# Influence of Prefermentation Clarification on Nitrogenous Contents of Musts and Wines

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A correct must clarification process improves fermentation and the quality of the wine obtained; however, if clarification is extensive, the wines obtained are of lower quality. The aim of this study was to determine the influence of two prefermentation clarification treatments (static sedimentation and vacuum filtration) on nitrogenous contents of musts (*Vitis* var. *garnacha* and *viura*), fermentation kinetics, and the resultant composition of the wines. The results show that neither static sedimentation nor filtration can alter the concentration of ammonium nitrogen, although both treatments increase amino nitrogen, and filtration decreases protein nitrogen remarkably. Consumption of amino nitrogen is not dependant on the degree of clarification, although in a more clarified must direct assimilation of amino acids (Thorne's mechanism) and other assimilation processes (Ehrlich's and Stickland's mechanisms) are possible.

**Keywords:** *Prefermentation clarification; alcoholic fermentation; nitrogenous contents; free amino acids*

## INTRODUCTION

Must is the raw material of winemaking, and any treatment used should improve the fermentation and the quality of the final product. Vinification can be done with nontreated must or sludge-free must (peels, seeds, polyphenols, tannins, etc.). The most frequent preclarification treatments (Troost, 1985) are sedimentation, clarification with separators, and vacuum filtration. Centrifugation has been used to a limited extent.

A desirable must clarification process should improve the quality of the wines, remove substances that produce unwanted flavors, favor the fermentation to dryness, and increase fermentation rates (Groat and Ough, 1978). An economic advantage is also obtained as clarification decreases waste products that have to be removed during the first rack. When clarification is too extensive, fermentation takes place with difficulty, the result often being wines with a sharp, poor, and stretched flavor. For example, in musts obtained from grapes in good condition that have been carefully harvested and immediately pressed, excessive clarification and mud separation can lead to high levels of acetic acid that destroy the quality of the wine. This takes place to a lesser extent when the must comes from grapes with botrytis or which are partially crushed (Delfini and Cervetti, 1987, 1988; Delfini and Costa, 1993). Consequently, it is of great interest to set up clarification techniques that avoid the decrease in nutrients, vitamins, and other growth factors to prevent unwanted technological results. It is especially important to study the effect of prefermentation clarification treatments on the nitrogenous components of must (ammonium ion, proteins, peptides, and free amino acids (Aa)) because they play an important role in the kinetics of fermentation and the quality of the wine and some of them are essential nutrients for the yeasts.

Ammonium ion is easily used by yeasts in metabolism, and it is rapidly consumed at the beginning of the fermentation process. Ammonium salt additions generally increase the fermentation rate. Peynaud (1984) has proposed the addition of ammonium nitrate when endogenous levels fall bellow 25 mg/L. In high-sugar musts, nitrogen supplementation may improve vigor, resulting in improved fermentation rates. Several workers have pointed out that nitrogen supplementation at these levels results in questionable increases in fermentative activity (Ingledew and Kunkee, 1985; Ingledew et al., 1987a; Zoecklein et al., 1988).

Free amino acids are very important in winemaking because they are a nitrogenous source for yeasts. Glutamic acid, arginine, serine, and alanine, together with ammonium ion, are superior nitrogenous sources for *Saccharomyces cerevisiae*. Others amino acids, on the other hand, are used with difficulty or not even used, depending on many factors such as pH, vitamins, and available oxygen (Cooper, 1982; Large, 1986). Although glycine and proline are poor nitrogen sources for yeast growth, they may serve directly or indirectly as osmoprotectants on very high gravity ethanolic fermentation by *S. cerevisiae* (Thomas et al., 1994). Usseglio-Tomasset and Bosia (1990) reported that, in the vinification of a 1984 Riesling must, the yeast completely assimilated all the Aa (except proline) after the fourth day of fermentation. In the same paper, they also reported that in vinification after maceration, in a 1984 Nebbiolo must, the yeast released nitrogen as proline and absorbed 92.8% of the remaining Aa by the fourth day of fermentation.

The aim of this study was to observe the influence of two prefermentation clarification treatments, static sedimentation and vacuum filtration, on fermentation kinetics and the nitrogenous composition of wines. Two different kinds of grapes were selected and subjected to the 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.

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## MATERIALS AND METHODS

**Materials.** *Vitis* var. *garnacha* and *Vitis* var. *viura* musts of Navarra Denomination of Origin (NDO) were collected; rosé and white wines were produced in the pilot plant. Musts and wines will be referred to by two letters and one number: the first letter stands for the cellar of origin (O, Olite; A, Arróniz), and the second corresponds to the grape variety in the must (G, *garnacha*; V, *viura*) or wine (R, rosé; W, white). The numbers indicate the prefermentation treatments: 1, control sample without treatment; 2, static sedimentation; 3, filtration with a rotary vacuum filter.

Standard solutions, for the analysis of the free Aa by HPLC, were prepared of 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., Inc. Craftsmen in Chemistry, 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 were purified through an HA 0.45 $\mu$m Millipore filter. Solvents were of HPLC quality, and reagents of analytical quality. Must was inoculated with 0.5 g/L Fermivin active dry *S. cerevisiae* from Gist brocades.

**Instruments.** A stainless crusher–stemmer, Marzola Marzinox (Marrodan and Rezola SA, Logroño, Spain), equipped with a rubber roller was used to press 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 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 $\mu$m, 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. The must's turbidity was determined using a 18900 Hach turbidimeter (Hach Co., Loveland, CO), prepared for colored samples.

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

**Methods.** *Vinification. (1) Rosé Wines.* Newly-cropped *V.* var. *garnacha* grapes were crushed and stemmed. The skins were not removed for 17 h. Must was later divided into three fractions. The first was treated with $SO_2$ (50 mg/L) but was not subjected to any prefermentation technique (treatment number 1). The other two, following refrigeration at 10 °C and the addition of $SO_2$ (50 mg/L), were clarified by two different prefermentation treatments: static sedimentation (treatment number 2) and filtration by rotary vacuum filter (treatment number 3). In static sedimentation, the must remained in stainless steel tanks for 24 h before racking. Then 400 L of the three musts was subjected to fermentation using *S. cerevisiae* (0.5 g/L) and controlled temperature. Average temperature was 17.7 °C and the standard deviation less than 2 °C. Finally, the wines were stabilized by refrigeration at −5 °C for 1 week and then filtered through a cellulose plate filter.

*(2) White Wines.* Newly-cropped *V.* var. *Viura* grapes were stemmed and crushed; the skins obtained were not removed for 5–8 h, and the same process as described above was followed for rosé vinification. Average temperature was 18 °C and the standard deviation less than 2 °C.

*Amino Acid Analysis.* The PICO·TAG method developed by Waters (Cohen et al., 1989) was followed. Samples were cleaned up 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 $\mu$L.

*Protein Nitrogen.* Must proteins were precipitated with tricholoroacetic 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 analyzed by the method described by the Office International de la Vigne et du Vin (1990), but modified by the addition of Se and $HgSO_4$ as catalysts instead of $CuSO_4$ and $K_2SO_4$. 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 performed according to the methods described by the Office International de la Vigne et du Vin (1990). 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 are shown in Table 1. Turbidity, which is a measure of the level of clarification obtained by different pretreatments, is higher for *garnacha* control must (1460 NTU) than for *viura* (695 NTU). As expected, filtered musts yielded the lowest turbidity levels, followed by static sedimentation and nontreated samples. Sugar content was hardly affected by prefermentation treatments. A small decrease in ash content was observed in those musts subjected to vacuum filtration (16.3% in *garnacha* and 11.8% in *viura* must), showing a decrease in cationic content. Ash alkalinity also decreased in both treatments, due to either sedimentation or retention in the filter of precipitated salts from organic acids.

Rosé wines reached the highest alcohol level; there was no difference in this parameter with prefermentation treatment. Volatile acidities of OR3 and AW3 wines obtained from filtered musts were 0.74 and 0.34 g of acetic acid/L, respectively, both higher than in the other wines. The high volatile acidity value in OR3 would endanger its preservation and lead to undesirable organoleptic characteristics. The increase of volatile acidity would be the consequence, among other factors, of the decrease in the fermentation rate and the accumulation of carbon dioxide. This $CO_2$ accumulation reduces long chain fatty acids to a minimum, producing an acetyl-CoA concentration that, when hydrolyzed, produces high concentrations of acetic acid. This does not happen in nonclarified musts (Delfini et al., 1992; Guilloux-Benatier and Feuillat, 1993).

Total acidity in the OR3 wine is the same as in the OG3 must (Table 1) which, together with its high volatile acidity, suggests the development of lactic bacteria that produce lactic and acetic acids from sugars while alcoholic fermentation takes place (Suarez and Leal, 1990). Microbiological assay of samples taken during the fermentative process revealed the presence of bacilli Gram (+), catalase (−), reinforcing the previous hypothesis. The development of lactic bacteria is favored in highly clarified musts by the presence of macromolecules of bacterial origin (Guilloux-Benatier and Feuillat, 1993).

**Nitrogenous Content of Musts and Wines.** Total nitrogen values (Table 2) are higher in control musts

Table 1. Characteristics of Musts and Wines (All Parameters Listed with Standard Error)

| sample | pH ± SE | turbidity (NTU ± SE) | reducing sugar (g/L ± SE) | ash (g/L ± SE) | ash alkalinity (mequiv/L ± SE) | alcohol (v/v % ± SE) | total acidity (g/L[a] ± SE) | volatile acidity (g/L[b] ± SE) | total $SO_2$ (mg/L ± SE) | free $SO_2$ (mg/L ± SE) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Musts | | | | | |
| OG1 | 3.31 ± 0.01 | 1460 ± 14 | 205 ± 1 | 2.89 ± 0.01 | 35 ± 1 | | 4.80 ± 0.03 | | 53.31 ± 0.01 | 3.1 ± 0.2 |
| OG2 | 3.30 ± 0.01 | 205 ± 5 | 202.3 ± 0.6 | 2.9 ± 0.3 | 30 ± 2 | | 5.30 ± 0.01 | | 40.77 ± 0.01 | 3.1 ± 0.2 |
| OG3 | 3.31 ± 0.01 | 66 ± 1 | 204 ± 3 | 2.42 ± 0.06 | 28.5 ± 0.5 | | 4.50 ± 0.01 | | 15.7 ± 0.8 | 1.6 ± 0.2 |
| AV1 | 3.51 ± 0.01 | 695 ± 7 | 179.7 ± 0.7 | 3.40 ± 0.06 | 42.1 ± 0.2 | | 4.11 ± 0.01 | | 25.1 ± 0.2 | 4.6 ± 0.2 |
| AV2 | 3.51 ± 0.01 | 200 ± 7 | 181.4 ± 0.5 | 3.33 ± 0.05 | 38.4 ± 0.5 | | 4.66 ± 0.01 | | 37.8 ± 0.9 | 5.3 ± 0.2 |
| AV3 | 3.47 ± 0.01 | 97 ± 3 | 181.6 ± 0.5 | 3.00 ± 0.08 | 37.9 ± 0.9 | | 4.32 ± 0.06 | | 24.8 ± 0.9 | 4.8 ± 0.2 |
| | | | | | Wines | | | | | |
| OR1 | 3.12 ± 0.01 | | 0.96 ± 0.01 | 1.06 ± 0.03 | 24.8 ± 0.4 | 12.4 ± 0.1 | 4.65 ± 0.01 | 0.27 ± 0.04 | 34.95 ± 0.01 | 3.2 ± 0.2 |
| OR2 | 3.10 ± 0.01 | | 0.99 ± 0.05 | 1.10 ± 0.01 | 24.5 ± 0.7 | 12.5 ± 0.1 | 4.95 ± 0.01 | 0.30 ± 0.02 | 37.4 ± 0.9 | 4.2 ± 0.2 |
| OR3 | 3.33 ± 0.01 | | 0.76 ± 0.02 | 1.06 ± 0.06 | 21.5 ± 0.1 | 12.8 ± 0.1 | 4.50 ± 0.01 | 0.74 ± 0.03 | 25 ± 1 | 3.2 ± 0.2 |
| AW1 | 3.35 ± 0.01 | | 0.27 ± 0.02 | 1.39 ± 0.07 | 30.0 ± 0.1 | 10.7 ± 0.1 | 3.94 ± 0.02 | 0.23 ± 0.02 | 34.9 ± 0.9 | 2.3 ± 0.3 |
| AW2 | 3.33 ± 0.01 | | 1.67 ± 0.01 | 1.16 ± 0.08 | 25.3 ± 0.3 | 10.6 ± 0.1 | 3.89 ± 0.01 | 0.27 ± 0.01 | 89.6 ± 0.9 | 3.2 ± 0.2 |
| AW3 | 3.40 ± 0.01 | | 0.13 ± 0.03 | 1.33 ± 0.02 | 28.8 ± 0.3 | 10.7 ± 0.1 | 3.28 ± 0.05 | 0.34 ± 0.04 | 65.60 ± 0.01 | 1.6 ± 0.2 |

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

than in those clarified by static sedimentation or vacuum filtration. This trend is reversed in wines since those obtained after vacuum filtration contain higher amounts than the others. Table 3 shows that percentages of nitrogen reduction during fermentation are 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 are around 52% in *viura* and 38% in *garnacha* musts.

Ammonium nitrogen concentrations in the two musts are similar, and due to the high solubility of ammonium salts, they seem to be independent of prefermentation treatment (Table 2). Ammonium almost disappears during fermentation, except in OR3 (Table 3). Yeasts preferentially incorporate ammonium to synthesize Aa, and this prevents the synthesis of general amino acid permease enzyme needed to incorporate Aa into proteins and polypeptides (Room et al., 1975; Prasad and Rose, 1986).

In the OG3 to OR3 fermentation, 24.4 mg/L ammonium nitrogen remain in the wine (Table 2); this value is much higher than the others. Ammonium nitrogen concentration was analyzed at different stages of the fermentation process, to verify if the transport of ammonium inside the yeasts finished at a certain level of ethanol concentration (Monteiro and Bisson, 1991). Ammonium nitrogen was not detected when concentration of ethanol reached 7%. The ammonium nitrogen present in OR3 could come from the ammonium released in the amino acids assimilation, either by Ehrlich's or Stickland's mechanism, through oxidation reduction. These two mechanisms are mainly used when the required Aa are not at the right levels or when they have to be synthesized from ammonium nitrogen (Usseglio-Tomasset and Bosia, 1990).

Filtering (treatment 3) considerably reduces protein nitrogen content in musts: 86.1% in OG3 and 65.8% in AV3 (Table 2). This important removal in vacuum-filtered musts is because proteins, with molecular weight between 13 000 and 65 000 Da (Yokotsuka et al., 1991), are retained in the filter. Proteins are one of the factors that favor fermentation because these macromolecules adsorb some fermentation inhibitors (Ollivier et al., 1987), for example, decanoic and octanoic acids and their corresponding ethyl esters (Lafon-Lafourcade et al., 1984).

Amino nitrogen content in filtered and sedimented musts significantly increases ($p = 0.05$) in relation to the control must (Table 2). This increase suggests that proteolysis, induced by proteases, has been produced. Proteolysis releases amino acids and small polypeptides assimilable by yeasts and may be favored by low $SO_2$ concentrations (Mareca, 1983).

**Fermentation Kinetics.** To characterize the kinetics, the process rates have been calculated from fermentation curves as an average percentage of the daily consumed sugar, in the ranges of 5−50% ($v_{f5-50}$) and 0−99% ($v_{f0-99}$) of total sugars (Houtman and Du Plessis, 1985). These results are shown in Table 3. In both vinifications, lower fermentation rates correspond to filtered musts (lower turbidity) because sludge influences yeast metabolism directly, although the mechanism is not widely known (Lafon-Lafourcade et al., 1984; Edwards et al., 1990). As for clarification by static sedimentation, the fermentation rate of *viura* must is also lower than the control must, while in the *garnacha* variety the initial fermentation rate is the same as in

**Table 2. Total Nitrogen and Nitrogen Concentrations in the Nitrogenous Fractions of Musts and Wines (All Parameters Listed with Standard Error)**

| sample | total nitrogen (mg/L ± SE) | ammonium nitrogen (mg/L ± SE) | protein nitrogen (mg/L ± SE) | amino nitrogen (mg/L ± SE) | other[a] nitrogens (mg/L) |
|---|---|---|---|---|---|
| | | | Musts | | |
| OG1 | 582.6 ± 0.6 | 107.4 ± 0.7 | 174.2 ± 0.4 | 168.2 ± 0.2 | 132.8 |
| OG2 | 553.4 ± 0.4 | 110.6 ± 0.6 | 150.0 ± 0.2 | 183.3 ± 0.3 | 109.5 |
| OG3 | 504.0 ± 0.5 | 116.0 ± 0.7 | 24.3 ± 0.1 | 183.1 ± 0.6 | 180.6 |
| AV1 | 412.6 ± 0.5 | 101 ± 10 | 80.5 ± 0.2 | 96.3 ± 0.6 | 135.2 |
| AV2 | 369.0 ± 0.5 | 110 ± 7 | 61.7 ± 0.3 | 116.9 ± 0.8 | 80.6 |
| AV3 | 332.6 ± 0.6 | 81 ± 10 | 27.6 ± 0.6 | 109.7 ± 0.5 | 114.4 |
| | | | Wines | | |
| OR1 | 197.5 ± 0.5 | 4.7 ± 0.1 | 19.8 ± 0.4 | 54.6 ± 0.3 | 118.4 |
| OR2 | 198.3 ± 0.2 | 4.7 ± 0.1 | 13.5 ± 0.7 | 54.0 ± 0.4 | 126.1 |
| OR3 | 310.5 ± 0.2 | 24.4 ± 0.1 | 13.5 ± 0.1 | 59.3 ± 0.2 | 213.3 |
| AW1 | 115.3 ± 0.3 | 2.8 ± 0.1 | 4.6 ± 0.4 | 26.8 ± 0.3 | 81.1 |
| AW2 | 100.4 ± 0.3 | *b* | 5.2 ± 0.1 | 27.4 ± 0.1 | 67.8 |
| AW3 | 160.5 ± 0.3 | 3.8 ± 0.1 | 4.4 ± 0.3 | 42.8 ± 0.1 | 109.5 |

[a] Obtained by difference. [b] Not detected.

**Table 3. Reduction of Nitrogen in the Different Fractions and Features of the Fermentation Kinetics in *garnacha* and *viura* Musts**

| | reduction during fermentation (%) | | | | sugar utilization | | fermentation |
|---|---|---|---|---|---|---|---|
| process | total nitrogen | ammonium nitrogen | protein nitrogen | amino nitrogen | $v_{f5-50}$[a] (%/days) | $v_{f0-99}$[b] (%/days) | time (days) |
| OG1–OR1 | 66.1 | 95.6 | 88.6 | 67.5 | 31.7 | 16.5 | 9 |
| OG2–OR2 | 64.2 | 95.8 | 91.0 | 70.5 | 31.7 | 10.0 | 10 |
| OG3–OR3 | 38.4 | 79.0 | 44.4 | 67.6 | 15.7 | 3.8 | 29 |
| AV1–AW1 | 72.1 | 97.2 | 94.3 | 72.2 | 31.8 | 14.1 | 16 |
| AV2–AW2 | 72.8 | 100.0 | 91.6 | 76.6 | 22.5 | 5.6 | 21 |
| AV3–AW3 | 51.7 | 95.3 | 84.1 | 60.9 | 14.0 | 4.3 | 25 |

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

the nonclarified ones but the rate slows down later on and gives a lower overall rate.

Reduction in the fermentation rates in filtered musts is not due to the deficiency of assimilable nitrogen. In fact, concentration of ammonium nitrogen in musts is similar and concentration of amino nitrogen is higher in the musts that have lower fermentation rates (Tables 2 and 3). When yeasts are devoid of nitrogen, the intracellular AMP/ATP ratio increases and inhibits sugar phosphorylation during glycolysis (Dombek and Ingram, 1986, 1988).

Sludge did not stimulate fermentation by providing assimilable nitrogen. The mechanism of such stimulation is not, however, clearly understood. Thomas et al. (1994) have suggested that particulate materials may serve as nucleation sites for the formation of $CO_2$ bubbles. When they reach a certain size, the bubbles escape into the atmosphere, thereby minimizing the inhibitory effect of this metabolic product on yeast growth. Another beneficial effect of particulate materials, put forward in the same paper, is the enlargement of the inner surface of the fermenting medium.

Protein nitrogen content decreases throughout the fermentative processes, and since it can not be assimilated by yeast, its reduction (Table 3) must be due to precipitation.

**Influence of Prefermentation Treatments in the Content of Free Aa.** *Aa in Musts.* The effect of prefermentative treatment in the concentration of amino acids in musts shows different trends, depending on the kinds of musts, and whether amino acids are found in proteins (AaP) or not found in proteins (AaNP).

In musts obtained from *garnacha* grapes that were in contact with skins for 17 h, AaP content increases in both treatments in relation to the control must (Figure 1a). There is a good correlation between AaP content in treatment 2 vs 1 (OG2 = 0.96OG1 + 5.6; $r^2 = 0.875$) and in treatment 3 vs 1 (OG3 = 1.22OG1 + 4.5; $r^2 = 0.718$). This increase is probably due to the hydrolytic activity of proteases of vegetable origin that appear in musts, released by the berry's pulp. This release of amino acids should increase the fermentation rates according to the literature (Monteiro and Bisson, 1991); however, an increase does not occur in this case (Table 3), probably because the AaP concentration is not the only restrictive factor in the kinetics of the process. Regarding AaNP (Figure 2a), treatment 2 hardly modifies AaNP content in comparison to treatment 1. Treatment 3 only raises the Gaba significantly. There is a good correlation between those AaNP concentrations for treatment 2 vs 1 (OG2 = 0.93OG1 + 0.15; $r^2 = 0.990$) and treatment 3 vs. 1 (OG3 = 1.44OG1 − 1.83; $r^2 = 0.974$).

Concentration of AaP in musts obtained from *viura* grapes subjected to pretreatments 2 and 3 is slightly higher than in the control must (Figure 1c). The variation in AaP concentrations in treatment 2 vs 1 is AV2 = 1.09AV1 + 0.06 ($r^2 = 0.988$) and for treatment 3 vs 1, AV3 = 1.23AV1 − 1.56 ($r^2 = 0.821$). The increase in AaP is smaller than that obtained in *garnacha* must; this could be attributed to a lower protease concentration in white vinification since the contact time between the must and the berry is lower (5–8 h). The small increase in AaP is not related to a higher fermentation rate (Table 3). Regarding AaNP concentration (Figure 2c), treatments 2 and 3 hardly modify the content in comparison to the control must: AV2 = 1.24AV1 − 0.32 ($r^2 = 0.974$) and AV3 = 0.97AV1 − 0.22 ($r^2 = 0.867$).

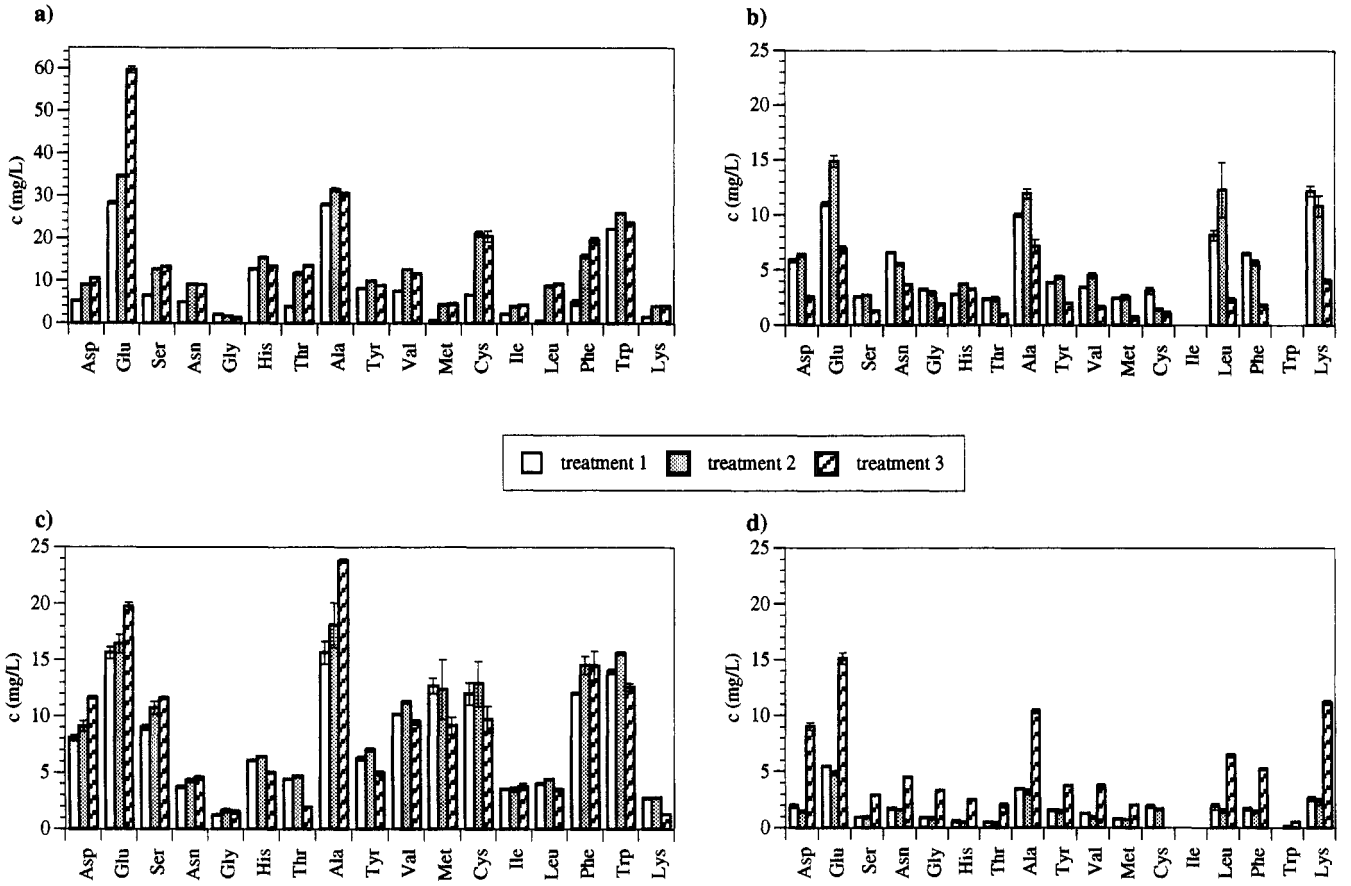


**Figure 1.** Free Aa found in proteins (AaP) of musts clarified by different prefermentation treatments and the corresponding wines: (a) musts from *V.* var. *garnacha*, (b) wine from *V.* var. *garnacha*, (c) musts from *V.* var. *viura*, and (d) wine from *V.* var. *viura*. (Arginine and proline are not included due to their high concentration.)

*Aa in Wines.* AaP contents are slightly more abundant in OR2 than in the control wine, as shown in Figure 1b. Regression analysis of AaP content in treatment 2 vs 1 gives OR2 = 1.15OR1 − 0.36 ($r^2$ = 0.842), which means that they follow the trend observed in the musts. In fact, the slope of both equations is similar and close to the unit. However, in the wine obtained from filtered must, AaP concentration (Figure 1b) is much lower than in the control wine (OR3 = 0.44OR1 + 0.25; $r^2$ = 0.613) and the data are not as well correlated as in previous cases.

AaNP content of the OR2 wine is generally lower than those in the control wine (Figure 2b), and the correlation is worse than in previous cases (OR2 = 0.53OR1 + 1.37; $r^2$ = 0.576). We found no correlation in AaNP concentrations between OR3 and OR1 Aa ($r^2$ = 0.157).

OR3 presents some anomalies in ornithine, arginine, and proline content when compared to OR1 and OR2 (Figure 2b and Table 4). Ornithine concentration in OR3 is significantly higher ($p$ = 0.05) than in other wine samples. The hydrolytic activity of lactic bacteria produces ornithine that blocks the transport of other essential Aa's like arginine (Suarez and Leal, 1990). This blocking could explain why only 68.8% of arginine was consumed in the process of OG3 to OR3, while arginine is consumed above 99% in the fermentative processes of OG1 to OR1 and OG2 to OR2. Arginine is an easily metabolizable Aa; yeasts use all four of its nitrogens for biosynthesis (Cooper, 1982; Monteiro and Bisson, 1991; Usseglio-Tomasset and Bosia, 1990). Proline concentration increases during the fermentation processes of OG1 to OR1 and OG2 to OR2 but decreases in the OG3 to OR3 process (62.5%). Proline is not taken up when the must has sufficient nitrogen, is also an excretion product of the catabolism of the yeasts (Usseglio-Tomasset and Bosia, 1990), and is only used under aerobic conditions (Ingledew et al., 1987b). To explain this anomaly, proline concentration along the fermentative process was analyzed. Proline was excreted in the three fermentative processes; the maximum values reached were OG1, 373 mg/L; OG2, 522 mg/L; and OG3, 223 mg/L. After stabilization, there was a consumption of proline reaching the values given in Table 4 for wines.

AaP concentration in white wine obtained from sedimented must is similar to the control wine, and as shown in Figure 1d, there is a good correlation between both results (AW2 = 0.90AW1 − 0.04; $r^2$ = 0.984). The concentration of AaP in white wine from filtered must (Figure 1d) is higher than in the control wine (AW3 = 2.94AW1 + 0.49; $r^2$ = 0.910); this is the same tendency as observed in musts. Table 4 shows that arginine is consumed almost completely in the fermentative processes of AV1 to AW1 and AV2 to AW2 and that proline concentration does not change from musts to wines.

Figure 2d shows that AaNP content in white wines obtained from sedimented must is similar to the control one, except Cyst2, which is higher in AW2 (AW2 = 1.46AW1 − 0.52; $r^2$ = 0.961). In white wines obtained from filtered musts, AaNP content is also similar to control wine (AW3 = 1.13AW1 + 0.49; $r^2$ = 0.896); the exceptions are Tau, Gaba, Creat, and Orn.

It can be concluded that the total nitrogen values were higher in control musts than in those clarified by static sedimentation or vacuum filtration. The two clarification treatments did not reduce the concentration of

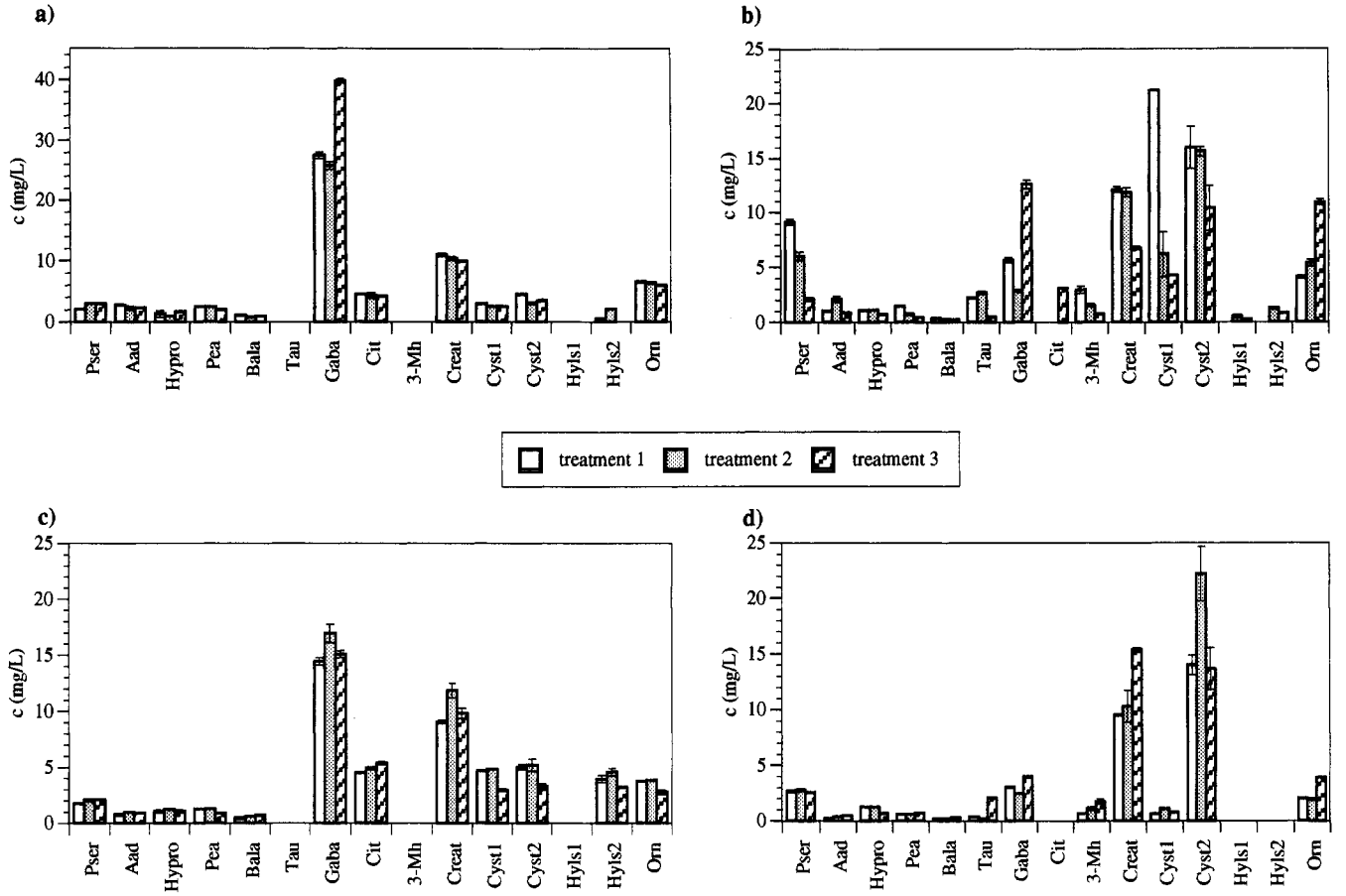


**Figure 2.** Free Aa not found in proteins (AaNP) of musts clarified by different prefermentation treatments and the corresponding wines: (a) musts from *V.* var. *garnacha*, (b) wine from *V.* var. *garnacha*, (c) musts from *V.* var. *viura*, and (d) wine from *V.* var. *viura*.

**Table 4. Concentration (Milligrams per Liter ± SE) of Arginine and Proline in Musts and Wines**

| | musts | | | | | | wines | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aa | OG1 | OG2 | OG3 | AV1 | AV2 | AV3 | OR1 | OR2 | OR3 | AW1 | AW2 | AW3 |
| Arg | 358 ± 11 | 372 ± 16 | 358 ± 8 | 148 ± 11 | 191 ± 16 | 179 ± 8 | 2.60 ± 0.01 | 1.90 ± 0.01 | 112 ± 3 | 0.80 ± 0.01 | 0.20 ± 0.01 | 0.8 ± 0.1 |
| Pro | 174 ± 10 | 173 ± 13 | 175 ± 7 | 147 ± 10 | 177 ± 14 | 197 ± 7 | 239 ± 15 | 266.9 ± 0.7 | 66 ± 8 | 136 ± 15 | 134.5 ± 0.7 | 185 ± 6 |

nitrogen that can be assimilated by the yeasts (ammonium and amino nitrogen). In fact, ammonium nitrogen concentration in clarified musts was similar to that in the control musts, and amino nitrogen concentration was significantly higher ($p = 0.05$). The vacuum-filtered musts present the slowest fermentation rates. Neither did we find indications that the sludges stimulate the fermentation rate, transferring assimilable nitrogen to the medium. The vacuum filtration reduced appreciably the protein nitrogen of the musts. The two pretreatments increased in musts the concentration of amino acids found in proteins (AaP), probably as a result of the action of vegetable proteases which pass into the must from the pulp. This increase in amino acids did not mean an increase in the must fermentation rate. Regarding the amino acids not found in proteins (AaNP), they were hardly modified by both treatments with respect to the control musts. Anomalies were observed in the evolution of ornithine, arginine and proline in the fermentation process of the most clarified *garnacha* must. Moreover, it appears that other amino acid assimilation processes occur which are different from direct assimilation (Thorne's mechanism).

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