Industrial Wine Making: Comparison of Must Clarification Treatments

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Three solid−liquid separation techniques, vacuum filtration, centrifugation, and settling, were used prior to fermentation to clarify musts from two different white grape varieties (*Vitis vinifera* var. Parellada and Macabeo) on an industrial scale. Solid content and ammonium and amino acid concentrations were analyzed before and after clarification, and sugar, ethanol, glycerol, *n*-propyl alcohol, isoamyl alcohol, and ethyl acetate compositions were monitored during the fermentations to determine how the clarification treatment affected the fermentation rate and higher alcohol production. The results show that vacuum filtration gives musts with the lowest solid content, and this means longer fermentation time, lower isoamyl alcohol production, and greater ethyl acetate formation. However, the three industrial clarification techniques studied caused no significant differences in the initial amount of ammonium nitrogen and total free amino acids.

Keywords: *Settling; centrifugation; vacuum filtration; higher alcohol; solid content*

INTRODUCTION

Must clarification is a very important operation for improving the quality of a white wine. Therefore, it should remove substances that produce unwanted flavors, favor the fermentation to dryness, and increase fermentation rates (Groat and Ough, 1978). An adequate must clarification has a favorable effect on wine aroma, due to alcohol production decreases and ester concentration increases (Groat and Ough, 1978; Van Wyk, 1978; Houtman et al., 1980; Houtman and du Plessis, 1986; Ancín et al., 1996). Nevertheless, a critical amount of suspended grape particulate is required for fermentation and ester production (Houtman et al., 1980) because if clarification is too extensive, it is difficult for fermentation to take place and the result is often wines with a sharp, poor, and stretched flavor.

It is well-known that must clarification slows fermentation (Groat and Ough, 1978; Van Wyk, 1978; Houtman et al., 1980; Houtman and du Plessis, 1986). This is because essential substances for yeast nutrition are removed during clarification. The effect of prefermentation clarification treatments on the nitrogenous components of must, vitamins, and other growth factors such as sterols has been studied in previous works. The main conclusions of these studies were that vitamins such as thiamin, pantothenic acid, and biotin are necessary if stalled fermentations are to be prevented, while yeasts need lipid and sterol unsaturations to maintain their growing capacity (Van Wyk, 1978; Houtman et al., 1980, 1981, 1986; Tromp, 1984; Ough et al., 1989; Trioli and Paronetto, 1992; Delfini et al., 1993; Ayestarán et al., 1995).

Tromp (1984) found that adding nitrogenous components increases the fermentation rate. On the other hand, Ayestarán et al. (1995) observed no differences in ammonium nitrogen concentration between clarified and unclarified musts, so a deficient nitrogen source was not the reason fermentation slowed when clarification was more rigorous.

Vinifications are frequently carried out on a smaller scale to find out what effects different treatments have on fermentation rates and the quality of the final product. The relevance of the results produced by scaled down fermentations is often questioned. It is well-known that it is difficult to maintain anaerobic conditions in small fermentations due to the large surface to volume ratio. Moreover, due to the size of the fermenting vessels, fewer volatile compounds are retained in a smaller fermenter (Ewart and Sitters, 1991; Güell and López, 1995). Güell and López (1995) reported that isoamyl and *n*-propyl alcohols are produced in greater quantities in laboratory scale fermentations and that less ethyl acetate and glycerol are produced.

The aim of this work was to observe and study, on an industrial scale, the influence of three prefermentation clarification treatments on the removal of solids and nitrogenous compounds, fermentation rates, and production of volatile compounds. Two white grape varieties, Macabeo and Parellada, were selected, and the clarification techniques compared were settling, vacuum filtration, and centrifugation. Solid content was obtained before and after each clarification treatment for both varieties, whereas amino acid concentration was analyzed before and after each treatment only for Macabeo musts. Fermentations of settled, filtered, and centrifuged musts were monitored to obtain data during fermentations on sugar and ethanol evolution and higher alcohol and ester production. The results of this study will enable the effectiveness of the most commonly used clarification treatments in Spanish cellars (settling and vacuum filtration) to be compared with centrifugation, which combines continuous operation and a small solid residue production.

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MATERIALS AND METHODS

Materials. *Vitis vinifera* var. Parellada and Viura (Macabeo) grapes from the 1994 harvest in the Tarragona Appellation were collected, and white wine vinification processes were carried out in a commercial cellar (Cooperativa Vila-Rodona, Alt Camp, Spain).

Equipment. The characteristics of the three sets of equipment used to clarify the musts are as follows: *static sedimentation*, stainless steel tanks with a capacity of 1200 hL; *vacuum filtration*, rotary vacuum filter (Cadalpe model 4258) with a surface filtration area of 30 m^2, suspension of perlites (3-4 Darcy) to build the filtration cake (average earth consumption = 1−1.5 kg/hL); *centrifugation*, carried out in a hermetic clarifier SC35 Westfalia centrifugal separator, with an automatic evacuation system for the sludge and a bowl volume of 18 L (flow rate ranged from 5000 to 7000 L/h, rotation velocity was 10000 G).

Industrial Vinification. Newly cropped Parellada and Macabeo grapes were crushed and pressed in a Willmess pneumatic press (50 000 kg capacity). The operation (prepress, press, and draining) lasted 4.5 h. The best quality must, which was given by a pressure of <0.2 atm, was used in the present study. The must was treated with SO_2 (5−7 g/hL) and clarified using one of the prefermentation treatments mentioned above.

Must clarified by filtration and centrifugation was previously collected in a stainless steel tank and continually fed to both sets of equipment. After prefermentation treatments, 460−470 hL of clarified must was fermented using a *Saccharomyces cerevisiae* inoculum (0.125 g of active dry yeast/L) while the temperature was maintained at 18 ± 1 °C. Ammonium biphosphate (10 g/hL) (Laffort & Cia S.A.) and a vitamin mixture with thiamin as the principal component (Actiferment, Laffort & Cia S.A.) (3 g/hL) were added to each tank prior to fermentation. The wine was racked after 50% of the sugar had been consumed, and fermentation was considered to be over when the total concentration of reducing sugars was <3 g/L. The wine was finally racked after fermentation, and the wines were sensorially evaluated to detect possible spoilage.

Methods. The total acidity, pH, and glycerol and sugar concentrations were determined in must before it was clarified. The total acidity was determined using AOAC method 962.12 (AOAC, 1984). Glycerol was determined using an enzymatic method (Ough and Amerine, 1988), and the initial sugar content was analyzed using the same liquid chromatographic procedure described below for fermentation samples.

To determine the solid content before and after each clarification treatment, five samples of 5 mL for unclarified musts and 15 mL for clarified musts were filtered (Whatman filter paper no. 1441110) and then dried (T = 70 °C) until of constant weight. The must's turbidity was determined using a 18900 Hach ratio turbidimeter (Hach Co., Loveland, CO).

Glucose, fructose, ethanol, and glycerol were analyzed by HPLC using a Beckman System Gold high-pressure liquid chromatograph, with a Spherogel carbohydrate column (7.5 mm × 30 cm) in an oven at 85 °C. The mobile phase was water with a flow rate of 0.45 mL/min. Detection was carried out using a refractive index detector at 20 °C. The amount of sample injected was 20 μL. Samples were cleaned up by centrifugation (10 000 rpm) and filtered with a 0.45 μm membrane before injection.

Ethyl acetate and *n*-propyl and isoamyl alcohol were determined using a Hewlett-Packard 5890 gas chromatograph, equipped with a Carbowax 1500 (4 m × $^1/_8$ in.) column and an FID detector. Nitrogen was used as mobile phase at a flow rate of 40 mL/min. The oven, the injector, and the detector temperatures were 80, 200, and 220 °C, respectively. Samples were centrifuged at 10 000 rpm before injection, and 4-methyl-2-pentanol (Aldrich 10991-6) was added as an internal standard.

Amino acid and ammonium nitrogen were determined with ionic exchange chromatography using the method of Spackman et al. (1958). A Pharmacia LKB Biotechnology chromatograph equipped with a polystyrene/divinylbenzene sulfonate column (200 × 4 mm) was used. Various lithium citrate buffers (Pharmacia Biotechtechnology) were used as the mobile phase to increase pH during analysis and improve the separation. The eluted sample was derived with ninhydrin (Fluka 72491) at 135 °C to form colored derivatives. These compounds have maximum absorption around 570 and 440 nm for amino acids and imino acids, respectively. Norleucine (Sigma N1398) was used as the internal standard. Before analysis, the samples were cleaned up by centrifugation (10 000 rpm), filtered through a 0.45 μm Millipore membrane, and ultrafiltered using a 10 000 Da membrane.

Four industrial vinifications, using the procedure described under Industrial Vinifications, were carried out for each variety: one for settled must, one for centrifuged must, and two for filtered must. Must samples of 1.5 L were immediately analyzed before and after each clarification. During fermentation, samples were collected and frozen every 12 h during the first 3 days and, after that, every 24 h.

Table 1. Effect of the Clarification Treatment on Solid Content, Turbidity, Free Amino Acids, and Ammonium Nitrogen

clarifn treatment	turbidity (NTU)		solid content (g/L ± SE)		fermentn time (h)
	before	after	before	after	
Parellada					
vac filtrn 1	1980	96	7.1 ± 0.1	1.8 ± 0.2	264
vac filtrn 2	1830	63	8.4 ± 0.3	1.3 ± 0.1	168
centrifn	1755	1065	6.2 ± 0.4	5.6 ± 0.2	120
settling	1290	760	6.6 ± 0.2	3.6 ± 0.3	168
Macabeo					
vac filtrn 1	1410	128	10.6 ± 0.4	1.7 ± 0.1	372
vac filtrn 2	1395	240	10.1 ± 0.3	1.9 ± 0.2	444
centrifn	1260	750	10.0 ± 0.7	2.8 ± 0.1	252
settling	1860	825	11 ± 2	3.7 ± 0.3	156

clarifn treatment	free amino acids (mM ± SE)		ammonium N (mM ± SE)	
	before	after	before	after
Macabeo				
vac filtrn 1	4.5 ± 0.2	4.2 ± 0.2	1.8 ± 0.2	1.8 ± 0.2
vac filtrn 2	4.0 ± 0.2	4.5 ± 0.2	1.8 ± 0.2	1.8 ± 0.2
centrifn	4.6 ± 0.2	4.8 ± 0.2	2.1 ± 0.3	1.8 ± 0.2
settling	5.0 ± 0.2	4.9 ± 0.2	1.8 ± 0.2	1.9 ± 0.2

RESULTS AND DISCUSSION

For each of the two varieties studied, four different musts were used because the grapes were harvested on four different days (due to the industrial scale of the experiment). Therefore, we give data for each of the four different batches for both varieties. The general parameters determined in the cellar were total acidity, pH, and glycerol. The pH and total acidity of the different must batches ranged from 3.36 to 3.54 and from 2.3 to 3.9 g/L (sulfuric acid), respectively. The glycerol content of the initial must batches was low except the high glycerol values of the Parellada must batch (0.95 g/L) used in vacuum filtration 2. This suggests the presence of grapes infected with *Botrytis cinerea*, which produces glycerol and gluconic acid from tartaric acid (Peynaud, 1984). Sugar content, determined by HPLC, was higher in the Macabeo must batches (197.7 ± 7.2 g/L) than in the Parellada batches (158.6 ± 7.7 g/L).

Influence of the Clarification Treatments on Must, Wine, and Fermentation Time. Table 1 shows that vacuum filtration is the clarification treatment that leads to the lowest solid content for both Macabeo and Parellada musts (removal of ≈80%). Free amino acid and ammonium nitrogen concentrations were determined only in Macabeo musts before and after clarifica-

Table 2. Final Concentration (± Standard Error) of Ethanol, Glycerol, *n*-Propyl Alcohol, Isoamyl Alcohol, and Ethyl Acetate for Wines Obtained with Musts at Different Levels of Clarification

	vac filtrn 1	vac filtrn 2	centrifn	settling
	Parellada			
ethanol (g/L)	74 ± 1	72 ± 2	79 ± 5	67.0 ± 0.7
glycerol (g/L)	3.9 ± 0.1	4.1 ± 0.1	4.5 ± 0.3	2.4 ± 0.1
n-propyl alcohol (mg/L)	18.6 ± 0.3	15.0 ± 0.4	27 ± 2	12 ± 3
isoamyl alcohol (mg/L)	76 ± 2	75.5 ± 0.3	97 ± 3	99 ± 3
ethyl acetate (mg/L)	32 ± 2	35 ± 2	17 ± 2	20.7 ± 0.3
	Macabeo			
ethanol (g/L)	80 ± 2	84 ± 2	84 ± 2	76.0 ± 0.4
glycerol (g/L)	4.3 ± 0.1	4.1 ± 0.1	4.8 ± 0.1	5.2 ± 0.1
n-propyl alcohol (mg/L)	10.9 ± 0.5	14.7 ± 0.6	10.8 ± 0.8	7.4 ± 0.5
isoamyl alcohol (mg/L)	93 ± 3	87 ± 2	139 ± 2	162 ± 4
ethyl acetate (mg/L)	23 ± 2	37.1 ± 0.4	11.8 ± 0.1	13.5 ± 0.6
threonine (μM)	123 ± 3	149 ± 3	116 ± 3	134 ± 3
leucine (μM)	31 ± 6	37 ± 7	68 ± 13	44 ± 6

tion (Table 1). The results show that the initial concentrations of the different musts vary slightly. This is probably due to the fact that different quantities of nitrogen are used in fertilization, which give different amounts of free amino acids and ammonium nitrogen in grapes (Sarimento et al., 1992). From the values in Table 1 it can be seen that there are no substantial differences between the ammonium nitrogen concentrations of clarified and unclarified musts, whichever clarification treatment was used. Therefore, the prefermentation treatment did not affect the initial ammonium nitrogen content. The main consequence of the previous results is that solid content and contact time between solids and must have no influence on initial free amino acid concentration. These results partially agree with those of Ayestarán et al. (1995), who found no substantial differences in the concentration of free amino acids that were not associated with proteins. Thus, the results obtained for Macabeo must during the present work show that, although vacuum filtration removes solids more rigorously, none of the industrial clarification techniques studied cause significant differences in the initial amount of ammonium nitrogen and total free amino acids. Table 1 presents the total fermentation time of wines from the two varieties and the three clarification methods studied. Significantly ($p = 0.05$) longer fermentation times were obtained for those must batches clarified using vacuum filtration. Hence, fermentation time increased as a consequence of a decrease in the solid content of the must. It should be noted that the filtered (filtration 2) Parellada must has an irregular and inexplicably short fermentation time.

Table 2 shows that the isoamyl alcohol concentration was significantly ($p = 0.05$) lower in wines from filtered must, independent of the variety studied. Second, the ethyl acetate content in wine was significantly ($p = 0.05$) higher when musts were treated by vacuum filtration. Finally, the results obtained in the present work indicate that the clarification treatment does not affect *n*-propyl alcohol production. The results for *n*-propyl alcohol and isoamyl alcohol match those obtained by Ancín et al. (1996) on a pilot plant scale. Figure 1 shows the ethyl acetate and isoamyl alcohol concentrations versus solid content for all of the musts studied. As can be seen from this plot, those musts that have a higher solid content and which are clarified by settling and centrifugation tend to produce more isoamyl alcohol and less ethyl acetate in both Macabeo and Parellada wines. In fact, a correlation can be established between the initial solid content of the musts and the final isoamyl alcohol and ethyl acetate contents in wines. As initial solid concentration increases, isoamyl alcohol production increases and ethyl acetate synthesis decreases.

Effect of the Clarification Treatment on Higher Alcohol Formation. It is interesting to study the effects of the clarification treatment on the formation kinetics of *n*-propyl, isoamyl alcohol, ethyl acetate, and glycerol. However, it is difficult to find out which mechanism is associated to a particular biosynthetic pathway, which means that it is not possible to find a single mathematical model to describe the kinetics. For these reasons an empirical kinetic model was chosen to compare *n*-propyl, isoamyl alcohol, ethyl acetate, and glycerol evolution during fermentation. This model defines two dimensionless parameters, t_R and P_R, where $t_R = t/t_F$ and $P_R = P/P_F$, with t_F and P_F being the final fermentation time and the product concentration at the end of fermentation, respectively. Dimensionless parameters are defined to compare the production rates of volatile compounds in fermentations of different lengths and levels of production. If the curve that fits

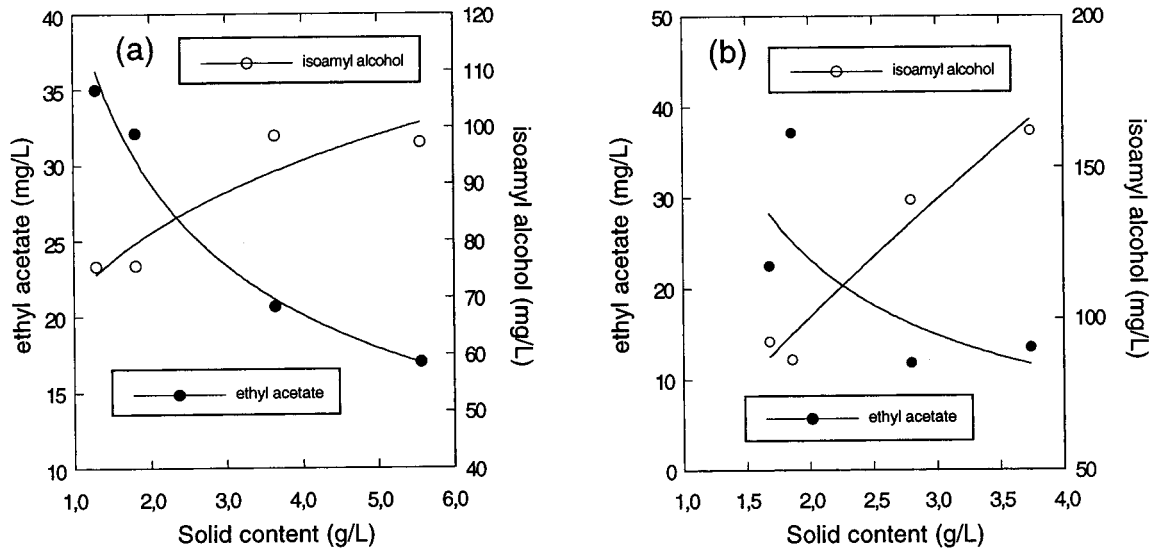

Figure 1. Final ethyl acetate and isoamyl alcohol concentrations attained by Macabeo (b) and Parellada (a) musts as a function of the initial solid content.

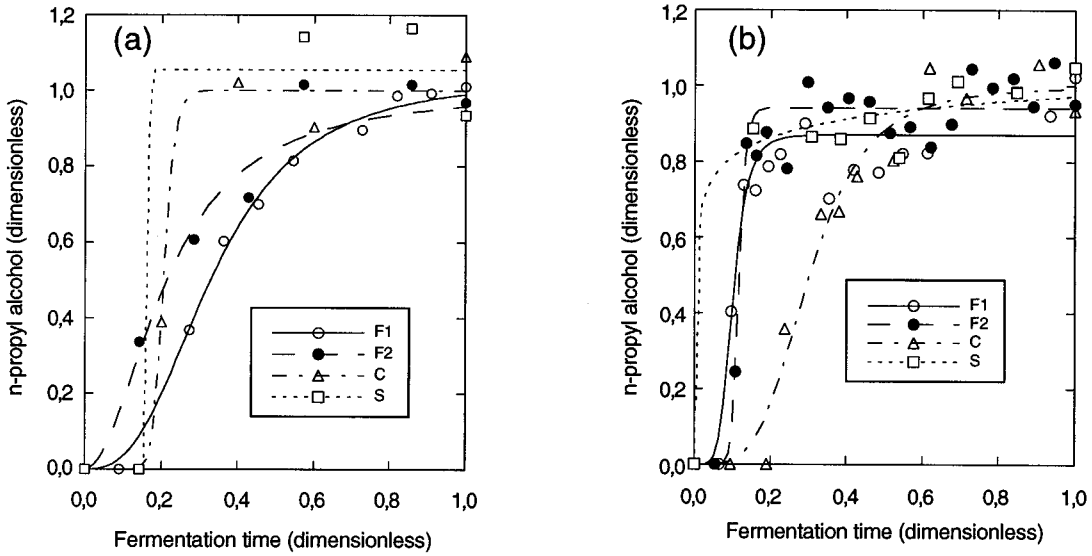

Figure 2. Dimensionless *n*-propyl alcohol concentration vs dimensionless time obtained during the fermentation of musts clarified using vacuum filtration (F), centrifugation (C), and settling (S) for the two white grape varieties studied: (a) Parellada; (b) Macabeo.

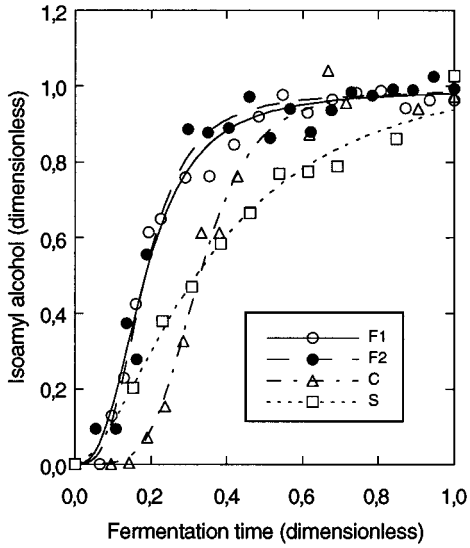

Figure 3. Dimensionless isoamyl alcohol concentration vs dimensionless time obtained during the fermentation of Macabeo musts clarified using vacuum filtration (F), centrifugation (C), and settling (S).

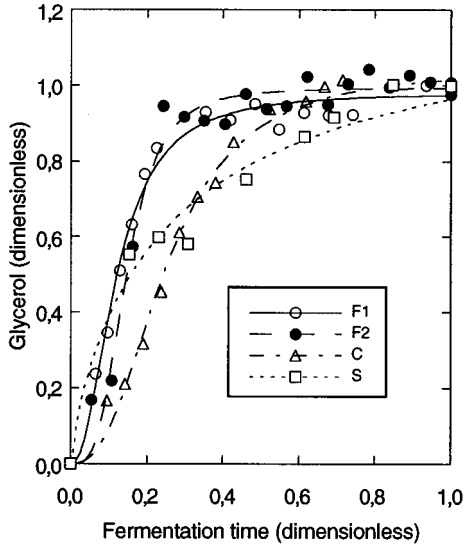

Figure 4. Dimensionless glycerol concentration vs dimensionless time obtained during the fermentation of Macabeo musts clarified using vacuum filtration (F), centrifugation (C), and settling (S).

the model has the same shape, the treatment can be expected to have no effect on the mechanism of formation. Moreover, the chosen model shows that P_R dependence on t_R can be described by a Moser-like equation (Atkinson, 1986)

$$P_R = P_{Rf}[(k_3 t_R)^m/[1 + (k_3 t_R)^m]] \quad (1)$$

where P_{Rf}, k_3, and m are empirical constants.

The model was previously tested with data about *n*-propyl, isoamyl alcohol, ethyl acetate, and glycerol formation during the laboratory and industrial scale fermentation of Macabeo and Parellada musts from the 1993 harvest (Güell and López, 1995). It was found that the model correlated with the data obtained during these fermentations (data not shown).

Figure 2 plots the dimensionless *n*-propyl concentration versus dimensionless time for both Macabeo and Parellada must fermentations. The symbols are the experimental data points obtained during industrial fermentations, and the lines are the correlation of these data points to eq 1. The profiles in Figure 2a show that, for Parellada musts, *n*-propyl forms throughout the fermentation in filtered must, while for settled and centrifuged musts *n*-propyl alcohol formation reaches its maximum values for $t_R = 0.2$. These results show that a lower initial solid content causes *n*-propyl alcohol to form more slowly. During the fermentation of Macabeo musts, *n*-propyl alcohol formation shows a different trend. All Macabeo wines, except for the centrifuged must, reach a maximum *n*-propyl alcohol concentration at the beginning of fermentation. The rate of *n*-propyl alcohol formation for the filtered musts of both varieties is seen to depend on the initial solid content.

Figures 3 and 4 plot the formation of isoamyl alcohol and glycerol for Macabeo wines, respectively. They show that the clarification treatment affects the formation rate of both compounds. The rate of isoamyl alcohol and glycerol formation is faster for filtered musts, and maximum concentration is reached in half the fermen-

Table 3. Contribution of the Different Ammonium Compounds to the Total Nitrogen Consumption during the First 48 h of Macabeo Must Fermentation

		concn (μM)		
compd	contribn (%)	$t = 0$ h	$t = 48$ h	consumpn (%)
		Vacuum Filtration 1		
NH_4	56.9	3292.9	590.0	82.1
Arg	13.0	884.7	264.8	70.1
Gaba	7.0	416.9	83.3	80.0
Ala	6.3	405.8	104.5	74.3
Glu	3.2	187.3	37.1	80.2
Gln	2.8	166.8	33.0	80.2
Ser	2.6	162.2	39.1	75.9
Asp	2.1	143.6	43.1	70.0
Thr	2.0	123.1	27.8	77.4
Val	1.3	128.1	64	49.9
His	0.8	49.6	12.9	73.9
Phe	0.7	39.2	4.5	88.5
Ile	0.6	30.3	0.0	100.0
Leu	0.6	30.5	2.9	90.6
total		6060.9	1306.9	
		Centrifugation		
NH_4	43.9	3247.8	1359.5	58.1
Arg	14.2	911.7	303.0	66.8
Ala	9.9	453.9	26.5	94.2
Glu	8.5	397.0	31.7	92.0
Val	3.7	159.5	0.0	100.0
Asp	3.8	180.5	18.1	90.0
Ser	3.5	163.7	11.2	93.2
Gaba	2.9	426.5	303.4	28.8
Thr	2.5	115.8	10.3	91.1
Lys	1.9	95.2	13.5	85.8
Leu	1.6	68.3	0.0	100.0
Phe	1.3	61.6	7.8	87.4
His	1.3	54.2	0.0	100.0
Gly	1.1	84.4	36.0	57.3
total		6419.9	2121.1	
		Settling		
NH_4	51.5	3436.0	135.2	96.1
Arg	15.3	992.5	14.2	98.6
Ala	7.7	505.9	13.7	97.3
Gaba	7.6	488.9	0.7	99.9
Glu	3.7	254.1	16.5	93.5
Ser	2.7	182.4	7.5	95.9
Gln	2.6	166.0	0.0	100.0
Asp	2.2	151.7	11.2	92.6
Thr	2.1	134.3	0.0	100.0
Val	1.7	152.6	43.5	71.5
His	0.9	56.2	0.0	100.0
Leu	0.7	44.4	0.0	100.0
Phe	0.7	47.4	5.1	89.2
Gly	0.6	50.8	10.6	79.2
total		6663.2	258.2	

tation time. In centrifuged musts, both alcohols form more slowly and reach their maximum at approximately $t_R = 0.6$. Finally, for settled musts (higher solid content), isoamyl alcohol and glycerol form throughout the fermentation and reach their maximum for $t_R = 1$. In Parellada musts, the clarification treatment was not seen to affect the formation rate of isoamyl alcohol and glycerol (data not shown).

The order in which the different nitrogen sources are depleted during the first 48 h of Macabeo must fermentations has been calculated. The contribution factor was determined as the ratio between the amount of a specific nitrogen source used and the total amount of nitrogen. The data in Table 3 show that there is a preference for ammonium nitrogen and for the amino acids arginine and alanine. So, on an industrial scale, the usual

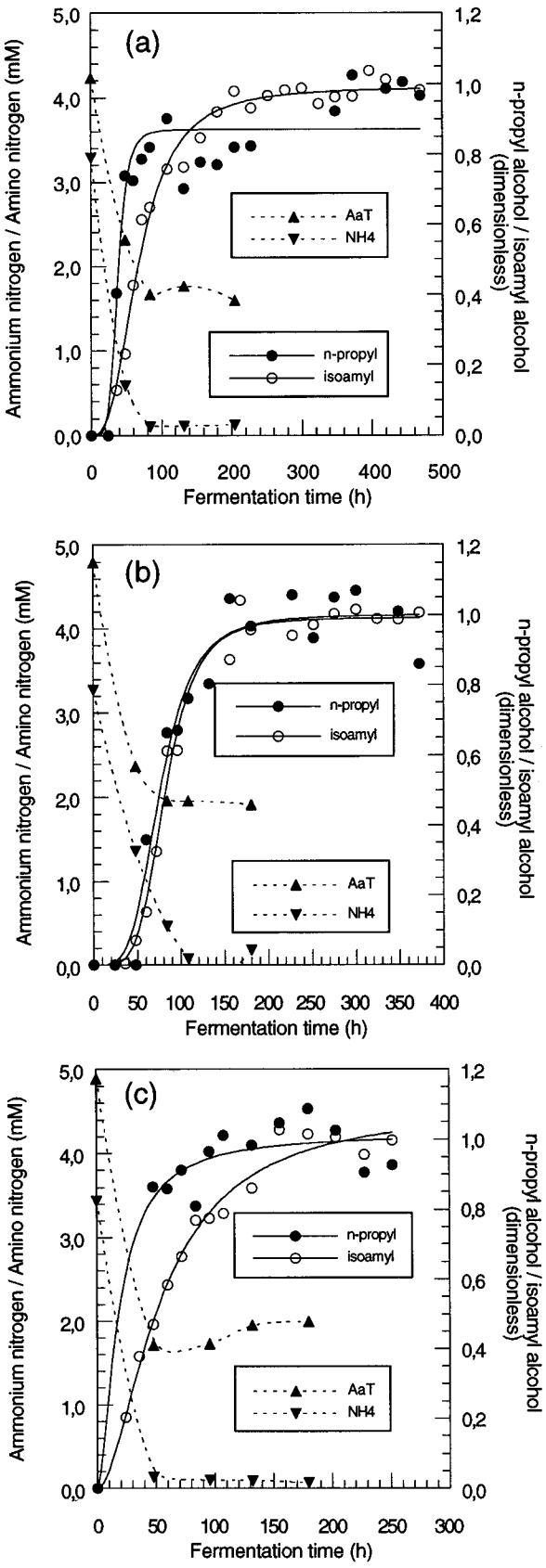

Figure 5. Total amino acid depletion and *n*-propyl and isoamyl alcohol formation vs time during the fermentation of Macabeo musts clarified using (a) vacuum filtration, (b) centrifugation, and (c) settling.

consumption sequence for nitrogen sources of *S. cerevisiae* reported by Trioli and Paronetto (1992) is used. As shown in Table 3, yeasts tend to use the nitrogen

compounds that are found in the highest concentrations and which are therefore more readily available.

During Macabeo must fermentation, it can be observed that amino acid and ammonium are consumed throughout the first 4 days of fermentation (Figure 5). Thus, the period of greatest amino acid and ammonium depletion is when the formation rate of isoamyl and *n*-propyl alcohol is at its fastest, as can be seen by comparing the plots in Figure 5. The total amino acid content shown in Figure 5 includes proline, which is scarcely consumed by *S. cerevisiae*. During the first 48 h of fermentation, the rate of isoamyl alcohol formation is highest when the consumption of free amino acids is highest. The fastest fermentation (settled must) used up to 65% of the total free amino acid concentration during the first 48 h of fermentation, and the next fastest (centrifuged must) used ≈50%. At the same time, 47% of isoamyl alcohol and 91% of *n*-propyl alcohol were produced during the fermentation of settled must. On the other hand, the slowest fermentation (filtered must) used up to 45% of the total free amino acids and produced 24 and 81% of isoamyl and *n*-propyl alcohol during the first 48 h of fermentation, respectively. From the results obtained in the present study there seems to be a relationship between amino acid consumption and the solid content of musts.

From an industrial point of view settling has the advantage of low cost and residue production, but it is a batch technique which needs long processing periods to be effective. Spanish cellars use this technique when grapes are brought to the cellar gradually and/or when they are not infected. When there is a big batch of grapes or they are infected, and therefore less able to withstand the required period for clarification through settling, vacuum filtration is mainly used as a prefermentation clarification technique. Vacuum filtration has the advantage of being a continuous process, but it generates a considerable amount of solid residue with a high organic content. Centrifugation combines the advantages of settling and vacuum filtration, since it is a continuous process that generates a fairly low amount of solid residue. The results obtained in the present study show that Parellada and Macabeo musts clarified using centrifugation lead to fermentation times that do not exceed the ones attained with filtered musts and do not affect the quality of the final product. Thus, the results of the present study suggest that it is positive to implement centrifugation as a clarification technique in Spanish cellars. Doing so will not only reduce environmental problems but also maintain wine quality.

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