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Effect of Simultaneous Inoculation with Yeast and Bacteria on Fermentation Kinetics and Key Wine Parameters of Cool-Climate Chardonnay

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Inoculating grape musts with wine yeast and lactic acid bacteria (LAB) concurrently in order to induce simultaneous alcoholic fermentation (AF) and malolactic fermentation (MLF) can be an efficient alternative to overcome potential inhibition of LAB in wines because of high ethanol concentrations and reduced nutrient content. In this study, the simultaneous inoculation of yeast and LAB into must was compared with a traditional vinification protocol, where MLF was induced after completion of AF. For this, two suitable commercial yeast-bacterium combinations were tested in cool-climate Chardonnay must. The time courses of glucose and fructose, acetaldehyde, several organic acids, and nitrogenous compounds were measured along with the final values of other key wine parameters. Sensory evaluation was done after 12 months of storage. The current study could not confirm a negative impact of simultaneous AF/MLF on fermentation success and kinetics or on final wine parameters. While acetic acid concentrations were slightly increased in wines after simultaneous AF/MLF, the differences were of neither practical nor legal significance. No statistically significant differences were found with regard to the final values of pH or total acidity and the concentrations of ethanol, acetaldehyde, glycerol, citric and lactic acids, and the nitrogen compounds arginine, ammonia, urea, citrulline, and ornithine. Sensory evaluation by a semiexpert panel confirmed the similarity of the wines. However, simultaneous inoculation led to considerable reductions in overall fermentation durations. Furthermore, differences of physiological and microbiological relevance were found. Specifically, we report the vinification of "super-dry" wines devoid of glucose and fructose after simultaneous inoculation of yeast and bacteria.

Alcoholic fermentation (AF) is indispensable for the production of alcoholic beverages, including grape wines. Most red and some white grape wines, especially those from cool climates, undergo a secondary fermentation, which is called malolactic fermentation (MLF) and most often is encouraged during the final phases of AF or after its conclusion (11). It is carried out by wine lactic acid bacteria (LAB) and leads to wine deacidification through conversion of dicarboxylic L-malic acid to monocarboxylic L-lactic acid and aroma modifications (34). While both AF and MLF may result spontaneously from the activity of yeast and bacteria naturally present in musts and wines, for some years highly concentrated freeze-dried preparations of yeast and bacteria have been used to induce AF and MLF, respectively (34). This, combined with better nutrient management, has led to faster and more predictable fermentations and wine quality. Yet, especially MLF remains difficult to accomplish in some wines or can be slow, mainly because of the strong combined inhibitory effect of ethanol and acidity in wines (52, 53). In order to encourage ideal conditions for the wine LAB necessary for MLF, wines have to be kept under conditions that increase the risk of spoilage by other microorganisms as well. Moreover, delayed or slow MLFs can be problematic for efficient fermentation tank utilization in the busy postharvest period and also for the early commercialization of wines.

Successfully inducing simultaneous AF and MLF, where both yeast and bacteria are inoculated into must, would thus be beneficial regarding microbiological and technical aspects. This would allow more efficient malolactic conversion in difficult wines (e.g., with low pH) because of the low alcohol concentrations and higher nutrient content present in fermented grape musts compared with wines. Also, wines obtained after successful AF/MLF would be immediately ready for downstream treatments, such as racking, fining, and sulfur dioxide addition, thus increasing microbiological stability and processing efficiency. However, and in spite of the considerable interest in this technique, its application is not very common because of fears of wine quality depreciation by LAB activity in musts and the limited scientific data available. Specifically, growth of certain wine LAB in grape musts can cause "stuck AF," i.e., interruption of AF before sugar depletion (14, 42), or wines with increased concentrations of acetic acid that render them unacceptable for consumption (11). Over the last years, several authors have researched yeast-bacterium interactions (3, 25, 30, 32), which have been recently reviewed by Alexandre et al. (1), and suitable yeast-bacterium combinations have been used to study simultaneous AF and MLF (20, 21, 43, 47). Most of these studies have concentrated on the microbial interactions, measured as cell numbers and viability, and a few wine parameters, such as sugar and malic acid levels. However, differences between consecutive and simultaneous AF and

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TABLE 1. Times to reach dryness (combined glucose and fructose levels of below 1 g liter^{-1}) and to reach L-malic acid concentrations of below 500 and 100 mg liter^{-1} during fermentations of Chardonnay must with *S. cerevisiae* CY3079 combined with *O. oeni* strain EQ54 or Alpha

Parameter	*Oenococcus oeni* EQ54			*Oenococcus oeni* Alpha		
	Simultaneous treatment, time since yeast/bacterial inoculation (days)	Consecutive treatment[a]		Simultaneous treatment, time since yeast/bacterial inoculation (days)	Consecutive treatment	
		Time since bacterial inoculation (days)	Total fermentation time (days)		Time since bacterial inoculation (days)	Total fermentation time (days)
Glucose + fructose <1 g liter^{-1}	19.5	21.6	NA[b]	20.5	21.6	NA
Malic acid <500 mg liter^{-1}	7.5	28	49.6	7.5	8	29.6
Malic acid <100 mg liter^{-1}	27	NR[c]	NR	20	44.4	66

[a] Consecutive treatments were inoculated with bacteria after reaching dryness at 21.6 days.
[b] NA, not applicable.
[c] NR, not reached.

MLF are still poorly documented or remain controversial (1), specifically with regard to the effect of this technique on wine composition.

The present study investigated the effect of the time of bacterial inoculation on vinification kinetics and important physiological parameters, with specific consideration of the concentrations of acetic acid and compounds with negative health effects. For this, a traditional vinification, where wine LAB were inoculated after completion of AF, was compared with a simultaneous vinification, where yeast and bacteria were inoculated concurrently. The vinifications were carried out in a pilot plant, that is, under practical multiseptic conditions. Two suitable commercial yeast-bacterium combinations were selected for this study based on the manufacturer's recommendations. A cool-climate Chardonnay must was chosen as a typical example of a white wine vinification with MLF. The time courses of glucose and fructose; malic, acetic, and other organic acids; acetaldehyde; and some nitrogenous compounds were tested, along with the final values of other chemical parameters.

MATERIALS AND METHODS

Microorganisms. *Saccharomyces cerevisiae* strain CY3079 and the two *Oenococcus oeni* strains EQ54 and Alpha are commercially available as pure freeze-dried cultures and were obtained from Lallemand (Montreal, Canada).

Grape must, vinification protocol, and sampling. Chardonnay grapes from a commercial Hawke's Bay (New Zealand) vineyard were harvested mechanically, destemmed, and crushed, and 620 kg of fruit was pressed at 1.3 bars in a bladder press (type 60; Willmes Anlagentechnik, Germany) to yield 400 liters of must that was cold-settled at 4°C for 24 h and racked. The racked must had a soluble solids content of 20.7 Brix (20°C), a pH of 3.28, and a total acidity of 10 g liter^{-1} as tartaric acid and contained 5.01 g liter^{-1} of malic acid. Diammonium hydrogen phosphate (Sigma) (300 mg liter^{-1}) was added as a yeast nutrient. Twelve 25-liter glass carboys were filled with the must. The four treatments (in triplicate) consisted of combining AF by *S. cerevisiae* CY3079 with MLF by *O. oeni* strain EQ54 or Alpha, where the malolactic bacteria (MLB) were inoculated either together with yeast (simultaneous AF/MLF) or after completion of AF (consecutive AF/MLF). The microorganisms were prepared and inoculated according to manufacturer's recommendations to give cell counts of 8×10^7 CFU ml^{-1} for the yeast and 1×10^6 CFU ml^{-1} for the wine LAB, which were confirmed by viable cell counts on YPD and MRS agars (Difco), respectively. The fermentation temperature was maintained at 19 to 20°C in a temperature-controlled room. Samples were taken periodically during fermentations and centrifuged at 10,000 × *g* for 5 min, and the supernatant was transferred into 15-ml screw-cap tubes and frozen at −18°C until analysis. After completion of AF and MLF as assessed by stable sugar and malic acid concentrations, the wines were racked of the primary yeast lees, fined with bentonite (0.5 g liter^{-1}) (Volclay WG, North Geelong, Australia), racked of the bentonite lees, cold stabilized (2°C, 1 week), and racked again. The concentration of free SO_2 was adjusted to 36 mg liter^{-1} in three rounds over a period of 2 weeks. Finally, the wines underwent depth filtration with filter sheets (EK grade; Seitz-Schenk, Germany) followed by 0.45-μm membrane filtration (142-mm nylon disk filter; Millipore) before bottling. CO_2 was used for inertion of headspaces throughout the entire winemaking process. The wines were stored in 750-ml bottles for 12 months for sensory evaluation.

Analytical methods. Organic acids were analyzed with formic acid as an internal standard by ion-exchange high-pressure liquid chromatography (HPLC) (Summit HPLC system; Dionex, Sunnyvale, Calif.). Samples were filtered through 0.2-μm nylon filters (Millipore), and 10 μl was directly injected. Separations took place on a 250-mm by 4.6-mm (inner diameter) Supelcogel H column, preceded by a 50-mm by 4.6-mm (inner diameter) Supelguard C610H (Supelco) precolumn with the same filling and a 0.5-μm in-line filter (Upchurch, Oak Harbor, WA). Separations were carried out under isocratic conditions with a 0.1% H_3PO_4 mobile phase (HPLC grade; Sigma) at a flow rate of 0.2 ml min^{-1}. UV detection of organic acids was carried out at 210 nm. Arginine, citrulline, and ornithine were quantified on a Shimadzu class VP system by reverse-phase HPLC. Amino acids were quantified after precolumn derivatization with o-phtaladehyde/3-mercaptopropionic acid according to the method of Bartók et al. (2). Chromatographic separations were performed with a 100-mm by 4.6-mm (inner diameter) column (Supelco) filled with 3 μm Hypersil ODS (Shandon, Cheshire, England). A cartridge-type zero-dead-volume guard column (Phenomenex Securityguard with two 4-mm by 3-mm [inner diameter] ODS cartridges) was attached directly to the analytical column, preceded by a 0.5-μm in-line filter (Upchurch, Oak Harbor, WA). Glucose, fructose, glycerol, acetaldehyde, ethanol, ammonia, and urea were analyzed with enzymatic test kits (Roche [now r-biopharm]). Total acidity (expressed as tartaric acid) and free and total SO_2 were measured by acid-base titration with standardized 0.1 M NaOH and by the aspiration-oxidation method, respectively (24). A triangle-type discrimination test (31) with a panel consisting of 20 semiexpert subjects selected from an enology class was carried out to determine if significant differences existed between wines from the four treatments. Replicates from same treatments were blended previously, and the composition of rounds and order of wines were randomized. Every subject completed three rounds of comparisons. Significance of results was determined as described by Roessler et al. (46) at a confidence interval of 0.05 (one-tailed test).

RESULTS

Inoculation of MLB in sequential treatments. Wines were considered to be "dry" and alcoholic fermentation concluded if the reducing sugar level fell below 0.1% (1 g liter^{-1}). This was the case after 21.6 days in consecutive AF/MLF treatments (Table 1), and thus, MLB were inoculated at this time point.

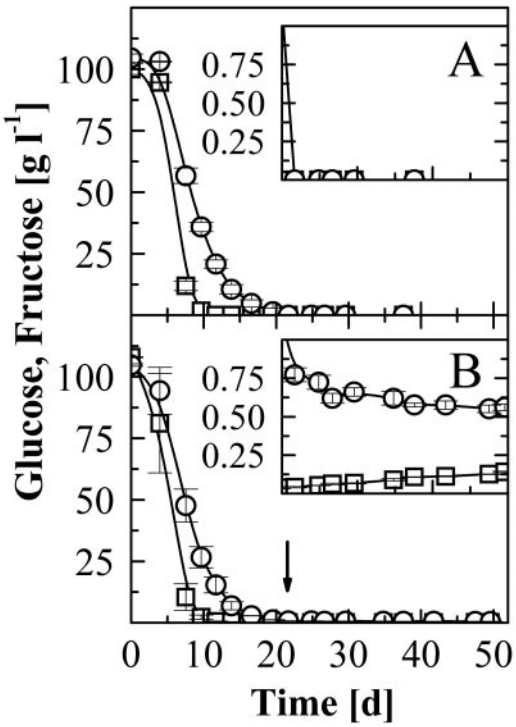

FIG. 1. Time courses of glucose and fructose concentrations during AF and MLF of Chardonnay with *S. cerevisiae* CY3079 and *O. oeni* Alpha. A, simultaneous inoculation of yeast and bacteria; B, inoculation of bacteria after completion of AF (the arrow indicates inoculation with MLB in consecutive treatment). □, glucose; ○, fructose. The insets magnify the last 30 days of fermentations. Values are means and standard errors.

Sugar and malic acid degradation. The degradations of glucose and fructose were similar during the first 3 weeks of AF regardless of the time of inoculation with MLB (Fig. 1A and B). However, after 3 weeks, the development of sugar concentrations differed greatly (insets in Fig. 1A and B). While wines with traditional, consecutive AF and MLF had combined glucose and fructose concentrations of approximately 700 mg liter^{-1} (inset in Fig. 1B and Table 2), wines produced after simultaneous inoculation of yeast and bacteria had no detectable glucose or fructose residues (inset in Fig. 1A and Table 2). These results could be confirmed with strain EQ54. Table 1 shows the fermentation times required to achieve malic acid concentrations of less than 500 and 100 mg liter^{-1}, with the latter being generally recognized as the threshold for a complete MLF (21). Overall, all treatments with simultaneous inoculation of yeast and bacteria led to faster (Table 1) and complete (Table 2) malic acid degradation. Even when the fermentation times after inoculation with MLB are compared (Table 1), MLF remained faster when bacteria were inoculated together with yeast. The difference was considerable for strain EQ54, which failed to reduce malic acid levels below 100 mg liter^{-1} after consecutive inoculation management.

Citric and acetic acids. Citric acid was degraded more rapidly in all treatments with simultaneous yeast and bacterium inoculation, and acetic acid formation was visibly correlated to citric acid degradation in these cases, which is shown for fermentations with strain Alpha in Fig. 2 (data for EQ54 not shown). During fermentations with bacterial inoculation after completion of AF, acetic acid levels increased during the first 2 weeks of AF without a strong correlation to citric acid degradation. Final citric acid concentrations in all wines were similar, while small differences could be measured for acetic acid concentrations, which were higher in treatments with simultaneous inoculation of yeast and bacteria (Table 2).

Acetaldehyde. There was a trend towards higher acetaldehyde residues in fermentations with consecutive AF and MLF, but the differences were not statistically significant (Table 2). However, the course of acetaldehyde levels during fermentations was influenced by the time of inoculation with bacteria. In all wines with bacterial inoculation after completion of AF, higher acetaldehyde maxima were reached, and acetaldehyde levels remained higher until inoculation with bacteria (strain Alpha) (Fig. 3).

Fumaric acid. Similar to the case for acetaldehyde levels, there was a trend towards higher fumaric acid residues in fermentations with consecutive AF and MLF, which was statistically significant in the case of strain EQ54 (Table 2). In all treatments, fumaric acid concentrations reached maxima of 10 to 15 mg liter^{-1} in the first quarter of AF and then decreased. However, after the initial peak, fumaric acid concentrations remained higher in consecutive treatments and decreased after inoculation with malolactic starter bacteria (strain Alpha) (Fig. 4).

Nitrogenous compounds. The time courses of the concentrations of nitrogenous molecules measured, that is, arginine and its metabolites ammonia, urea, citrulline, and ornithine, were very similar in all treatments. Ammonia, which had been supplemented by addition of diammonium phosphate to the must, was exhausted after 10 days in all treatments. Arginine, from an average initial concentration of 508 ± 13 (standard error) mg liter^{-1}, was depleted in all treatments within 14 days. Urea could be detected only in the first 4 days of fermentations and was always below 1 mg liter^{-1}. Citrulline could be detected only in the first 7 days of fermentations and never exceeded 3.4 mg liter^{-1}. Ornithine could be detected in consecutive treatments until day 12, with a maximum values of 11 mg liter^{-1}, whereas it disappeared in simultaneous AF/MLF treatments after 4 days of vinification. None of these nitrogenous compounds could be detected in the final wines (Table 2).

Other wine parameters. No statistically significant differences were found for any combination of treatments for the concentrations of lactic acid (7.88 ± 0.04 g liter^{-1} [mean ± standard error]), glycerol (5.27 ± 0.05 g liter^{-1}), ethanol (13.7% ± 0.25% [vol/vol]), or bound SO_2 per total SO_2 (46.7% ± 0.56%); the total acidity (6.53 ± 0.08 g liter^{-1}); or the pH (3.53) in the final wines (Table 2).

Sensory evaluation. Sensory evaluation of all wines by a triangle discrimination test with a semiexpert panel revealed no statistically significant differences for any treatment combination regardless of the bacterial strain used and the timing of malolactic fermentation.

DISCUSSION

In this work, the simultaneous inoculation of yeast and bacteria into must was compared with a traditional vinification protocol, where MLF was induced by inoculation of bacteria

TABLE 2. Values of several wine parameters after stabilization with sulfur dioxide

Parameter (unit[a])	Value (mean ± SE)[b]			
	Oenococcus oeni EQ54		*Oenococcus oeni* Alpha	
	Simultaneous treatment	Consecutive treatment	Simultaneous treatment	Consecutive treatment
pH	3.54	3.52	3.53	3.53
Total acidity (g liter^{-1})	6.38 ± 0.08	6.75 ± 0.06	6.46 ± 0.08	6.55 ± 0.06
Ethanol (%, vol/vol)	13.0 ± 0.3	13.9 ± 0.3	13.6 ± 0.2	14.1 ± 0.3
Acetaldehyde	5.5 ± 1.8	10.9 ± 1.7	6.8 ± 1.6	9.5 ± 1.9
Bound SO_2/total SO_2 (%)	46.1 ± 0.06	45.6 ± 0.17	46.7 ± 0.07	48.2 ± 0.37
Glycerol (g liter^{-1})	5.3 ± 0.08	5.3 ± 0.02	5.2 ± 0.15	5.3 ± 0.2
Ammonia	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Urea	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Arginine	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Citrulline	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Ornithine	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Malic acid	0.0 ± 0*	356 ± 4.6*	0.0 ± 0*	40 ± 3.8*
Acetic acid	196 ± 2*	149 ± 1.2*	188 ± 7.5	168 ± 7.0
Citric acid	120 ± 20.7	201 ± 3.6	141 ± 8.6	165 ± 0.3
Lactic acid (g liter^{-1})	7.97 ± 0.07	7.78 ± 0.05	7.83 ± 0.05	7.93 ± 0.02
Fumaric acid	1.41 ± 0.01*	1.84 ± 0.01*	1.35 ± 0.13	1.69 ± 0.09
Glucose	0.0 ± 0*	140 ± 17.6*	0.0 ± 0*	140 ± 5*
Fructose	0.0 ± 0*	517 ± 17*	0.0 ± 0*	561 ± 21*
Glucose + fructose	0.0 ± 0*	657 ± 47*	0.0 ± 0*	702 ± 17*

[a] Units are milligrams liter^{-1} unless otherwise stated.
[b] Wines from simultaneous and consecutive alcoholic and malolactic fermentations with two different malolactic strains (EQ54 and Alpha) are compared. Student's *t* test was used to ascertain statistically significant differences (*, statistically significant difference at a confidence interval of 0.01).

after completion of AF. Studies regarding fermentation kinetics and success after inducing AF and MLF simultaneously have been contradictory. It has been reported that at elevated sugar concentrations and after malic acid depletion, formation of high acetic acid concentrations through the sugar metabolism of the mostly heterofermentative wine LAB, i.e., oenococci and lactobacilli, may occur (8, 28, 29, 35). In fact, in the presence of external electron acceptors, such as fructose, acetyl-phosphate could be hydrolyzed to acetic acid with the net formation of an additional ATP from heterofermentation (18, 36), and this physiological response may be partly responsible for the vigorous growth and spoilage caused by certain LAB (13). However, it is now common practice to induce MLF with bacterial strains selected for their beneficial properties with regard to wine quality (26, 38). In addition, even wines considered to be dry after AF, in which MLF is traditionally carried out, can contain significant amounts of glucose and especially fructose, as shown in this study, and thus could provide sufficient substrate for considerable acetic acid formation. Finally, results from other studies suggest that simultaneous AF/MLF both was practical and did not affect acetic acid levels (4–6, 27, 48). Besides making wines unpalatable, the main concern about formation of acetic acid is the inhibition of yeast (23) and thus alcoholic fermentation.

In this study, we used two suitable yeast-bacterium combinations for carrying out simultaneous AF/MLF. Neither an inhibitory effect of simultaneous inoculation of yeast and bacteria on AF nor a negative effect of this technique on final wine quality could be substantiated: While acetic acid levels were slightly increased in simultaneous treatments (Table 2), the differences observed were of neither practical nor legal significance, since sensory thresholds (10) and maximum legal levels for acetic acid range around 1 g liter^{-1}. The higher acetic acid values in wines from simultaneous fermentation management were correlated with a more important degradation of citric acid, which was delayed with regard to the malolactic conversion, suggesting that besides heterofermentation, the metabolism of citric acid significantly contributed to acetic acid formation and confirming recent observations from Chardonnay vinifications (39).

The values obtained for other wine parameters, including pH, total acidity, and the concentrations of acetaldehyde, glycerol, and several nitrogenous compounds, further confirm the similarity of the wines obtained by the different vinification strategies, regardless of the bacterial strain employed. While there was a trend towards higher ethanol concentrations in consecutive vinifications, it was not of statistical significance. Moreover, sensory evaluation did not allow discrimination between any of the treatments.

Nevertheless, some technical and chemical differences of statistical and practical significance were encountered. In fact, overall fermentation times were greatly reduced by inoculating bacteria and yeast concurrently into must. For the *O. oeni* strain Alpha, MLF was completed (malic acid concentration of ≤100 mg liter^{-1} [21]) 46 days sooner in vinifications with simultaneous inoculation of yeast and bacteria. In addition, malolactic conversion was not complete in all consecutive treatments, namely, in vinifications with strain EQ54, where an average malic acid residue of 356 mg liter^{-1} remained, which could lead to reduced microbiological stability during postfermentation handling and in the bottle. Among known potential factors leading to decreased malolactic activity (52, 53), the relatively high ethanol concentration (>13% by volume) in the wines was notable. Fumaric acid, which is present in trace amounts in grape berries (44) and is formed by yeast as a fermentation by-product (15, 45), is degraded by strains of *O. oeni* (12), but it also exerts an inhibitory effect on them (9, 41). The course of fumaric acid concentrations observed in this

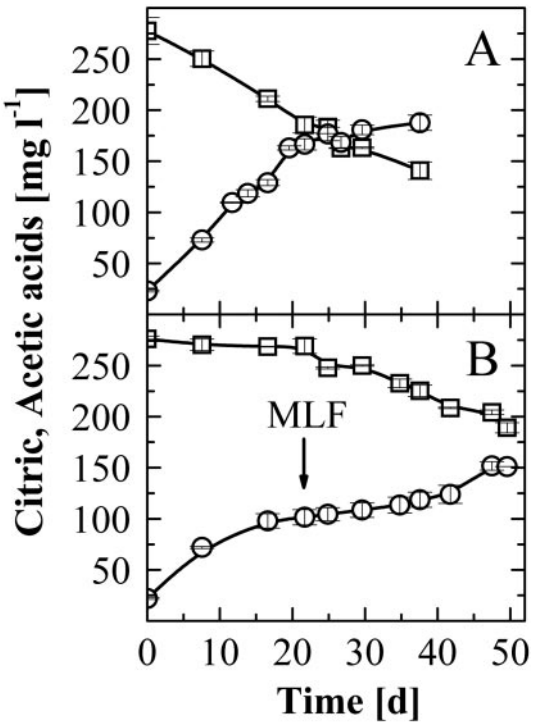

FIG. 2. Time courses of citric acid degradation and acetic acid formation during AF and MLF of Chardonnay with *S. cerevisiae* CY3079 and *O. oeni* Alpha. A, simultaneous inoculation of yeast and bacteria; B, inoculation of bacteria after completion of AF (the arrow indicates inoculation with MLB in consecutive treatment). □, citric acid; ○, acetic acid. Values are means and standard errors.

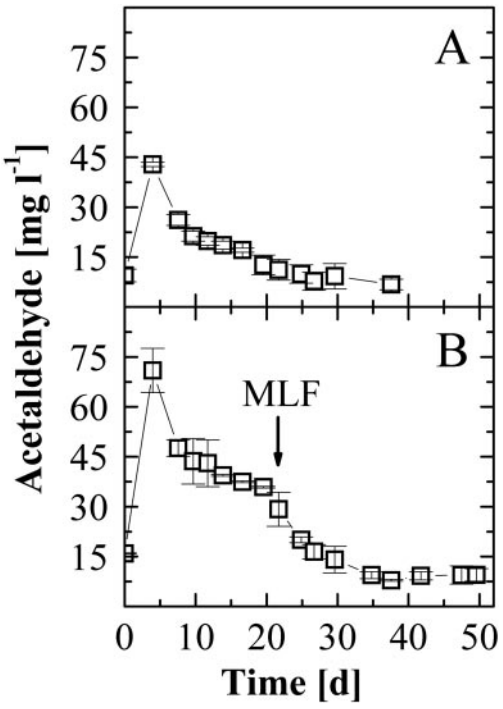

FIG. 3. Time courses of acetaldehyde levels during AF and MLF of Chardonnay with *S. cerevisiae* CY3079 and *O. oeni* Alpha. A, simultaneous inoculation of yeast and bacteria; B, inoculation of bacteria after completion of AF (the arrow indicates inoculation with MLB in consecutive treatment). Values are means and standard errors.

study was in accordance with results from cider (22), but the overall concentrations make an inhibitory effect unlikely. However, with regard to compounds with nutritional value, it could be observed that arginine, which has been reported to be essential for growth of some wine lactic acid bacteria (16, 17) and plays an important role in bacterial growth and pH resistance (7, 37, 51), was depleted in all treatments after 14 days, while MLF was induced only after 21.6 days in consecutive treatments.

Other chemical parameters were greatly different and of microbiological relevance. Both the ratio and final concentrations of glucose and fructose in wine made by traditional, consecutive AF/MLF were typical, with combined concentrations of approximately 700 mg liter^{-1} made up mostly of fructose. The slight increase of glucose concentrations during MLF is likely due to the activity of bacterial glycosidases (19). However, no previous report of the complete depletion of glucose and fructose during vinifications, which was manifest in all treatments where bacteria were inoculated simultaneously with yeast (i.e., six separate fermentations), was found, and these wines were termed "super-dry." Degradation of these amounts of hexoses by heterofermentative lactic acid bacteria would have led to significantly higher acetic and lactic acid concentrations in wines from simultaneous treatments (Table 2), which was not the case. Thus, it is suggested that the yeast metabolism was influenced by the simultaneous inoculation technique.

Acetaldehyde is the most important carbonyl formed during fermentation. It is an important flavor-active compound and a strong binding partner of SO_2, which is essential for wine preservation as an antioxidant and antimicrobial (33). In this work, final concentrations for acetaldehyde were similar across treatments, which was also visible in the percentage of bound SO_2 per total SO_2 at the adjusted free SO_2 level (36 mg liter^{-1}). However, simultaneous AF/MLF displayed lower overall acetaldehyde concentrations during AF and lower maxima, likely due to the rapid degradation of yeast-produced acetaldehyde by bacteria. This is in agreement with recent work showing that acetaldehyde and SO_2-bound acetaldehyde were degraded by wine lactic acid bacteria (40). Since acetaldehyde also is a factor in copigmentation reactions and contributes to red wine color development (49), the effect of simultaneous yeast and bacterial activity on wine chemistry and color will have to be considered more carefully in red wine fermentations. While lower overall acetaldehyde concentrations could be compensated for by aerations, which are usual during skin maceration, oxygen is known to influence the kinetics of bacterial metabolites with strong sensory impact, such as diacetyl (39), and thus, application in red wines will require specific consideration.

Arginine has received considerable attention in the past, as the main yeast fermentable amino acid and also because of its role as a substrate for the formation of the carbamoyl compounds urea and citrulline by yeast and bacteria, respectively. The latter compounds lead to the formation of carcinogenic ethyl carbamate in wines over time (50). The results presented

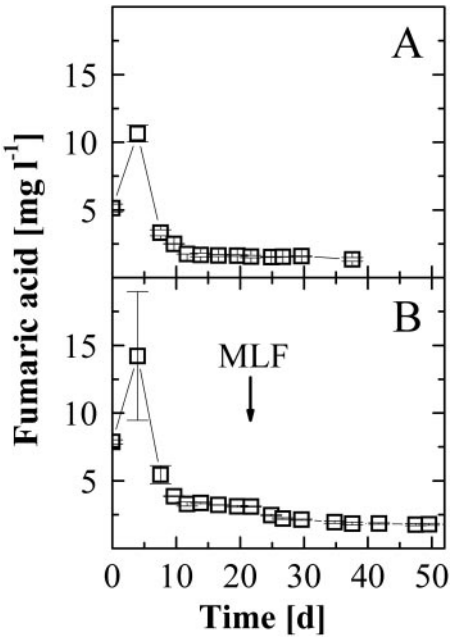

FIG. 4. Time courses of fumaric acid levels during AF and MLF of Chardonnay with *S. cerevisiae* CY3079 and *O. oeni* Alpha. A, simultaneous inoculation of yeast and bacteria; B, inoculation of bacteria after completion of AF (the arrow indicates inoculation with MLB in consecutive treatment). Values are means and standard errors.

here demonstrate that the vinification method chosen had only a small effect on the time courses of arginine and its degradation products, and all metabolites were depleted in the final wines.

In contrast to earlier studies, the current work has found no evidence of a negative impact of simultaneous AF/MLF on fermentation success and kinetics or on final wine parameters. Instead, several aspects suggest a microbiological and technological advantage of applying this fermentation protocol. Specifically, simultaneous AF/MLF may be advantageous for low-pH, cool-climate white musts with high potential alcohol. Successful simultaneous vinification protocols will strongly depend on the selection of suitable yeast-bacterium combinations (1), and future studies should investigate vinification performance with other grape varieties, including red grapes. Further work will address these factors, with a special focus on the occurrence of "super-dry" wines, reproducibility, and physiological significance.

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