wines

General parameters of original musts and stabilized wines are shown in Table 1. Turbidity, which is a measure of the level of clarification obtained by different pretreatments, was higher for the garnacha control must (1460 NTU) than for viura (695 NTU). As expected, filtered musts yielded the lowest turbidity levels, followed by static sedimentation and non-treated samples.

Total nitrogen values were higher in control musts than in decanted musts, and both were greater than in the filtered musts. This trend was reversed in wines since those obtained after vacuum filtration contain higher amounts than the others. The percentages of nitrogen reduction during fermentation were around 70% in viura and about 65% in garnacha musts, these values being much higher than those obtained by Gorinstein *et al.* (1984). For vacuum filtration those percentages were around 52% in viura and 38% in garnacha musts.

Filtering considerably reduced protein nitrogen content in musts: 86.1% in garnacha must and 65.8% in viura must. This important decrease in vacuum-filtered musts is due to the fact that proteins, with molecular weights between 13 000 and 65 000 Da (Yokotsuka *et al.*, 1991) are retained in the filter.

In rosé and white wines obtained from filtered musts, stabilized and kept for a year, the volatile acidity was 0.74 and 0.34 acetic acid g/litres, respectively, both higher than in the other wines. The increase of volatile acidity would be the consequence, among other factors, of the decrease in the fermentation rate (Ayestarán *et al.*, 1995). To characterize the kinetics, the process rates have been calculated from fermentation curves as an average percentage of the sugar consumed daily, in the ranges 5–50% (v_{f5-50}) and 0–99% (v_{f0-99}) of total sugars (Houtman & Du Plessis, 1985). These results are shown in Table 2. As for clarification by static sedimentation, the fermentation rate of viura must was also lower than for control must, while in the garnacha variety, the initial fermentation rate was the same as in the non-clarified one, but slowed down later and gave a lower overall rate. Filtered musts showed a reduction in sugar consumption rate (v_{f5-50} and v_{f0-99}) with respect to control must and sedimented must.

Influence of clarification on ethanol and higher alcohol concentration

Concentrations of ethanol and HA of stabilized wines are shown in Table 3. For the same grape variety, wine ethanol concentration did not vary with prefermentation treatments, while total HA concentration decreased as the level of clarification of the initial must increased.

Rosé wines obtained from vacuum-filtered musts showed a higher reduction in total HA concentration (58.9%) when compared to control wines than white wines (50.2%) obtained under similar conditions. In the first case, the decrease in turbidity of the filtered must

Table 1. Characteristics of musts and wines (all parameters with their standard error)

Samples		pH ± SE	Turbidity (NTU ± SE)	Reducing sugar (g/litre ± SE)	Ash (g/litre ± SE)	Total acidity (g/litre[a] ± SE)	Volatile acidity (g/litre[b] ± SE)	Total SO_2 (mg/litre ± SE)	Total nitrogen (mg/litre ± SE)	Protein nitrogen (mg/litre ± SE)
Garnacha	Control	3.31 ± 0.01	1460 ± 14	205 ± 1	2.89 ± 0.01	4.80 ± 0.03	—	53.3 ± 0.01	583.6 ± 0.6	174 ± 0.4
	Decanted	3.30 ± 0.01	205 ± 5	202 ± 0.6	2.9 ± 0.3	5.30 ± 0.01	—	40 ± 0.01	553.4 ± 0.4	150 ± 0.2
	Filtered	3.31 ± 0.01	66 ± 1	204 ± 3	2.42 ± 0.06	4.50 ± 0.01	—	15.7 ± 0.8	504 ± 0.5	24.3 ± 0.1
Viura	Control	3.51 ± 0.01	695 ± 7	180 ± 0.7	3.40 ± 0.06	4.11 ± 0.01	—	25.1 ± 0.2	412.6 ± 0.5	80.5 ± 0.2
	Decanted	3.51 ± 0.01	200 ± 7	181 ± 0.5	3.33 ± 0.05	4.66 ± 0.01	—	37.8 ± 0.9	369 ± 0.5	61.7 ± 0.3
	Filtered	3.47 ± 0.01	97 ± 3	181 ± 0.5	3.00 ± 0.08	4.32 ± 0.06	—	24.8 ± 0.9	332.6 ± 0.6	27.6 ± 0.6
Rosé wine[c]	Control	3.12 ± 0.01	—	0.96 ± 0.01	1.06 ± 0.03	4.65 ± 0.01	0.27 ± 0.04	35.0 ± 0.01	198 ± 0.5	19.8 ± 0.4
	Decanted	3.10 ± 0.01	—	0.99 ± 0.05	1.10 ± 0.01	4.95 ± 0.01	0.30 ± 0.02	37.4 ± 0.9	198 ± 0.2	13.5 ± 0.7
	Filtered	3.33 ± 0.01	—	0.76 ± 0.02	1.06 ± 0.06	4.50 ± 0.01	0.74 ± 0.03	25 ± 1	310 ± 0.2	13.5 ± 0.1
White wine[c]	Control	3.35 ± 0.01	—	0.27 ± 0.02	1.39 ± 0.07	3.94 ± 0.02	0.23 ± 0.02	34.9 ± 0.9	115 ± 0.3	4.6 ± 0.4
	Decanted	3.33 ± 0.01	—	1.67 ± 0.01	1.16 ± 0.08	3.89 ± 0.01	0.27 ± 0.01	89.6 ± 0.9	100 ± 0.3	5.2 ± 0.1
	Filtered	3.40 ± 0.01	—	0.13 ± 0.03	1.33 ± 0.02	3.28 ± 0.05	0.34 ± 0.04	65.6 ± 0.01	16 ± 0.3	4.4 ± 0.3

[a]As g/litre tartaric acid.
[b]As g/litre acetic acid.
[c]Wines produced from control, decanted and filtered musts.

Table 2. Features of the fermentation kinetics in Garnacha and Viura musts

Samples	Process	Sugar utilization v_{f5-50}[a] (%/day)	v_{f0-99}[b] (%/day)	Fermentation time (days)
Garnacha	Control	31.7	16.5	9
	Decanted	31.7	10.0	10
	Filtered	15.7	3.8	29
Viura	Control	31.8	14.1	16
	Decanted	22.5	5.6	21
	Filtered	14.0	4.3	25

[a]Average percentage of sugar used daily during the time required to ferment from 5 to 50% of the total.
[b]Average percentage of sugar used daily during the time required to ferment from 0 to 99% of the total.

Table 3. Ethyl and higher alcohols concentrations in wines obtained with musts with different levels of clarification (all parameters with their standard error)

	Rosé wines[a]			White wines[a]		
	Control	Decanted	Filtered	Control	Decanted	Filtered
Ethyl alcohol (v/v ± ES)	12.4 ± 0.1	12.5 ± 0.1	12.8 ± 0.1	10.7 ± 0.1	10.6 ± 0.1	10.7 ± 0.1
Isoamyl alcohol (mg/litre ± ES)	186 ± 13	152 ± 8	68 ± 2	212 ± 2	190 ± 3	83 ± 6
Isobutyl alcohol (mg/litre ± ES)	36 ± 1	33 ± 1	17.0 ± 0.6	45 ± 1	46.9 ± 0.5	34 ± 2
n-Propyl alcohol(mg/litre ± ES)	19.0 ± 0.7	18.0 ± 0.8	14.0 ± 0.5	15.0 ± 0.4	20.0 ± 0.6	17.0 ± 0.8
Total higher alcohols (mg/litre ± ES)	241 ± 13	203 ± 8	99 ± 2	272 ± 2	257 ± 4	134 ± 6

[a]Wines produced from control, decanted and filtered musts.

when compared to the control (Table 1) was 95.5%, and in the second case, it was lower (86.0%). The HA concentration in wines obtained from decanted musts was lower than the control wines; however it was higher than the wines obtained from filtered musts. The reduction was less in white than in rosé wines. Therefore, an increase in must turbidity results in greater HA concentrations, a detrimental factor for wine quality. This agrees with the paper of Groat and Ough (1978).

There is a relationship between isoamyl and isobutyl alcohol concentrations (Table 3) and the clarification level of the initial must (Table 1). A reduction in the turbidity of the initial must produced lower concentrations of these alcohols in stabilized wines. In rosé wines obtained with filtered must, *n*-propyl alcohol decreased slightly. These results coincide with those of Klingshirn *et al.* (1987), who observed the same tendency with insoluble solids concentration. He found that wines with 0.8% (v/v) insoluble solids resulted in much higher isobutyl and isoamyl alcohol values than wines with 0.2–0.4% (v/v); no significant differences in *n*-propyl alcohol levels were found in either case.

Relationship between nitrogenous content and higher alcohol concentration

In the presence of increasing concentration of ammonium nitrogen, deamination of amino acids, and the ensuing formation of HA through Ehrlich's pathways, notably decreased; thus, the greater the consumption of ammonium nitrogen, the lower the final concentration of HA should be (Ough & Bell, 1980; Usseglio-Tomasset, 1985). Figure 1(a) and (b) shows the evolution which ammonium nitrogen undergoes during fermentation for garnacha and viura must. In our results, ammonium nitrogen was completely consumed in all samples during the first half of fermentation; afterwards, small concentrations were excreted to the medium, except in filtered garnacha must where excretion was greater (20 mg/litre). Therefore, concentration variations of HA with prefermentation treatments of must cannot be explained by ammonium nitrogen consumption data, indicating that this consumption was not a determining factor in the formation of HA in our case.

Amino nitrogen was obtained from the concentrations of the amino acids analysed, expressed in mg/litre of nitrogen. Clarified musts showed significantly higher ($P = 0.05$) concentrations of amino nitrogen [Fig. 2(a) and b] than control musts in all cases, as a result of the action of vegetable proteases which pass into the must from the pulp (Ayestarán *et al.*, 1995). During the first fermentation stage, a large part of this nitrogen is consumed, as the yeasts use amino acids as nitrogenous sources (Large, 1986; Bezenger & Navarro, 1987). Amino nitrogen consumption was much lower in the vinification of rosé wines when the initial must has been filtered. Consumption of this nitrogen did not differ between the control must and clarified musts for whites. Herráiz *et al.* (1989) observed that, using a synthetic medium and nonclarified musts (*Vitis* var. *verdejo* and *albillo*) in fermentations produced by *S. cerevisiae*, HA concentration increases (except for *n*-propyl alcohol) when amino acid content increases.

In this study, control musts (with lower amino nitrogen content but with higher turbidity) produced wines with higher isoamyl and isobutyl alcohol concentrations

than clarified musts (with a greater concentration of amino nitrogen but lower turbidity). This seems to indicate that, in this case, the degree of clarification of the must is a more determining factor than amino nitrogen in higher alcohols formation.

Initial concentrations and consumption of HA precursor amino acids examined in this study (Leu, Val, Gaba and Thr) are shown in Fig. 3. The consumption of these amino acids was calculated by the difference between the initial concentration and that which remained at maximum depletion. Leucine is the main precursor of isoamyl alcohol through Ehrlich's pathway (Usseglio-Tomasset, 1985). In Fig. 3(a) it can be seen that, for the garnacha must, clarification processes resulted in a considerable increase both in initial leucine concentration and in its consumption during fermentation. No major changes in this amino acid were observed with prefermentation clarification in the white vinification [Fig. 3(b)]. In synthetic media, when leucine is the only nitrogenous source, isoamyl alcohol concentration is much higher than that obtained in the presence of any other amino acid or of ammonium ions in the medium (Usseglio-Tomasset, 1985). Ouchi *et al.* (1980) also found that leucine concentration increases final isoamyl alcohol concentrations in wine. In our results, the garnacha control must with very low leucine concentrations showed higher isoamyl alcohol concentration than clarified musts, whereas the viura musts had similar leucine concentration but a different isoamyl alcohol contents (Table 3). Therefore, according to these data, the relationship between leucine concentration and consumption and isoamyl alcohol formation is not as direct in real, complex media such as the one studied.

Valine is the main precursor of isobutyl alcohol through Ehrlich's pathway (Henschke & Jiranek, 1993). Figure 3(a) shows that initial concentrations of valine in clarified garnacha musts, and its consumption during fermentation, increased substantially with respect to the control. However, the more clarified musts resulted in a lower production of isobutyl alcohol (Table 3). On the other hand, in synthetic media, the

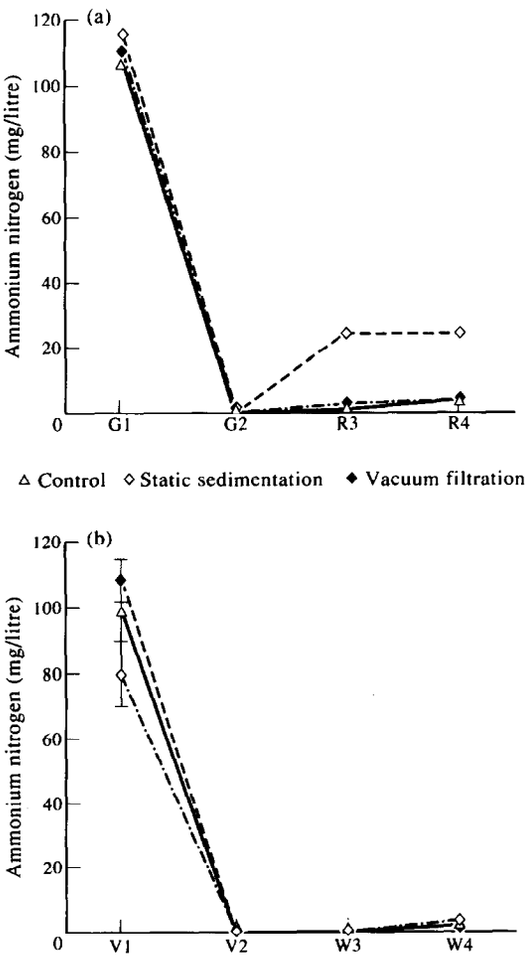

Fig. 1. Evolution of ammonium nitrogen concentration during fermentation, and in stabilized wines made with musts from (a) *Vitis* var. garnacha and (b) *Vitis* var. viura (G1, V1—initial must; G2, V2—must half-way through fermentation; R3, W3—newly made wine; R4, W4—wine which has been stabilized and kept for 1 year). (Standard errors are included but may not be perceptible in the same cases.)

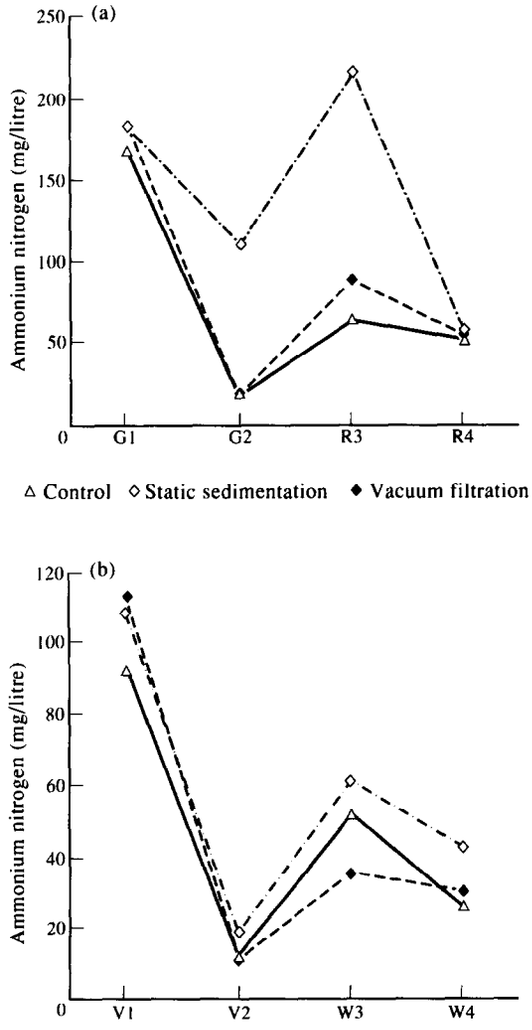

Fig. 2. Evolution of amino nitrogen concentration during fermentation and in stabilized wines made with musts from (a) *Vitis* var. garnacha and (b) *Vitis* var. viura (G1, V1—initial must; G2, V2—must half-way through fermentation; R3, W3—newly made wine; R4, W4—wine which has been stabilized and kept for 1 year). (Standard errors are included but may not be perceptible.)

greatest concentration of isobutyl alcohol is obtained when valine is supplied as the only nitrogenous source (Usseglio-Tomasset, 1985). In the viura must (Fig. 3b), initial concentration and consumption of this amino acid increased slightly in decanted musts compared to control musts, while in filtered musts, there was practically no variation in initial concentration and consumption. Wines from control and decanted musts showed similar concentrations of isobutyl alcohol, while in wine from filtered musts, the concentration of this alcohol was lower.

In the case of *n*-propyl alcohol, its main precursors are threonine and γ-aminobutyric acid (Usseglio-Tomasset, 1985). In Fig. 3(a) it can be observed that, in garnacha musts, initial threonine concentrations, as well as their consumption during the fermentation, were higher in the clarified musts than the control musts. However, Gaba was not consumed during vinification of rosé with filtered must. In viura must (Fig. 3b), clarification treatment hardly affected initial concentrations and consumption of Gaba during fermentation, although both values were somewhat higher in wines from sedimented musts. Values of *n*-propyl alcohol concentration found in all wines were independent of the concentration and consumption of these amino acids, and of the clarification level of initial musts. This coincides with the results obtained by Ough and Bell (1980) and Vos (1981).

Therefore, no correlation has been found between the higher alcohols studied and the concentration and consumption of the precursor amino acid present in the must. These results are similar to those of Chen (1978). That author points out that fermentation studies using dual-labelled amino acids and sugars have demonstrated that no simple relationships exist between concentration of the parent amino acid and the corresponding alcohol. Additionally, Knatchbull and Slaughter (1987) stated that it is unlikely that the solids affect fusel oil production through its effect on amino acid uptake.

Glucose utilization and higher alcohol formation

The presence of glucose in the medium is essential for the yeasts to synthesize higher alcohols; from glucose, α-ketoglutarate is formed, an acceptor of amino groups and driver of transaminations of amino acids. In the case of certain hydrophobic amino acids, this transamination leads to the appearance of carbonated derivatives that can change into higher alcohols through Ehrlich's pathway (Woodward & Cirillo, 1977). Furthermore, the glucose can change into some of these higher alcohols through intermediate α-keto acids formed from the pyruvate (Cooper, 1982).

Table 2 shows that, in filtered must fermentation, glucose consumption was much slower than in the fermentation of less clarified musts (control or sedimented). This slower glucose consumption would explain a lower production of the higher alcohols, owing to lower formation of precursor α-keto acids. On the other hand, a slow glucose consumption would imply a decrease in the synthesis of α-ketoglutarate,

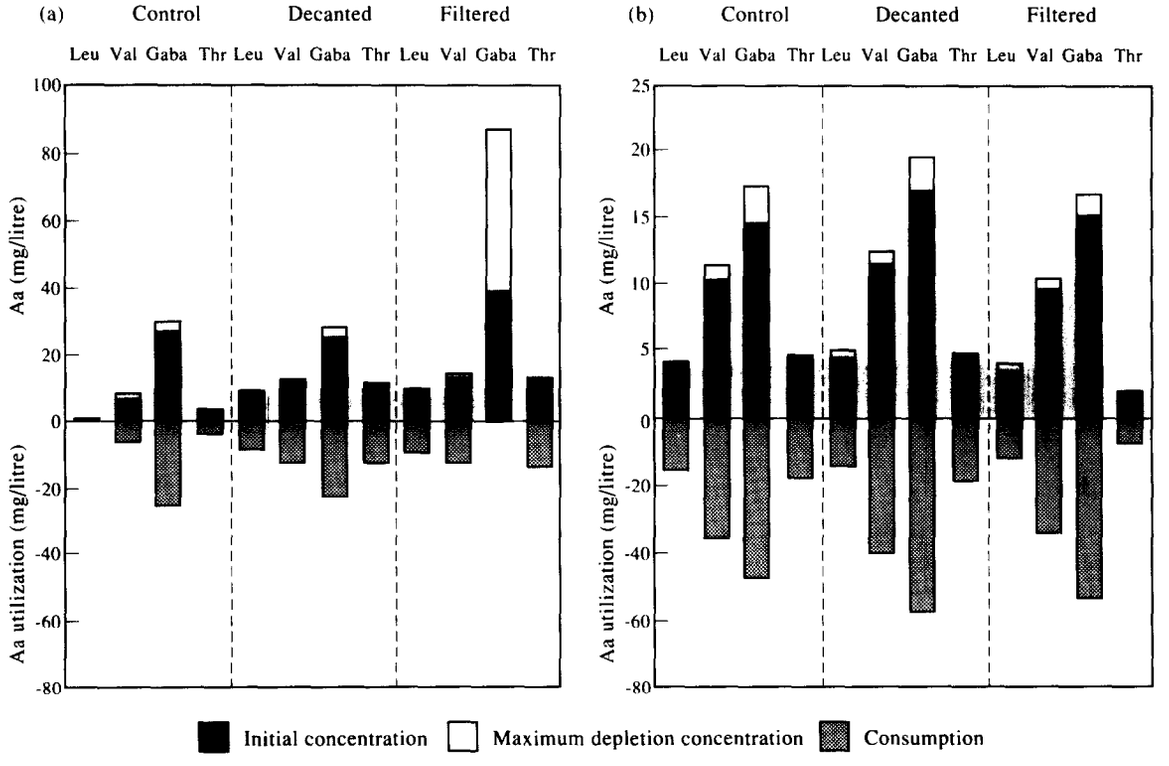

Fig. 3. Initial concentration, concentration at the moment of greatest depletion and consumption during fermentation of precursor amino acids of higher alcohols in wines made from musts subjected to different clarification processes: (a) rosé wines and (b) white wines.

deamination of parent amino acids of higher alcohols thereby being less intense. This reduced transamination leads to an accumulation of amino acids inside the yeast cell and probably explains its excretion to the medium due to an inability to metabolize. This could be the reason why higher concentrations of amino nitrogen were found in wines made with filtered musts, especially rosés, than in control wines [Fig. 2(a) and (b)]. Furthermore, ethanol's toxicity on the yeast's membrane is greater in well-clarified media, and inhibits the transport of amino acids to the cell's interior by altering the membrane potential (Casey & Ingledew, 1986). Also contributing to the increased amino acid liberation in the filtered musts would be the yeast's increased proteolytic activity caused by oversaturation of carbon dioxide, a gas that escapes with difficulty in the absence of trubs (Kruger *et al.*, 1992).

Pyruvate conversion, arising from glucose, into α-keto-isovalerate (precursor of isobutyl alcohol) and into α-keto-isocaproate (precursor of isoamyl alcohol), requires the expenditure of an NADPH molecule and an NADH molecule (Cooper, 1982). Fermentation of filtered musts occurred with slower glucose consumption (Table 2), pointing to retarded cellular growth (Bely *et al.*, 1990). Under these conditions, the NADPH, necessary in growth processes, would be oriented with a preference towards biosynthesis and cellular development, and would probably not be used in the formation of higher alcohols, which would also be a contributing factor towards the reduction in the synthesis of these compounds.

Ethanol and *n*-propyl alcohol are formed from pyruvate through not very demanding processes in relation to glucolysis or cellular biosynthesis. This would probably lead to similar final concentrations of both alcohols in all wines analyzed.

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