Production of Decanoic Acid and Other Volatile Compounds and the Growth of Yeast and Malolactic Bacteria During Vinification

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Ultrafiltered Aurore grape juice (pH 3.5, 20° Brix) was inoculated with *Saccharomyces cerevisiae* and *Leuconostoc oenos* PSU-1 with and without the addition of insoluble grape solids (13 g/L, dry wt) and/or yeast ghosts (1 g/L). Wine made without insoluble materials (control) attained higher levels of decanoic acid (~5 mg/L) during alcoholic fermentation than treatments with insoluble grape solids, (~1 mg/L), yeast ghosts (~2.5 mg/L), or grape solids and yeast solids (~0.8 mg/L). Alcoholic fermentation stuck and malolactic fermentation (MLF) occurred most rapidly in control wines, while addition of grape solids and/or yeast ghosts stimulated alcoholic fermentation but delayed MLF. These results suggest that inhibition of malolactic bacteria by yeast was probably due to factors other than production of decanoic acid and other medium-chain fatty acids. Presence of insoluble materials during alcoholic fermentation further influenced the formation of higher alcohols and esters and altered the sensory quality of the finished wine.

KEY WORDS: alcoholic fermentation, decanoic acid, malolactic fermentation, yeast ghosts

The antagonism between yeast and malolactic bacteria grown together in both grape juice and synthetic media has often been observed (2,3,17,19,37,46). Although the nature of this microbial antagonism remains unknown, inhibition of malolactic bacteria by wine yeast has been generally thought to be the result of nutrient depletion or production of inhibitory compounds. King and Beelman (17) and Wibowo *et al.* (46) concluded that yeast-produced ethanol and sulfur dioxide, two known inhibitors of malolactic bacteria, did not account for the observed bacterial antagonism, and production of other antibacterial compounds by yeast was hypothesized. The interaction between these organisms is further dependant on yeast and bacterial strain (46).

Medium-chain fatty acids, present in many alcoholic beverages (35), are toxic to both yeast (23,37) and malolactic bacteria (8,27,40). Edwards and Beelman (8) suggested that these yeast-produced compounds may result in bacterial antagonism during alcoholic fermentation. One acid, decanoic acid, was found to suppress the growth of *Leuconostoc oenos* PSU-1 at 5- and 10 mg/L, levels reported to be present in some wines depending on must composition and winemaking conditions (12). At 30 mg/L, decanoic acid was lethal to the bacteria. Thus, the first objective of this study was to determine the relationship between production of medium-chain fatty acids and the growth of yeast and malolactic bacteria in grape juice.

Production of medium-chain fatty acids and other major volatiles during alcoholic fermentation is dependent on must composition, grape cultivar, yeast strain, fermentation temperature, and winemaking practices (12,13,21,33,44). Concord wine fermented on the skins was shown by Nelson and Acree (33) to have a lower level of decanoic acid (0.6 mg/L) compared to cold-pressed (2.2 mg/L) or thermally vinified (2.3 mg/L) wines. Other winemaking practices, such as must clarification prior to alcoholic fermentation, also affect the formation of volatile compounds (5,10,12,18,26). Houtman *et al.* (12) noted that wines made from sterile-filtered (deaerated) Chenin blanc juice (20°Brix) had higher amounts of decanoic acid than those made from settled juice (13 and 7.5 mg/L, respectively). Most studies concerning formation of higher alcohols (fusel oils) demonstrated results similar to those of Guymon *et al.* (11) in that the presence of insoluble grape solids during alcoholic fermentation resulted in increased levels of these compounds in the finished wine. The effect of insoluble solids on ester formation is less clear. For example, Groat and Ough (10) reported that ester production was enhanced in one must but retarded in another, even though both were fermented with insoluble grape solids. Thus, the second objective of this study was to determine the influence of insoluble materials, both insoluble grape solids and yeast ghosts, on the formation of major volatile compounds and on the growth of the wine microorganisms during vinification.

Materials and Methods

White French-hybrid grapes (cv. Aurore) were harvested in New York State and pressed at the Taylor

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Wine Company (Hammondsport, NY). The juice (pH 3.2; 14°Brix) was ultrafiltered at the Taylor Wine Company using a commercial Romican unit (Woburn, MA) with 50 000 molecular weight cut-off membranes to remove grape solids and wild yeasts and bacteria. Removal of the natural microflora from the juice allowed fermentations to proceed without addition of sulfur dioxide, a potent inhibitor of malolactic bacteria (25).

Once ultrafiltered, the juice was transferred to 208-L (55 gal) plastic drums. Insoluble grape solids, collected from the ultrafiltration unit, were steamed at 100°C and placed into plastic buckets and sealed. The juice and grape solids were transported to The Pennsylvania State University and frozen at -10°C until used.

The juice was thawed at room temperature and then placed into eight 18.9-L (5-gal) glass carboys. The soluble solids and pH were adjusted to 20°Brix and pH 3.5 using granular sucrose and $KHCO_3$, respectively. Two carboys (without insoluble material) served as controls while insoluble grape solids (13 g/L, dry wt), yeast ghosts (1 g/L, Nutrex 370, Universal Foods Corp., Milwaukee, WI), or a combination of insoluble grape solids and yeast ghosts (13 g/L and 1 g/L, respectively) were added to the remaining carboys.

Inoculation was accomplished using an active-dry form of yeast, *Saccharomyces cerevisiae* Montrachet strain No. 522 (Universal Foods Corp.), and using freeze-dried cultures of *Leuconostoc oenos* PSU-1, prepared as described by Duke (6). Yeast cultures were rehydrated in 100 mL of lukewarm phosphate buffer (pH 7.0, 0.023 *M* NaH_2PO_4, and 0.030 *M* $Na_2HPO_4 \cdot 7H_2O$) for *ca* 20 to 30 minutes prior to inoculation at an initial viable population of 1×10^6 cfu/mL. Bacterial cultures were rehydrated in Aurore grape juice (25°C) for *ca* 20 to 30 minutes and inoculated at an initial population of 6×10^5 cfu/mL. Fermentations were carried out in the carboys at room temperature (25°C). The wines were racked twice, 19 and 43 days after inoculation, and 50 mg/L sulfur dioxide was added at the second racking. The wines were then clarified with bentonite and bottled in preparation for further chemical and sensory analysis.

During fermentation, viable yeast and bacterial populations were determined in triplicate (per carboy) using the spread plate method. Yeast were plated on wort agar (17) prepared by steaming 150 g diamalt (Premier Malt Products, Inc., Milwaukee, WI) and 850 mL distilled water at 100°C for 10 minutes. The suspension was filtered through pellon (interface) cloth and 20 g agar added prior to autoclaving. Bacteria were plated on modified apple Rogosa agar (17). All plates were incubated at 25°C and counted after three days (yeast) or 10 days (bacteria). The progress of MLF was determined by paper chromatography as outlined by Kunkee (20).

Extraction, analysis, and identification of major volatile compounds present in fermenting juice and in finished wine was accomplished by using the XAD-2 resin extraction technique, GLC, and GC-MS described by Edwards and Beelman (8). At various times during fermentation, a 200-mL aliquot of juice from each carboy was transferred to a 250-mL centrifuge bottle and centrifuged at $900 \times g$ (2000 rpm) for 20 minutes using a Damon/IEC International Model UV centrifuge (Needham Heights, MA) to remove insoluble material before extraction. For finished wine analysis, aliquots from one bottle produced from each fermentation carboy were extracted without prior centrifugation. Extraction and analysis of fermenting juice and finished wine was performed in triplicate. Statistical analysis was accomplished using Fisher's protected least significant difference test at $p = 0.05$ (41).

To determine the influence of medium-chain fatty acids on the viability of both yeast and malolactic bacteria in pure culture under model conditions, commercially bottled white grape juice (Welch Foods, Westfield, NY) was diluted 1:1 (v/v) with distilled water and adjusted to pH 3.5 with $KHCO_3$ and to 10°Brix with granular sucrose. Aliquots of the juice (100 mL) were transferred to milk-dilution bottles and steamed for 10 minutes at 100°C. Pure cultures of *Saccharomyces cerevisiae* and *Leuconostoc oenos* PSU-1, rehydrated in the pH 7.0 phosphate buffer previously described, were inoculated separately into the juice at initial populations of 1×10^6 cfu/mL. Medium-chain fatty acids were dissolved in absolute ethanol and added to the juice to yield concentrations of 5 mg/L hexanoic acid, 10 mg/L octanoic acid, and 5 mg/L decanoic acid. Juice without medium-chain fatty acids had equivalent amounts of absolute ethanol added. The viable populations of yeast and bacteria were determined by the spread plate method described earlier.

Results and Discussion

The growth of yeast in grape juices with insoluble materials, either insoluble grape solids and/or yeast ghosts, peaked at viable populations exceeding 10^7 cfu/mL following short lag periods (Fig. 1, Table 1). These fermentations were considered to be normal as indicated by the fermentation curves (Fig. 2) where dryness was achieved after approximately seven days. On the other hand, the yeast population in juice fermented without insoluble material (control) peaked at a significantly lower population of 4×10^6 cfu/mL before entering a gradual death phase (Fig. 1). Poor yeast growth resulted in a stuck fermentation at 3° Brix to 4° Brix, indicated in Figure 2.

Stuck fermentation can be the result of variations in winemaking practices, nutritional deficiencies, or the presence of inhibitory substances (12,14,22). One winemaking practice that often leads to incomplete fermentation is early must clarification. Although wines made from clarified musts can be higher in quality than those made from unclarified musts (12,29), this practice increases the probability for stuck fermentations. Explanations for stuck fermentations of this nature are not known, but recently, Lafon-Lafourcade *et al.* (22) and Ribéreau-Gayon (37) indicated that fermentations can prematurely cease due to elevated levels of medium-

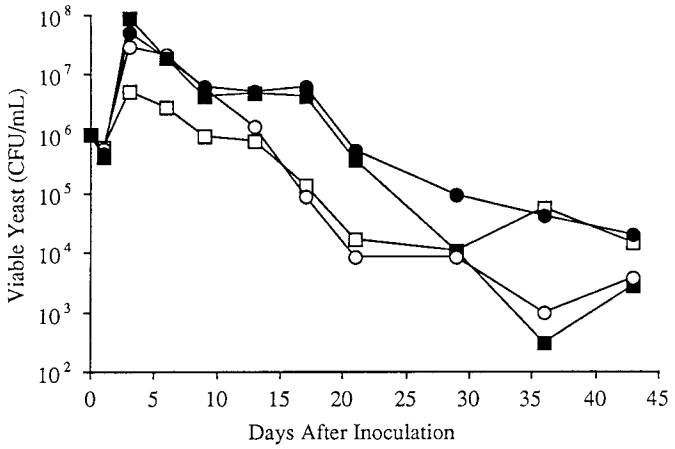

Fig. 1. Growth of *Saccharomyces cerevisiae* in Aurore grape juice without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●). Replicates having fewer than 30 colonies at the 10^{-1} dilution were assumed to have a population of 300 CFU/mL for calculation purposes.

Table 1. Yeast growth (log_{10} cfu/mL) in Aurore grape juice (data from Fig. 1).*

Day	Without insoluble material	Insoluble grape solids	Yeast ghosts	Insoluble grape solids & yeast ghosts
1	5.8[a]	5.6[a]	5.7[a]	5.7[a]
3	6.6[a]	7.8[b]	7.4[ab]	7.6[b]
6	6.4[a]	7.3[b]	7.3[b]	7.3[b]
9	5.9[a]	6.6[b]	6.8[b]	6.8[b]
13	5.8[a]	6.6[a]	5.9[a]	6.7[a]
17	5.1[a]	6.6[b]	4.7[a]	6.8[b]
21	4.0[ab]	5.5[b]	3.3[a]	5.6[b]
29	4.0[a]	4.0[a]	3.9[a]	4.7[b]
36	4.4[a]	2.5[a]	2.9[a]	3.7[a]
43	3.9[a]	3.3[a]	3.2[a]	3.5[a]

*Log_{10} of population means within a row (same day) followed by different letters are significantly different at $p = 0.05$.

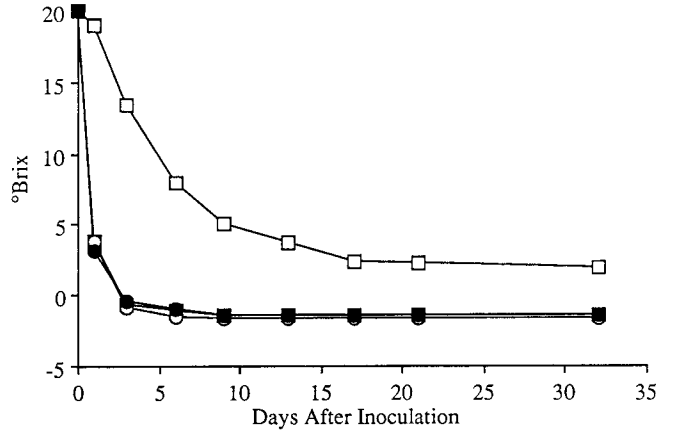

Fig. 2. Rate of alcoholic fermentation in Aurore grape juice measured by the decline in °Brix. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●).

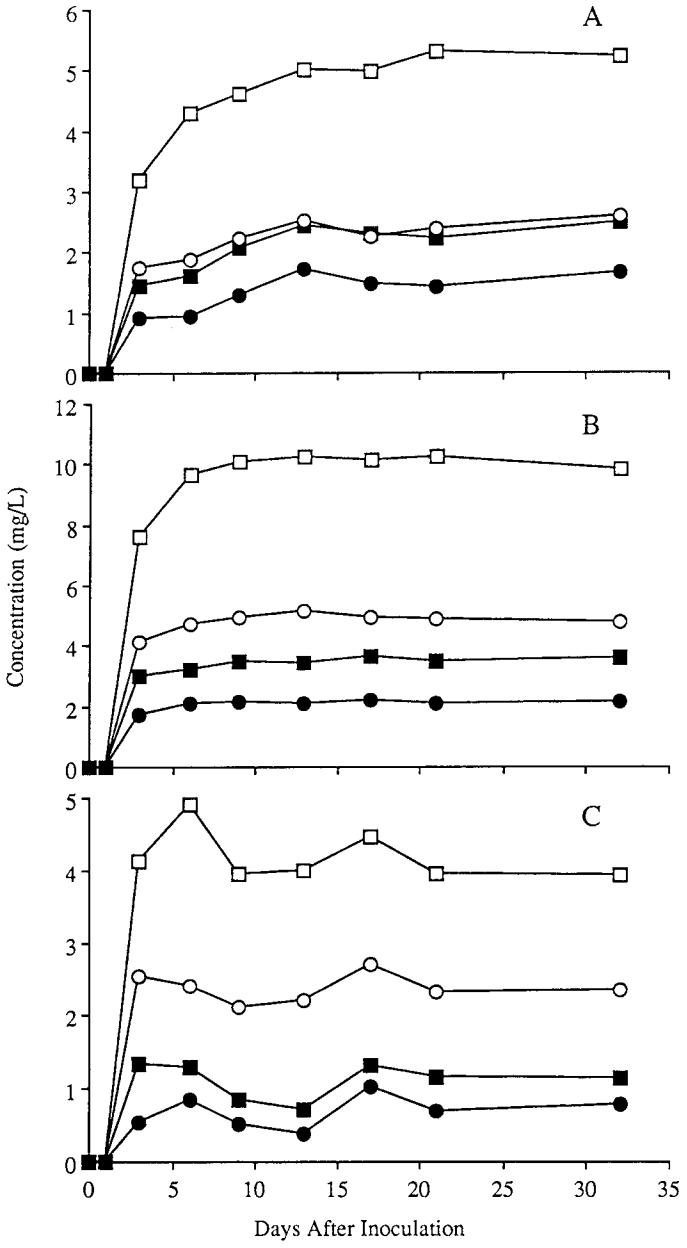

Fig. 3. Production of hexanoic (a), octanoic (B), and decanoic (C) acids by *Saccharomyces cerevisiae* during fermentation. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●).

chain fatty acids, compounds produced naturally by yeast during alcoholic fermentation (38). In the present work, wines in which the alcoholic fermentation stuck (control wines) contained higher levels of hexanoic (Fig. 3A), octanoic (Fig. 3B), and decanoic (Fig. 3C) acids than wines that completed fermentation. Concentrations of these acids reached maximum levels of approximately 5 mg/L hexanoic, 9 mg/L octanoic, and 5 mg/L decanoic acids during vinification. Wines fermented with insoluble materials contained less than half these levels and completed alcoholic fermentation (Fig. 2). While these data support the suggestion of Lafon-Lafourcade *et al.* (22) and Ribéreau-Gayon (37), poorer yeast growth may also have been due to other unknown factor(s) with

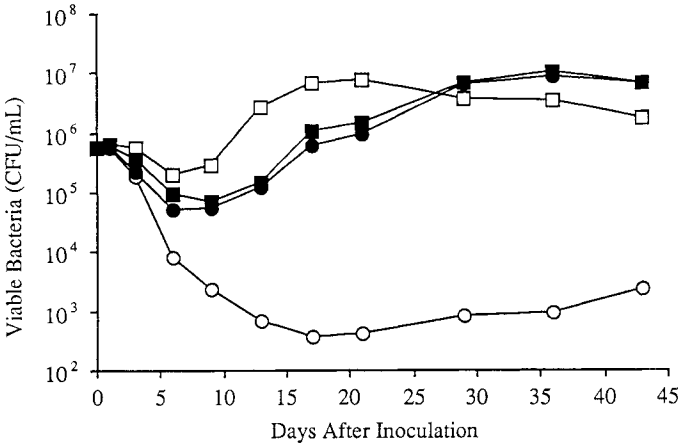

Fig. 4. Growth of *Leuconostoc oenos* PSU-1 in Aurore grape juice. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●). Replicates having fewer than 30 colonies at the 10^{-1} dilution were assumed to have a population of 300 cfu/mL for calculation purposes.

Table 2. Bacterial growth (log_{10} cfu/mL) in Aurore grape juice (data from Fig. 4).*

Day	Without insoluble material	Insoluble grape solids	Yeast ghosts	Insoluble grape solids & yeast ghosts
1	5.8[a]	5.6[a]	5.7[a]	5.7[a]
3	5.7[a]	5.6[a]	5.2[a]	5.3[a]
6	5.3[a]	4.9[bc]	3.9[a]	4.7[b]
9	5.4[b]	4.9[b]	3.1[a]	4.7[b]
13	6.3[c]	5.1[b]	2.8[a]	5.1[a]
17	5.1[a]	5.9[b]	2.5[a]	5.6[b]
21	6.8[c]	6.1[b]	5.6[a]	5.9[b]
29	6.9[c]	6.8[b]	2.8[a]	6.8[b]
36	6.4[b]	7.0[b]	2.8[a]	6.9[b]
43	6.2[b]	6.7[c]	3.1[a]	6.7[c]

*Log_{10} of population means within a row (same day) followed by different letters are significantly different at $p = 0.05$.

subsequent changes in yeast metabolism resulting in increased acid production.

In considering beer fermentation, Clarke *et al.* (4) noted that yeast strain, wort composition (including original gravity), and the nitrogen/fermentable carbohydrate ratio all influence the formation of medium-chain fatty acids. Indicating the importance of air (oxygen) to synthesis of these acids by wine yeast, Houtman *et al.* (12) reported that wines made from deaerated, sterile-filtered juice had higher levels of fatty acids than those made from aerated juice. In the present study, since the control wines contained higher levels of hexanoic, octanoic, and decanoic acids (Fig. 3), less oxygen could have been available to yeast growing in these musts due to the absence of insoluble materials containing entrapped air. However, these differences also could be the result of adsorption of the acids by the insoluble materials, since data from Ribéreau-Gayon (37) indicate that >50% of added decanoic acid (3 mg/L) was adsorbed after 24 hours by the addition of yeast ghosts (1 g/L) in a model system.

In addition to inhibition of the alcoholic fermentation, data presented by Lonvaud-Funel *et al.* (28) and Edwards and Beelman (7) demonstrated that MLF also could be retarded by medium-chain fatty acids. These studies supported the suggestion that the often observed bacterial antagonism during alcoholic fermentation was due to production of these compounds by actively growing yeast. An attempt, therefore, was made to relate the production of these acids to the growth of malolactic bacteria. However, as shown in Figure 4 and Table 2, malolactic bacteria grew better and entered a stationary phase significantly faster in the control wines than in wines fermented with insoluble materials in spite of the fact that these wines contained higher levels of medium-chain fatty acids (Fig. 3). In fact, MLF was completed six to seven days faster in the absence of insoluble grape solids and/or yeast ghosts (Table 3). Better growth of the malolactic bacteria in the control wines was probably a direct result of poorer yeast growth with a corresponding reduction in production of antagonistic factor(s).

Table 3. Time to complete malolactic fermentation in Aurore wine with and without additions of insoluble grape solids and/or yeast ghosts.

Treatment	Days to complete MLF
Without insoluble material	25
Insoluble grape solids	31 - 32
Yeast ghosts	No conversion*
Insoluble solids and yeast ghosts	32

*No conversion by second racking.

To confirm that medium-chain fatty acids inhibit yeast to a higher degree than malolactic bacteria, a mixture of medium-chain fatty acids in concentrations similar to those produced in the control wine (Fig. 3) was added to commercially bottled grape juice inoculated with pure cultures of *Saccharomyces cerevisae* or *Leuconostoc oenos* PSU-1. As illustrated in Figure 5, yeast was apparently more inhibited by the acids than the bacteria. Yeast growth in the presence of the acids underwent a prolonged lag phase, with a peak population significantly lower than that in the absence of acids (Table 4). Conversely, bacterial growth in the presence or absence of the acids was statistically similar during the lag phase. In fact, malolactic bacteria entered logarithmic growth faster and reached significantly higher populations in juice with the acids, indicating that the acids may have been slightly stimulatory to the bacteria at the concentrations used. Although inhibition of *Saccharomyces* spp. and lactic acid bacteria by medium-chain fatty acids have been reported in grape products and silage by other researchers (7,36,40,47), stimulation of malolactic bacteria by medium-chain fatty acids has not been previously observed. As noted by Kabara (15,16), microbial toxicity of medium-chain fatty acids varies with the organism, chain length of the acid, and

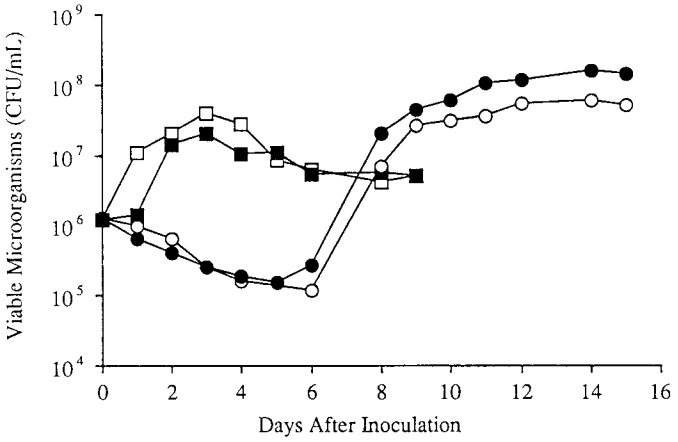

Fig. 5. Growth of *Saccharomyces cerevisiae* and *Leuconostoc oenos* in pure culture in commercially bottled grape juice with and without a mixture of hexanoic (5 ppm), 0ctanoic (10 ppm), and decanoic (5 ppm) acids. Yeast (❑, ■) and bacterial (○, ●) or without (❑, ○) acids.

Table 4. Viable yeast and bacteria populations (log_{10} cfu/mL) grown in pure culture in commercially bottled grape juice with and without a mixture of hexanoic (5 ppm), octanoic (10 ppm), and decanoic (5 ppm) acids (data from Fig. 5).*

	Yeast		Bacteria	
Days after inoculation	Without acids	With acids	Without acids	With acids
1	7.0[a]	6.2[b]	6.0[a]	5.8[a]
2	7.3[a]	7.2[a]	5.8[a]	5.6 b
3	7.6[a]	7.3[b]	5.4[a]	5.4[a]
4	7.4[a]	7.0[b]	5.2[a]	5.3[a]
5	6.9[a]	7.0[a]	nd	5.2
6	6.8[a]	6.7[a]	5.1[a]	5.4[a]
6	6.6[a]	6.8[a]	6.8[a]	7.3[a]
9	6.7[a]	6.7[a]	7.4[a]	7.6[a]
10	nd	nd	7.5[a]	7.8[a]
11	nd	nd	7.6[a]	8.0[b]
12	nd	nd	7.7[a]	8.1[b]
14	nd	nd	7.8[a]	8.2[b]
15	nd	nd	7.7[a]	8.2[b]

*Log_{10} of population means for yeast or bacteria within a row (same day) followed by different letters are significantly different at $p = 0.05$ as determined by Student's t test.

nd = not determined.

pH of the medium. Observed differences in sensitivity between yeast and malolactic bacteria may be related to differences in cell wall structure (9).

The formation of other bacterial inhibitors has been postulated by Lonvaud-Funel *et al.* (28) and Wibowo *et al.* (36). Fatty acids not analyzed in the present study, including decanoic acid (C12) and palmitoleic acid (C16:1), have been reported to be inhibitory to malolactic bacteria by Lonvaud-Funel *et al.* (28). In their study, low concentrations of decanoic or palmitoleic acid (*ca* 0.5 mg/L) inhibited MLF. The authors suggested that these acids may therefore be responsible for variations in the malolactic fermentability of wines, especially those fermented with different yeast strains. Wibowo *et al.* (46) suggested the presence of other inhibitory mechanism(s) including productions of toxic proteins. Direct evidence is currently lacking for either hypothesis.

In agreement with Barillere *et al.* (1), Lafon-Lafourcade *et al.* (22) and Minárik (30), addition of yeast ghosts was found to enhance alcoholic fermentation (Fig. 2). Similar results have also been observed by A. C. Rice and J. Lucia (personal communication, 1987) performing experiments at a New York State winery using musts of *Vitis labrusca* (cv. Delaware). Explanations for this observation are not currently known, but addition of nutrients (31) or adsorption of toxic inhibitors such as medium-chain fatty acids (22,37) have been proposed. Recent work by Munoz and Ingledew (32) suggests that stimulation of stuck or sluggish fermentations by yeast ghosts cannot be completely attributed to adsorption of these toxic acids.

Unlike the alcoholic fermentation, the influence of insoluble materials on the rate of MLF is less clear. In the present study, malolactic bacteria grew best in wines fermented without insoluble material (Fig. 4). Liu and Gallander (24), however, observed that wines experienced faster MLF when fermented with insoluble grape solids with bacterial inoculations made after alcoholic fermentation. The difference between these two studies is probably related to the poor yeast growth observed in the control wines (Fig. 1) through reduction of the bacterial antagonism. Another insoluble material, yeast ghosts, has been previously reported to stimulate the growth of malolactic bacteria (7,27). On the other hand, Test and Bannister (42) reported that low levels of yeast ghosts (0.24 g/L) slightly decreased the rate of MLF in barrel-fermented Chardonnay at a California winery. At a higher level of 0.96 g/L, the fermentation was markedly slowed, in agreement with the results in Table 3, where MLF did not occur by the time of second racking. Similarly shown in Figure 3, the bacterial population in juice fermented with yeast ghosts declined at a rapid rate and did not enter logarithmic or stationary phase by the second racking.

In response to the results presented in Table 3, R. E. Kunkee (personal communication, 1988) suggested that inhibition of MLF could have been the result of bacteria settling with the yeast ghosts. Observations which lend support to his suggestion include the fact that the fermentation carboys were not physically agitated during fermentation and that wines fermented with yeast ghosts appeared to be highly clarified after alcoholic fermentation in comparison to other treatments. The discrepancy between these results and earlier work (7) may in fact be due to agitation, since the fermentation vessels in the earlier study (100-mL milk dilution bottles) were swirled or agitated prior to sampling, thus keeping the bacteria in suspension.

Interestingly, the relationship between yeast ghosts and malolactic bacteria was found to be influenced by the presence of insoluble grape solids (Fig. 4, Table 2). Bacterial growth in wines fermented with insoluble grape solids alone or with insoluble grape

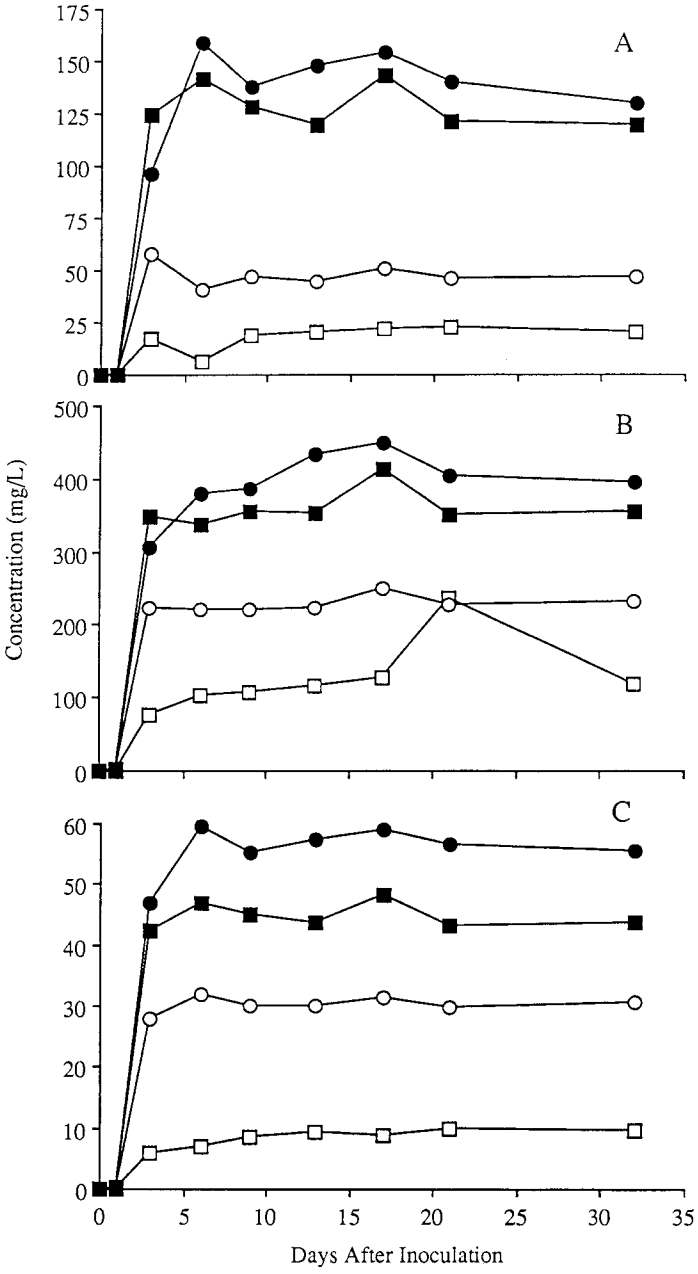

Fig. 6. Production of isobutyl (A), isoamyl (B), and phenyl ethyl (C) alcohols by *Saccharomyces cerevisiae* during fermentation. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●).

solids and yeast ghosts were similar, while bacterial growth with yeast ghosts alone was very poor, indicating that the inhibitory influence of yeast ghosts on the bacteria was negated by insoluble solids. Since insoluble grape solids influence the growth of yeast, differences in bacterial inhibition could be a direct consequence of changes in yeast growth and metabolism. The mechanism by which insoluble materials affect yeast metabolism is not known but may involve addition of oxygen (5,11). Additional explanations include: *a)* adsorption or removal of toxic inhibitors by insoluble materials shown for the interaction between yeast ghosts and medium-chain fatty acids (22,37); *b)* the presence or absence of

Table 5. Concentration of volatile compounds (mg/L) in bottled Aurore wine fermented with and without insoluble grape solids and/or yeast ghosts.*

Compound	Without insoluble material	Insoluble grape solids	Yeast ghosts	Insoluble grape solids & yeast ghosts
Ethyl acetate	24.9[a]	33.6[bc]	38.9[c]	29.1[ab]
Isobutyl alcohol	11.0[a]	108[c]	37.5[b]	129[b]
Isoamyl acetate	3.79[a]	7.83[c]	6.50[b]	8.21[c]
Isoamyl alcohol	110[a]	304[c]	211[b]	363[d]
Ethyl hexanoate	1.40[b]	1.19[a]	1.19[a]	1.16[a]
Ethyl octanoate	2.34[c]	1.75[b]	1.86[b]	1.62[a]
Phenyl ethyl acetate	1.14[a]	1.60[b]	1.56[b]	1.75[b]
Hexanoic acid	5.21[c]	2.26[b]	2.41[b]	1.57[a]
Phenyl ethyl alcohol	8.90[a]	39.1[c]	28.5[b]	5.10[d]
Octanoic acid	8.41[d]	3.08[b]	4.23[c]	2.06[a]
Decanoic acid	2.48[d]	0.53[b]	1.35[c]	0.33[a]

*Treatment means within a row (same day) followed by different letters are significantly different at $p = 0.05$.

unknown substance(s) that influence yeast production of antagonistic factor(s); or *c)* the amount of insoluble material present during fermentation, since two different levels of solids were used (13 g/L insoluble grape solids and 1 g/L yeast ghosts). Obviously, the interaction between insoluble materials and the growth and metabolism of yeast and bacteria is complicated and firm conclusions can not be made without additional research.

Besides medium-chain fatty acids, yeast produce many other volatile compounds during fermentation which are important for wine flavor and bouquet. Production of other volatiles, including higher alcohols (fusel oils) and esters, was also found to be dependant on the presence of insoluble materials during fermentation. Wines fermented with insoluble grape solids with or without yeast ghosts contained higher levels of isobutyl alcohol (Fig. 6A), isoamyl alcohol (Fig. 6B), and phenyl ethyl alcohol (Fig. 6C) than control wines made with yeast ghosts alone. Statistical analysis revealed significant differences between treatments in the bottled wines (Table 5). These findings are in agreement with those of Crowell and Guymon (5), Klingshirn *et al.* (18), and Liu *et al.* (26). Crowell and Guymon (5) determined that wines produced from turbid grape musts contained higher levels of higher alcohols, especially isobutyl and isoamyl alcohols, than those made from clarified juice. The authors further noted that addition of other inert solids such as cellulose and starch to fermenting musts also leads to an increase in the production of higher alcohols.

Some researchers have proposed that insoluble material influences higher alcohol formation by incorporation of small quantities of air into the must. Guymon *et al.* (11) suggested that insoluble materials aerate a must by adding entrapped air which results in increased higher alcohol production. Later work by Crowell and Guymon (5) revealed that fusel oil produc-

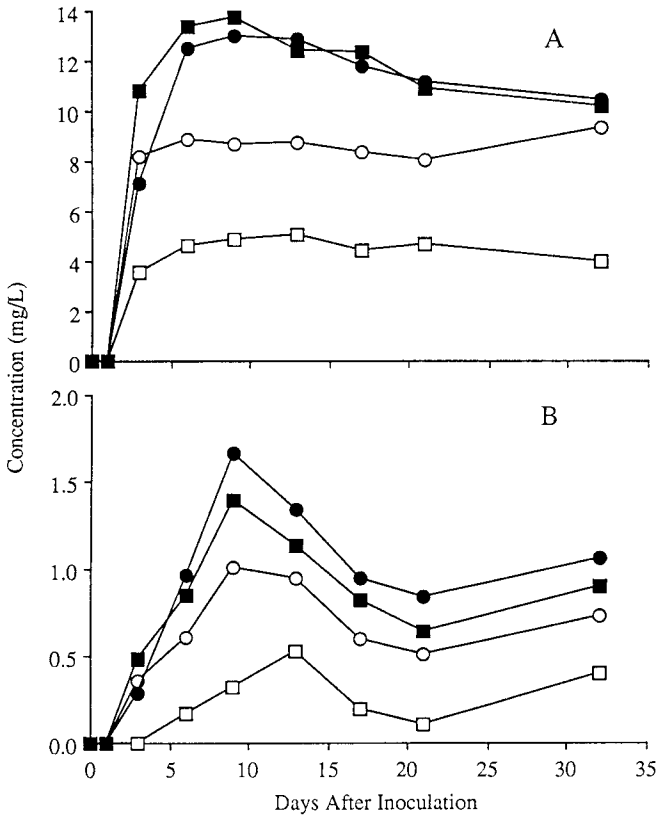

Fig. 7. Production of isoamyl (A), and phenyl ethyl (B) acetates by *Saccharomyces cerevisiae* during fermentation. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●).

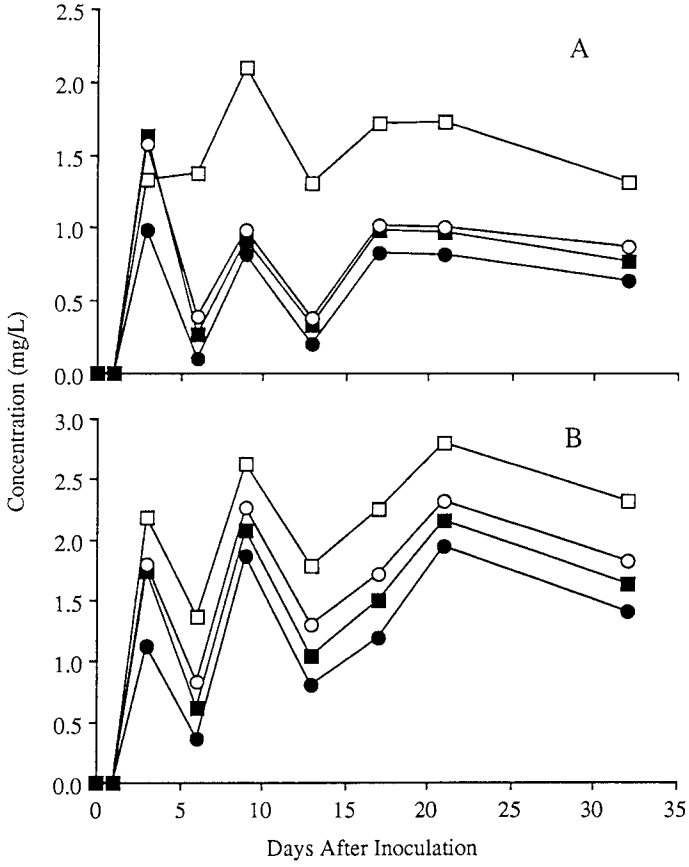

Fig. 8. Production of ethyl hexanoate (A), and ethyl octanoate (B) by *Saccharomyces cerevisiae* during fermentation. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●).

tion was reduced in the presence of deaerated insoluble solids. However, aeration of the must by insoluble solids does not appear to be the only explanation in that the authors noted that wines fermented without the soluble solids had lower levels of higher alcohols than wines made using deaerated juice and insoluble solids. Firm conclusions can not be made with regards to these data because statistical analysis was not applied in their study. Other proposed mechanisms include increased enzymatic activity (18) or addition of precursory compound(s) to higher alcohols, since these alcohols can be formed from amino acids and other carbon sources (38).

Unlike medium-chain fatty acids or higher alcohols, interpretation concerning the relationship between ester formation and the presence of insoluble material during fermentation is less clear. Wines fermented without insoluble material generally had lower levels of isoamyl acetate (Fig. 7A) and phenyl ethyl acetate (Fig. 7B) and higher levels of ethyl hexanoate (Fig. 8A) and ethyl octanoate (Fig. 8B) than other treatments, particularly wines fermented with insoluble grape solids. The production of ethyl acetate (Fig. 9) followed a different pattern in that wines fermented with yeast ghosts had the highest levels. Statistical analysis revealed few significant differences between treatments (Table 5). Houtman (13) similarly reported that the amount of insoluble material present during fermentation greatly affected volatile ester production, with maximal amounts of esters produced with 1% to 2% juice lees present.

Wines made by fermentation of clarified grape juice are generally considered to be more "fruity" and have fewer off-odors (10,39), although the risk of stuck fer-

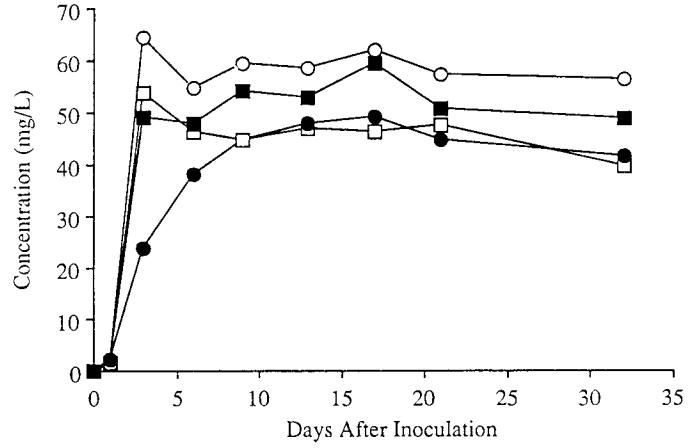

Fig. 9. Production of ethyl acetate by *Saccharomyces cerevisiae* during fermentation. Juice fermented without insoluble material (❑), with insoluble grape solids (■), with yeast ghosts (○), or with insoluble grape solids and yeast ghosts (●).

mentation is often greater when fermenting these musts. Enologists can attempt to reduce this potential problem by using massive yeast inoculation or by aeration of the juice (12), two practices not always desirable. An alternative solution indicated by the present study is the use of yeast ghosts. Addition of yeast ghosts to ultrafiltered juice stimulated the growth of yeast (Fig. 1), resulting in a completed alcoholic fermentation (Fig. 2). Furthermore, wines made with yeast ghosts had significantly lower levels of undesirable fusel oils, and levels of the major esters were either similar or slightly higher in concentration than wines produced by the more traditional method with insoluble grape solids (Table 5). Yeast ghosts have been recommended by Wahlstrom and Fugelsang (45) as a means for preventing stuck fermentations.

Before recommending commercial use of yeast ghosts, sensory analysis of these wines needed to be performed in order to ascertain the influence of yeast ghosts on the overall quality of the finished wine. Some workers have noticed that yeast ghosts did not adversely affect the flavors and bouquets of wines, while others have reported the opposite. For instance, Barillere *et al.* (1) and Lafon-Lafourcade *et al.* (22) reported that French wines treated with yeast ghosts did not have a "yeasty" flavor and were of comparable quality to untreated wines. On the other hand, Usseglio-Tomasset (43) indicated that yeast ghosts had an unfavorable influence on wine aroma. In fact, this author recommended using small amounts of decolorizing carbon for stuck fermentations rather than yeast ghosts to avoid undesirable sensory effects. Because of this potential problem, an attempt was made to characterize the sensory quality of the Aurore wines using the aroma descriptors defined by Noble *et al.* (34).

The results of sensory evaluation indicated that control wines were generally more fruity and had fewer off-odors than wines fermented with insoluble grape solids, in agreement with the results of Singleton *et al.* (10,39). Wines made with yeast ghosts were of comparable quality to control wines although some panelists did detect an off-odor described as "musty". These trends should be analyzed with caution because statistical analysis of the data revealed significant ($p = 0.05$) judge-wine interactions, indicating that the training sessions for the judges were insufficient. Interpretations and conclusions regarding the influence of insoluble material on wine aroma are therefore limited. Interactions between aroma compounds, especially among higher alcohols, may have contributed to panel confusion (29). Certainly, further research is needed concerning the sensory analysis of wines made with yeast ghosts before recommending commercial use to wineries.

Conclusions

Insoluble grape solids and yeast ghosts were found to influence the formation of medium-chain fatty acids and other volatile compounds as well as the growth of yeast and malolactic bacteria. Wines made without added insoluble materials (control) contained higher levels of decanoic acid and other medium-chain fatty acids than treatments with insoluble materials. In these wines, stuck fermentations were observed and MLF occurred most rapidly. Addition of insoluble grape solids and/or yeast ghosts resulted in lower levels of medium-chain fatty acids, while stimulated alcoholic fermentation and delayed MLF. Moreover, decanoic acid and other medium-chain fatty acids were more inhibitory to yeasts than to malolactic bacteria. Thus, the often observed inhibition of malolactic bacteria by yeast was probably due to factor(s) other than production of these compounds.

Yeast ghosts added to ultrafiltered grape juice resulted in a faster alcoholic fermentation than in juice without insoluble materials. In addition, lower levels of higher alcohols and equal or higher levels of the major esters were produced in these wines than wines made with insoluble grape solids. Although the sensory analysis of these wines was inconclusive, addition of yeast ghosts to highly clarified musts may be an attractive alternative method to reduce the risk of stuck fermentations while maintaining wine quality.

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