Relation between Fatty Acid Content and Its Evolution during Fermentation and Utilization of Free Amino Acids in Vacuum-Filtered Viura Must

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The activity of proteins that transport amino acids changes depending on the unsaturated residue which is introduced into the phospholipids of *Saccharomyces cerevisiae* membrane. The aim of this study was to observe the influence of fatty acid content and its evolution during fermentation on the utilization of free amino acids in *Vitis vinifera* var. Viura must clarified by vacuum filtration. The results demonstrate that filtration of the must did not reduce the concentration of free amino acids, but it did reduce fatty acids, especially the unsaturateds. In the first half of fermentation (up to 50% sugar consumption), fatty acid utilization was different in each sample without involving an alteration in the uptake of amino acids. In the second half of fermentation (from 50% sugar consumption until the end of fermentation), the clarified sample had greater consumption of saturated fatty acids than unsaturated, in contrast to the control; this, along with other factors, produced a greater liberation of free amino acids in the filtered sample.

Keywords: *Free amino acids; fatty acids; vacuum filtration; Viura musts*

INTRODUCTION

Nitrogenous compounds of must exert an important influence on the growth and metabolism of yeast and, consequently, on the fermentation rate (Henschke and Jiranek, 1993). In this respect, prefermentative clarification treatments of must can alter yeast development since they decrease total nitrogen content (Bidan et al., 1986; Lagunas, 1986). It has been observed that vacuum filtration and sedimentation of Garnacha musts diminished protein nitrogen, did not alter ammonial nitrogen concentration, and increased slightly amino nitrogen with respect to unclarified must (Ancín et al., 1996a,b).

Ammonia and amino acids form the principal nitrogenous source for yeast (O'Connor-Cox and Ingledew, 1989). The key step in the metabolism of these compounds in *Saccharomyces cerevisiae* is their transport to the cell cytosol (Bisson, 1991). The uptake of ammonia occurs by active transport utilizing two specific permeases; yeast obtains the necessary energy for this transport by degradation of glucose existing in the medium (Roon et al., 1975; Dubois and Grenson, 1979; Egbosimba and Slaughter, 1987). The uptake of amino acids also occurs by active transport utilizing a nonspecific transport system such as general amino acid permease (GAP) and different specific transporters of some amino acids. These transport systems introduce protons and amino acids into the cell cytosol (Cooper, 1982; Bisson, 1991); the protons, which enter the cell by this system and by simple diffusion, are excreted through the plasma membrane by means of ATPase proton transporter that requires energy (Serrano, 1978).

The uptake and metabolism of nitrogenous compounds by microorganisms during fermentation not only depend on the strain and its physiological condition but also depend on the chemical composition and physical characteristics (Henschke and Jiranek, 1993). Therefore, excessive must clarification causes yeast to modify its utilization of nitrogenous compounds; in filtered musts of var. Garnacha, amino nitrogen was consumed only 39.7% during the first half of fermentation (up to 50% sugar consumption), whereas in unclarified must 88.5% was consumed. In the same study, it was confirmed that ammonia was consumed slower in musts with high levels of clarification (Ancín et al., 1996a).

Lipids are also nutrients for yeast and affect the activity of the proteins that transport amino acids and of enzymes united to the membrane such as ATPase; these activities depend on lipid composition of the cellular membrane of these microorganisms (Keenan and Rose, 1979; Keenan et al., 1982; Prasad and Rose, 1986; Rose, 1989). Thus, Calderbank et al. (1984) observed that enrichment of the membrane with oleic and linoleic acids modified the accumulation of L-arginine, L-lysine, L-histidine, L-threonine, and L-valine in yeast cytosol, although other amino acids were not affected. It seems that the activity of proteins that intervene in the transport systems of these compounds is altered differently according to the unsaturated residue introduced into the phospholipids. Similarly, transport of amino acids and certain membrane functions require the presence of saturated residues (Otoguro et al., 1981). Membrane enrichment with different phospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) affects also GAP (Calderbank et al., 1985).

In a previous study (Ancín et al., 1996d), it was evident that vacuum filtration of Viura musts, among other things, drastically reduced the total content of fatty acids and eliminated totally the acids arachidic, behenic, and linolenic. The aim of this study was to

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observe the influence of fatty acid contents of the must and its evolution during fermentation on the utilization of free amino acids in *Vitis vinifera* var. Viura must clarified by vacuum filtration. Must, the object of this study, was obtained, and its maceration was done in a wine cellar, and the fermentation was done in a pilot plant to simulate industrial conditions. Thus, musts and wines obtained were analyzed and compared with control must (unclarified).

MATERIALS AND METHODS

Samples and Vinification. *V. vinifera* var. Viura grapes of Navarra Denomination of Origin (NDO) were collected, crushed, and destemmed in order to make white wines in a pilot plant.

The skins were not removed for 5−8 h. Must was later divided into two fractions. The first one was treated with SO_2 (50 mg/L) but was not subjected to any prefermentation technique. The other, following refrigeration at 10 °C and addition of SO_2 (50 mg/L), was clarified by rotary vacuum filtration. Then 400 L of each of the two musts were subjected to fermentation using 0.5 g/L of Fermivin active dry *S. cerevisiae* from Gist brocades. The temperature was controlled at 18 °C with a standard deviation of less than 2 °C. In both cases, the fermentation was continued until the concentration of reducing sugars fell below 2.5 g/L.

A stainless crusher-stemmer, Marzola Marzinox (Marrodan and Rezola SA, Logroño, Spain), equipped with a rubber roller was used to destem and crush the grapes. The must was filtered through a rotary vacuum filter, Espal V-20 (Temavinsa, Logroño, Spain), with a 6500-L measuring barrel, equipped with 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 L/h.

Vinification was carried out in stainless steel (AISI 316-18/8/2) vertical tanks. Tank dimensions were 0.76 m diameter and 1.1 m height, and the capacity was 400 L. Must was inoculated with 0.5 g/L Fermivin active dry *S. cerevisiae* from Gist brocades (F. Lafford and Ca., Pasajes, Spain).

Preparation and HPLC Analysis of Free Amino Acids. Analysis was performed with a Waters high-pressure liquid chromatograph (Waters Chromatography Div., Milford, MA) 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 × 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 workstation.

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

The Pico·Tag method developed by Waters (Cohen et al., 1989) was followed. Samples were cleaned by ultrafiltration with a Millipore ultrafree MC cartridge, and L-norleucine and L-methionine sulfone 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 Fatty Acids. Determination was performed on a Perkin-Elmer 8420 gas chromatograph (Perkin-Elmer Corporation, Norwalk, CO) equipped with flame ionization detector and a fused silica capillary column (Supelcowax 10; 30 m × 0.25 mm i.d.).

Standard solutions for the analysis of the fatty acids (Matreya Inc., Pleasant Gap, PA) by GC were prepared for different concentrations at intervals from 10 to 250 mg/L. Internal standards were methyl heptadecanoate and methyl undecanoate (Sigma Chemical Co., St. Louis, MO). For the preparation of methyl esters, sodium methoxide 0.2 M (0.5 g of sodium metal in 100 mL of anhydrous methanol) and hydrochloric acid at 4% (w/w) in methanol (prepared by passing a stream of hydrogen chloride through anhydrous methanol) were used. Methyl esters were extracted with a mixture of hexane/H_2O 2:10 (v/v). Reagents employed were analytical quality and from Panreac (Montcada i Reixac, Barcelona, Spain).

The lipid fraction was extracted by the procedure of Darné and Madero-Tamargo (1979); to 20 mL of sample were added 10 mL of ethanol and 10 mL of double distilled water. The sample was homogenized with an Ultra-Turrax T25 (8000 rpm for 2 min), and then the fatty acids were extracted with 20 mL of chloroform by homogenizing at 8000 rpm for 1.5 min; this operation was done at 0 °C and repeated five times. Fatty acid derivatization was done following the method recommended in the Código Alimentario Español (1977), which is based on the formation of methyl esters by transesterification of the esters present and esterification of free fatty acids and their subsequent extraction. The identification and quantification of fatty acid methyl esters was done by GC. The temperature of the injector and detector was 230 °C and the initial oven temperature was 120 °C, increased at 3 °C/min up to 200 °C and was then maintained for another 35 min. Helium was the carrier gas. The volume injected, equally for the samples or standards, was 1 μL.

Protein Nitrogen and Enological Parameters. Must proteins were precipitated with tricholoacetic 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 was analyzed by 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 catalyst instead of Se and $HgSO_4$. Soluble protein nitrogen analysis of the wines was performed by Bradford's modified method (Waters et al., 1991).

Total nitrogen, ammonium nitrogen, and enological parameters were measured according to the methods described by the Office International de la Vigne et du Vin (1990). Amino nitrogen was calculated by determining free amino acids by HPLC following the Pico.Tag method.

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

The turbidity of the must was determined using a Model 18900 Hach turbidimeter (Hach Co., Loveland, CO) prepared for colored samples.

All determinations were performed in quadruplicate on representative samples of musts and wines. The results given in tables are with standard errors (SE). To improve clarity, the results represented in histograms do not include SE; however, the coefficients of variation for amino acid data obtained by the method described were between 1% and 13%.

RESULTS AND DISCUSSION

General Parameters and Nitrogenous Fractions. The turbidity of the clarified must decreased 86% (Table 1), and the ash content decreased by 14.7% with respect to the control; this was due to vacuum filtration eliminating organic salts and grape solids from the must. Additionally, filtration modified slightly the total acidity value and pH, but it did not alter the initial sugar content.

Fermentation of the clarified must was slower than the control, and the percentage of sugar consumed per

Table 1. Evolution of General Parameters of Viura Musts and the Wines Produced after Fermentation (n = 4, All Parameters Are Given with Standard Error, SE)

		turbidity (NTU ± SE)	ash (g/L ± SE)	reducing sugars (g/L ± SE)	pH ± SE	total acidity (g/L[a] ± SE)	volatile acidity (g/L[b] ± SE)	alcohol (v/v % ± SE)
filtered	must	97 ± 3	2.9 ± 0.1	181.6 ± 0.5	3.47 ± 0.01	4.3 ± 0.1		
	midpoint of fermentation		2.05 ± 0.04	73.4 ± 0.6	3.31 ± 0.01	4.36 ± 0.02		
	wine recently produced		1.44 ± 0.05	0.73 ± 0.06	3.37 ± 0.01	3.44 ± 0.02	0.25 ± 0.01	10.5 ± 0.1
control	must	695 ± 7	3.4 ± 0.1	180 ± 2	3.51 ± 0.01	4.11 ± 0.01		
	midpoint of fermentation		3.1 ± 0.2	74 ± 2	3.32 ± 0.01	4.52 ± 0.01		
	wine recently produced		1.6 ± 0.1	0.45 ± 0.08	3.28 ± 0.01	4.85 ± 0.02	0.14 ± 0.01	10.7 ± 0.1

[a] As g/L tartaric acid. [b] As g/L acetic acid.

Table 2. Evolution of Nitrogenous Fractions and Total Nitrogen of Viura Musts and the Wines Produced after Fermentation (n = 4, All Parameters Are Given with Standard Error, SE)

		N-protein[a] (mg/L ± SE)	N-protein[b] (mg/L ± SE)	N-NH_4^+ (mg/L ± SE)	N-amino (mg/L ± SE)	N-total (mg/L ± SE)
filtered	must	27.6 ± 0.6	6 ± 1	81 ± 10	109.8 ± 0.5	332.6 ± 0.6
	midpoint of fermentation		3.9 ± 0.2	*c*	19.1 ± 0.1	314 ± 3
	wine recently produced		2.5 ± 0.5	*c*	62.0 ± 0.1	176 ± 1
control	must	80.5 ± 0.2	12 ± 2	101 ± 10	96.3 ± 0.6	412.6 ± 0.5
	midpoint of fermentation		11.8 ± 0.1	*c*	12.80 ± 0.04	410 ± 1
	wine recently produced		2.7 ± 0.3	*c*	52.6 ± 0.2	125 ± 4

[a] Total protein nitrogen from trichloroacetic acid method. [b] Soluble protein nitrogen from Bradford's method. [c] Not detected.

Table 3. Evolution of Arginine and Proline during Fermentation of Viura Musts and Recently Wine (n = 4, All Parameters Are Given with Standard Error, SE)

	concentration (mg/L)			[a]assimilation/excretion[b] (mg/L)	
amino acids	initial must	midpoint of fermentation	recently produced wine	first half of fermentation	second half of fermentation
control					
arginine	148 ± 11	0.71 ± 0.04	2.7 ± 0.9	−147.3	+2
proline	147 ± 10	48 ± 5	282 ± 22	−99	+234
filtered					
arginine	179 ± 8	1.4 ± 0.2	5.4 ± 0.3	−177.6	+4
proline	197 ± 7	79 ± 3	305 ± 22	−118	+226

[a] Assimilation, negative value. [b] Excretion, positive value.

day, up to 99% consumption of initial sugars, was 14.1 in the control and 4.3 in the filtered must; the control needed 16 days to ferment to dryness whereas the filtered required 25 days (Ayestarán et al., 1995; Ancín et al., 1996c) even though the alcoholic degree was similar (Table 1). The volatile acidity of the wine derived from filtered must was superior to that of the control, but in both cases it was between 0.12 and 0.30 g of HAc/L, values described by Amerine and Ough (1976) for wines not presenting any problems for conservation nor for organoleptic characteristics.

Clarification reduced total nitrogen of the must (19.4%) with respect to the control sample (Table 2). Among the different nitrogenous fractions, protein nitrogen in filtered must decreased most (65.7%). In the first half of fermentation (up to 50% sugar consumption), soluble protein nitrogen in clarified must decreased 35%, and the control did not suffer any variation. In filtered must, the rate of ammonium ion uptake was the same as the control, and in both media it disappeared in the first hours of fermentation. Amino nitrogen decreased equally in both samples (filtered, 82.6%; control, 86.7%) during the first half of fermentation.

Free Amino Acids and Fatty Acids in Initial Must. Filtration of Viura must did not reduce the concentration of free amino acids. Amino acid constituents of proteins (AaP) increased significantly ($p = 0.05$) in the filtered must with respect to the control while the amino acids not constituents of proteins (AaNP) scarcely changed (Ayestarán et al., 1995). The majority of amino acids in both samples were arginine, proline, alanine, glutamic, and γ-aminobutyric acids (Figure 1 and Table 3), which agrees with the results for other grape varieties (Kliewer, 1970; Juhász et al., 1984; Ooghe and Kastelijn, 1988). Arginine and proline had the largest concentrations, as occurs in an ample variety of musts (Kluba et al., 1978; Amerine et al., 1980; Sponhloz, 1991).

The total concentration of fatty acids in the control viura must was 32.2 mg/L (Table 4), palmitic being the most abundant saturated acid and linoleic the most abundant unsaturated; both constituted 68.3% of the total content. Filtration reduced the total concentration of fatty acids by 81.7% of the initial must concentration (Table 5) eliminating especially unsaturated fatty acids (linolenic 100%; linoleic 97.1%); in the filtered must, this produced a decrease in the relation of unsaturated/saturated fatty acids (0.2) in comparison with the control (1.2) (Ancín et al., 1996d).

Utilization of Free Amino Acids and of Fatty Acids during the First Half of Fermentation (up to 50% Sugar Consumption). In this fermentation step, the major part of amino acids present was consumed in both musts although there were differences in utilization of some of them depending on the initial must turbidity. Basic amino acids (histidine, ornithine, δ-aminobutyric acid, and arginine), transported to the yeast cytosol by specific permeases and by GAP (Cooper, 1982; Horák, 1986), were consumed in similar percent-

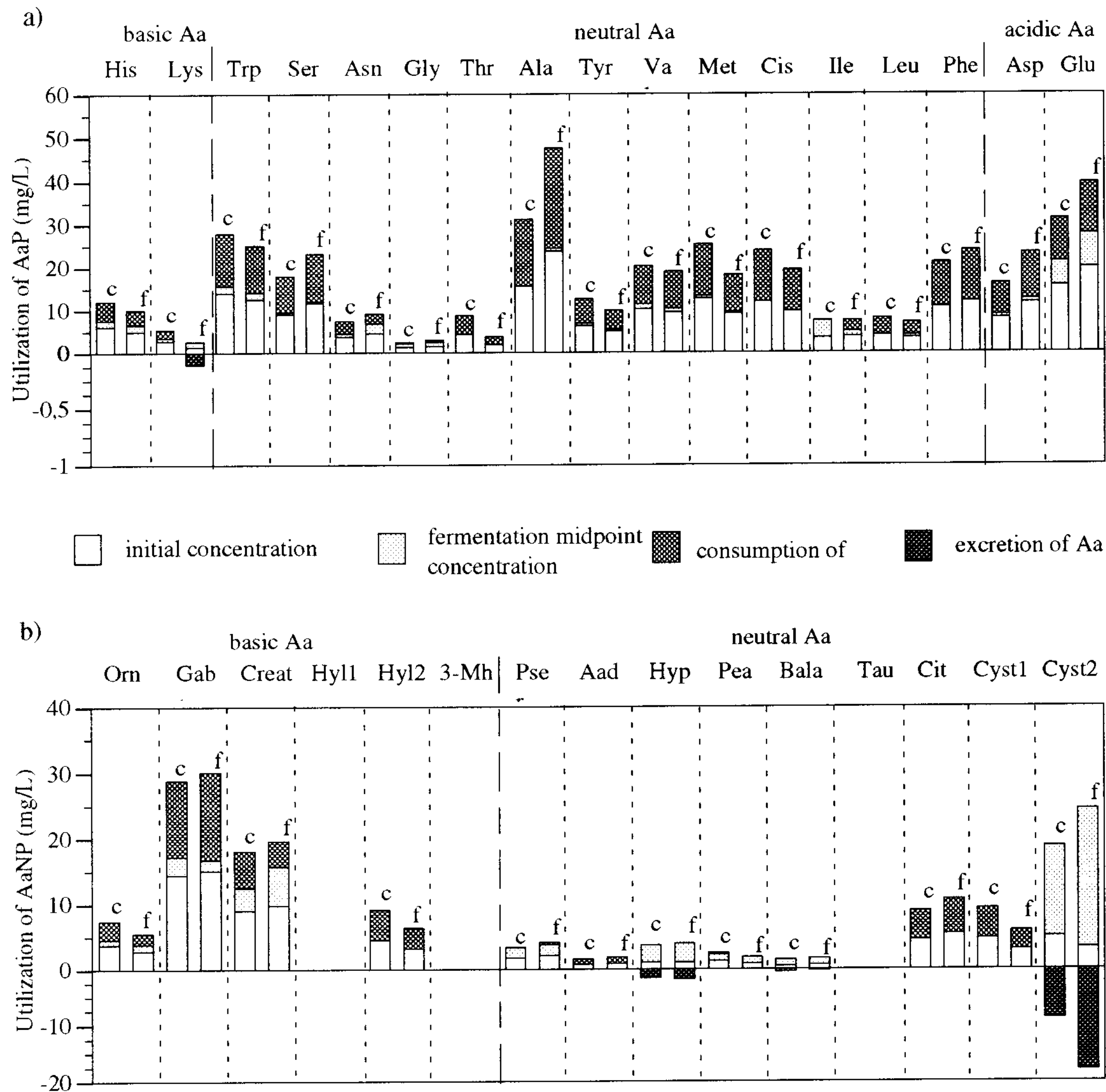

Figure 1. Initial concentration of amino acids in the musts and at fermentation midpoint. Assimilation or excretion of amino acids during the first half of fermentation. Values are for comparison and not additive; (a) amino acids of proteins (AaP); and (b) amino acids not found in proteins (AaNP); (c) control must sample; (f) filtered must sample.

ages in the filtered sample and in the control (Figure 1 and Table 3); however, lysine, a minor component in these samples, was consumed in the control but not in the filtered in spite of both samples using the same transport systems as for the other amino acids.

Neutral amino acids followed a similar pattern in both samples, and a large part of them were consumed in high percentages with respect to their initial concentrations. Hydroxyproline and β-alanine were excreted in both samples. Asparagine, glycine, and leucine were consumed more in the control than in the filtered. However, isoleucine and phosphoserine were consumed in the filtered but not in the control (Figure 1 and Table 3). The transport of neutral amino acids by GAP did not seem to be inhibited in this clarified medium, equal to the transport of basic amino acids. Among the sulfur amino acids, methionine was consumed in comparable percentages in both samples (Figure 1). Cystine and cystathionine-1 were consumed practically in totality in both samples, and cystathionine-2 was excreted. Among acidic amino acids, aspartic was consumed in high percentage in both samples (control, 91.7%; filtered, 94%) and glutamic was consumed 65% in the control and 59% in the filtered sample. Both acidic amino acids are good nitrogenous sources for an ample number of yeast (LaRue and Spencer, 1967; Cooper, 1982).

The evolution of fatty acid concentrations in this fermentation step was different in each sample (Ancín et al., 1996d); in the clarified must, the total concentration of these compounds increased (77.9%) whereas in the control it decreased (46.9%). Unsaturated fatty acids were excreted 77.8% in the clarified sample and were consumed 72.2% in the control (Tables 4 and 5). On the other hand, filtration eliminated linolenic acid ($C_{18:3}$) from the must, and linoleic ($C_{18:2}$) was not consumed in this sample. This was important since neither fatty acid can be synthesized by *S. cerevisiae* (Rosi and Bertuccioli, 1992). The saturated long-chain fatty acids disappeared equally in both musts (filtered, 40%; control, 48.4%), consuming, principally, $C_{16:0}$ and $C_{18:0}$. Therefore, the degree of plasma membrane saturation of yeast ought to be different in the two samples.

The GAP transport system is very sensitive to changes in lipid composition (phospholipids and their degree of unsaturation) of the plasma membrane of *S. cerevisiae* produced in very clarified musts (Kruger et al., 1992; Thomas et al., 1994). However, in our case, the clarification treatment by vacuum filtration had modified

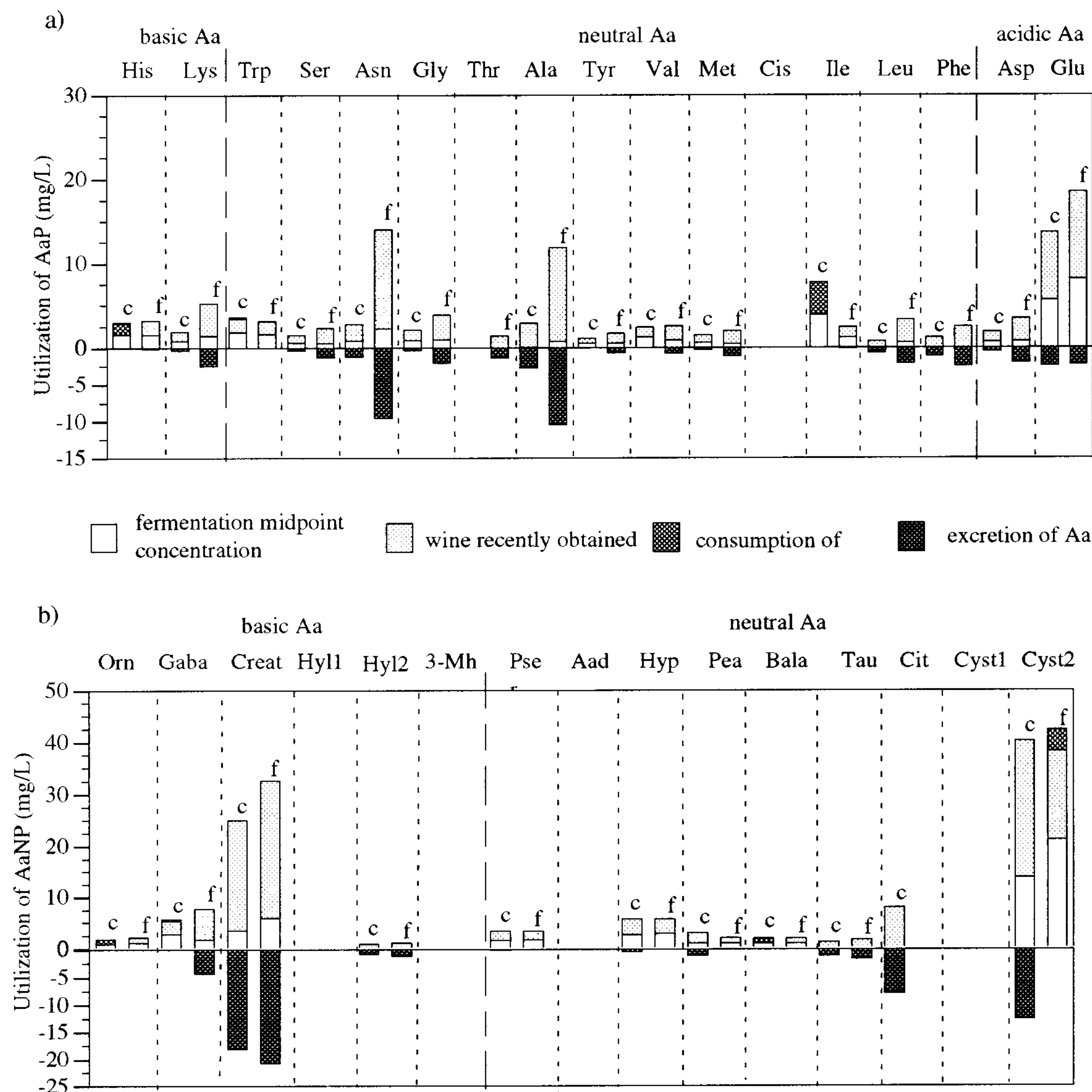

Figure 2. Concentration of amino acids in the musts at fermentation midpoint and in the wine recently obtained. Assimilation or excretion of amino acids during the second half of fermentation. Values are for comparison and not additive; (a) amino acids of proteins (AaP); and (b) amino acids not found in proteins (AaNP); (c) control must sample; (f) filtered must sample.

the lipid composition of the must and its evolution in this fermentation step, but it probably had not altered sufficiently the saturation degree of the plasma membrane of *S. cerevisiae* so as to vary importantly amino acid uptake. In contrast, vacuum filtration of Garnacha must altered the utilization of amino acids (Ancín et al., 1996a).

Utilization of Free Amino Acids and of Fatty Acids during the Second Half of Fermentation (from 50% Sugar Consumption until the End of Fermentation). The majority of amino acids were excreted in both wines in quantities independent of their initial concentrations (Figure 2); this agrees with the results of Monteiro and Bisson (1991a,b). However, excretion of these compounds was greater in the wine produced from filtered must; with the exception of glutamic acid and proline, which were liberated in similar concentrations, all AaP were excreted in greater amounts than in the control. In the control sample, histidine was consumed (Figure 2a). In the case of AaNP, an irregular evolution occurred in both samples (Figure 2b).

In the wines, the large excretion of proline was noteworthy surpassing even the initial concentration (Table 3); this agrees with results obtained for rosé wines produced from filtered Garnacha must (Ancín et al., 1996a). During the anaerobic steps of fermentation, the degradative enzyme of proline (proline oxidase) does not activate since it requires oxygen; thus, this amino acid does not degrade, but it accumulates in the cell cytosol, and when it reaches a high concentration, it is excreted to the medium (Ingledew et al., 1987; Jiranek et al., 1990; Monteiro and Bisson, 1991b).

With respect to the utilization of fatty acids in this fermentative step, there was a notable difference depending on the initial must turbidity (Ancín et al., 1996d). In wine obtained from filtered must, a smaller percentage (37.1%) was consumed than in the control (77.8%) (Tables 4 and 5). In the clarified sample, yeast utilized greater quantities of saturated acids (38.9%) than unsaturated (26.7%) in contrast to the control, where the consumption was similar (saturated, 76.9%; unsaturated, 80.1%) and greater than the clarified sample. This probably means a greater saturation of yeast plasma membrane in the filtered sample.

The final concentration of amino acids in the wine was due to excretion or reabsorption of these substances. Both processes are modified in media where yeast have

Table 4. Concentration (mg/L) of Fatty Acids in Control Viura Must, Initial, Midpoint of Fermentation, and in Wine Recently Produced ($n = 4$, All Parameters Are Given with Standard Error, SE)

	fatty acids	initial must	midpoint of fermentation	wine recently finished
medium chain	$C_{8:0}$	0.74 ± 0.03	1.34 ± 0.04	0.71 ± 0.01
	$C_{10:0}$	0.8 ± 0.02	2.5 ± 0.1	0.6 ± 0.03
	$C_{12:0}$	0.58 ± 0.05	1.9 ± 0.1	0.15 ± 0.01
	total	2.12 ± 0.06	5.74 ± 0.14	1.46 ± 0.03
long chain	$C_{13:0}$	0.07 ± 0.02	0.08 ± 0.02	0.06 ± 0.01
	$C_{14:0}$	0.32 ± 0.04	0.70 ± 0.04	0.06 ± 0.01
	$C_{15:0}$	0.006 ± 0.004	0.030 ± 0.001	0.020 ± 0.004
	$C_{16:0}$	9.7 ± 0.6	4.5 ± 0.6	0.82 ± 0.06
	$C_{18:0}$	1.42 ± 0.04	1.2 ± 0.1	0.41 ± 0.03
	$C_{20:0}$	0.53 ± 0.01	*a*	*a*
	$C_{22:0}$	0.69 ± 0.01	*a*	*a*
	$C_{16:1}$	0.64 ± 0.03	0.29 ± 0.03	0.25 ± 0.03
	$C_{18:1}$	1.9 ± 0.1	0.7 ± 0.1	0.27 ± 0.02
	$C_{18:2}$	12.3 ± 0.5	3.0 ± 0.4	0.34 ± 0.02
	$C_{18:3}$	2.5 ± 0.1	0.83 ± 0.09	0.100 ± 0.002
	total	30.07 ± 0.79	11.33 ± 0.74	2.33 ± 0.08

[a] Not detected.

Table 5. Concentration (mg/L) of Fatty Acids in Filtered Viura Must, Initial, Midpoint of Fermentation, and in Wine Recently Produced ($n = 4$, All Parameters Are Given with Standard Error, SE)

	fatty acids	initial must	midpoint of fermentation	wine recently finished
medium chain	$C_{8:0}$	0.75 ± 0.04	1.83 ± 0.02	2.3 ± 0.2
	$C_{10:0}$	0.18 ± 0.06	2.80 ± 0.04	1.4 ± 0.1
	$C_{12:0}$	0.11 ± 0.01	1.9 ± 0.1	0.39 ± 0.01
	total	1.04 ± 0.07	6.53 ± 0.11	4.09 ± 0.22
long chain	$C_{13:0}$	0.07 ± 0.01	0.16 ± 0.02	0.21 ± 0.03
	$C_{14:0}$	0.07 ± 0.01	0.27 ± 0.01	0.11 ± 0.01
	$C_{15:0}$	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
	$C_{16:0}$	2.9 ± 0.2	1.2 ± 0.2	0.7 ± 0.1
	$C_{18:0}$	0.92 ± 0.06	0.7 ± 0.1	0.29 ± 0.06
	$C_{20:0}$	*a*	*a*	*a*
	$C_{22:0}$	*a*	*a*	*a*
	$C_{16:1}$	0.24 ± 0.03	0.79 ± 0.04	0.53 ± 0.03
	$C_{18:1}$	0.29 ± 0.06	0.47 ± 0.04	0.46 ± 0.08
	$C_{18:2}$	0.36 ± 0.04	0.31 ± 0.06	0.16 ± 0.02
	$C_{18:3}$	*a*	*a*	*a*
	total	4.88 ± 0.22	3.94 ± 0.24	2.5 ± 0.15

[a] Not detected.

altered conformations of plasma membrane. Excretion will be favored in the case of wine produced from filtered must, where yeast plasma membranes will be more saturated and thus have less resistance to the toxic action of ethanol (Ingram et al., 1986). Reabsorption of amino acids from the medium will be greater in the control wine since yeast will have membranes with an adequate level of fatty acids and, as such, will have a greater tolerance to ethanol favoring reabsorption of these compounds (Ferreras et al., 1989).

CONCLUSIONS

Vacuum filtration of Viura must decreased turbidity 86% with respect to the initial sample; this involved an important elimination of fatty acids, especially unsaturateds, from the must as well as a different evolution of these compounds throughout fermentation. Although, probably, this will have produced a certain alteration in the plasma membrane of yeast, it was not sufficient to alter the uptake of amino acids during the first half of fermentation. On the contrary, in the second half of fermentation, greater concentrations of free amino acids remained in the wine produced from filtered must; this probably was due to yeast having less capacity to reabsorb these compounds and less resistance to the toxic action of ethanol.

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