Contribution to the Knowledge of Malolactic Fermentation Influence on Wine Aroma

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In this work, we study two aspects of malolactic fermentation: the use of industrial lactic acid bacteria starter-cultures in a white Sauvignon wine and the effects of the bacterial activity on the composition of wines in terms of volatile substances from oakwood during partial malolactic fermentation in barrels. A sensory evaluation was made by the use of discriminative and descriptive methods. The consequences of bacterial development are thus limited, but it is nevertheless possible to make characteristic observations. The carbonyl substances were formed in connection with a more or less fast bacterial growth and a degradation of the citric acid. However, the influence of the bacterial starter-culture is difficult to establish. The concentration of the compounds resulting from wood was higher in the wines after malolactic fermentation compared to a wine not having undergone bacterial development. Greater complexity was perceived during the sensory analysis, with buttered, spiced, roasted, vanilla, and smoked notes. On the other hand, the intensity of the descriptors characteristic of grape variety decreased.

Keywords: *Malolactic fermentation; aroma; sensorial analysis; lactic acid bacteria*

INTRODUCTION

Malolactic fermentation (MLF) is necessary for the aging of red wines and also for certain white wines. Indeed, the bacterial activity plays a role in the stabilization of wine and ensures a decrease in acidity and an enrichment of aromatic composition. This latter is not well-known. The importance attached to the organoleptic contribution of fermentation varies according to authors (Davis et al., 1985). More recently Sauvageot and Vivier (1997) indicated a very low impact of malolactic fermentation on the tasting profile of Chardonnay and Pinot Noir wines.

The main substrates of the metabolism of lactic acid bacteria are malic acid, citric acid, and pentose and hexose traces (Kunkee,1974; Davis et al., 1985). Among the products of the various reactions which occur during malolactic fermentation, only the compounds with lactic or buttered-like odors such as diacetyl or other dicarbonyl compounds have been well studied. Some research is related to the conditions of the synthesis of these compounds (de Revel et al., 1989) and to their contents in wines and their organoleptic consequences (Bertrand et al., 1984; de Revel and Bertrand, 1993, 1994; Henick Kling, 1995). The reduction of dicarbonyl compounds into hydroxy ketones and diols has also been studied (de Revel, 1992). The nature and the quantity of the other substances associated with the activity of lactic acid bacteria in wine remain largely unknown factors. The aromatic impact of bacterial activity is undoubtedly very variable according to types of wines.

Currently, a small number of commercial lactic acid bacteria starter-cultures are able to control the release of malolactic fermentation with a high probability of success. It is necessary to continue research into new starter-cultures in order to improve not only their establishment in wine but also their performance and their consequences on the organoleptic quality of wines.

The present research concerns experiments carried out in 1996 in the area of Bordeaux to investigate the influence of malolactic fermentation conditions in a white Sauvignon wine. They are preliminary observations on a mode of wine making little studied up to now. We particularly considered the nature of the container (new oak-barrels, old oak-barrels after containing one wine, or stainless steel tank) and the origin of the commercial starter-culture. Methods of sensory evaluation (Afnor, 1995) were used to study the consequences of the conditions of malolactic fermentation on the aromatic richness of the wines.

MATERIALS AND METHODS

Experimental Design. All of the wine used in this experiment came from the same must. This must was from the Graves area, vintage 1996. Alcoholic fermentation was carried out with a Sauvignon must in various containers: a stainless steel tank (1.5 HL), three new barrels, and a barrel already used for one year. Malolactic fermentation was started by using two commercial starter-cultures called SBX and SBY.

Sampling was performed during a 64-day period starting 7 days after yeasts addition (alcoholic fermentation start) and ending 71 days after stuck of MLF. Sample-taking points for the analyze (before MLF and after MLF) was carried out before (10-day) and after partial malolactic fermentation in barrels (51-day) and in the tank (51-day). One of the commercial starter-cultures of lactic acid bacteria (SBY) was reactivated for 48 h in 1.5 L of must, and the other was added directly

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Table 1. Experimental Design and Sensory Tests Applied for Wine Tastings

malolactic fermentation	wine storage	bacteria starter-cultures	profile wine no.	triangle test no.
before	stainless-steel tank	SBX	1	1
after	stainless-steel tank	SBX	2	1
before	new oak barrel	SBX	3	
after	new oak barrel	SBX	4	2
after	new oak barrel	SBY	5	2
before	one-year-old barrel	SBX	6	
after	one-year-old barrel	SBX	7	

(SBX) to the wine. At the time of bacterial addition, the temperature of the wine was 19 °C. In addition, the wine in one of the new barrels was sulfited to 4 g/HL after alcoholic fermentation and was used as control without undergoing malolactic fermentation. The experimental design is presented in Table 1.

Moreover, one wine was matured on lees in the new barrels for 8 months and was divided into three batches; one was the control batch not receiving bacteria, while the others were the wines with SBX and SBY added.

Microbiological and Physicochemical Analyses. The microflora was counted three times per week, and the temperature was regularly measured during alcoholic and malolactic fermentation. Traditional analyses (total acidity, volatile acidity, alcohol content, free and total SO_2, reducing sugars) were carried out by the official methods or the usual methods recommended by the International Organization of the Vine and wine (OIV). Malic, citric, and acetic acids were quantified using a sequential analyzer Kone Progress plus version 4.1.

Chromatography was used to measure the content of dicarbonyl compounds (diacetyl and methylglyoxal) by derivation using diaminobenzene (Guillou et al., 1997) and other substances by direct injection: acetoin, acetol, butane-2,3-diol, and propane-1,2-diol (de Revel and Bertrand, 1994), the volatile compounds of fermentation higher alcohols, acetaldehyde, ethyl acetate (Bertrand, 1988) and fatty acids, ethyl esters of fatty acids, hexyl acetate, phenylethyl acetate, isoamyl acetate, ethyl lactate, diethyl succinate (Bertrand, 1981). Volatile compounds resulting from oak wood, *trans*-3-methyloctano-4-lactone, *cis*-3-methyloctano-4-lactone, eugenol, *trans*-isoeugenol, and vanillin, were determined after extraction according to the method described by Barbe and Bertrand (1996).

Sensory Evaluations. Tastings of the wines were carried out by wine experts (teachers and students of the faculty of Œnology). Eleven of them established the sensory profile of the wines, and seventeen took part in the discrimination tests. All the wines were tasted at a temperature of 12 °C and were evaluated by sniffing and tasting. The wines were tasted at the end of alcoholic fermentation, after malolactic fermentation had stopped, and after 8 months of maturing; after clarification and sulfiting, they were stored at 4 °C.

Seven wines (Table 1) were evaluated by quantitative descriptive analysis (AFNOR NF V 09-016). A card of 16 descriptors was proposed, accompanied by a scale of intensity in 8 categories (0−7). The wines were presented in a monadic way (one after the other) in a random order for each subject.

Two triangle tests (AFNOR NF V 09-013) were carried out in order to confirm certain results of the sensory profile. The first consisted in comparing the wine ferments, some before and some after partial malolactic fermentation, and the second in evaluating the influence of the bacterial starter-culture (SBX, SBY) after malolactic fermentation in the new barrels (Table 1). The wines were presented at random with regard to the nature of the repeated wine and to the order of the wines within each triad. Black glasses and a monochromatic light (sodium vapor lamp) were used to mask any possible difference in aspect.

Finally a preference test was carried out to compare the influence of SBX and SBY in new barrels. To approximate the usual conditions of consumption, this type of wine, in general undergoes aging in oak barrels for 8−10 months before bottling. The test was carried out after an 8-month stay in new barrels. The test consisted in a classification of preference of the two wines inoculated by lactic acid bacteria and a control wine not having undergone MLF but having remained in a barrel the same time. This test was carried out by 125 subjects: 63 students and 62 professionals.

Statistical Analyses. The notes were interpreted using the results of a variance analysis (ANOVA) to two factors (wines, judges) carried out on each of the 16 descriptors.

The results of the triangle tests were analyzed by the probability theory that the number of right answers follows a binomial distribution B (n, 1/3) where n is the size of the panel ($n = 17$). Three Friedman tests were applied to the results of the test of preference: all subjects ($n = 125$), students panel ($n = 63$), and panel of professionals ($n = 62$). The products were regarded as differently perceived or preferred for a probability lower than 5%.

RESULTS

Development of Microorganisms. Alcoholic fermentation lasted 7 days in barrels and 10 days in the tank. Yeast populations were not significantly different. The temperature reached a maximum of 23 °C in the tank and 28 °C in the barrels. Thereafter, all of the wines were stored at 22 °C.

In the control wine, after a fall due to sulfiting, the residual indigenous bacterial population was 10^2 to 10^3 cells/mL. In the wines added with commercial bacterial starter-cultures, the maximum population after innoculation was about 5×10^7 to 8×10^8 cells/mL for SBX and SBY (reactivated starter-culture), respectively. After 30 days, a loss of viability led to a stop in malolactic activity in all of the wines (Table 2).

The percentage of malic and citric acids varied from one wine to another (Table 3). In the one added with SBY, the degradation of the acids was greater, and preliminary reactivation allowed for a longer survival of the bacteria.

Table 2. Wine Composition before and after Malolactic Fermentation

	alcohol[a]	T.A.[a]	V.A.[a]	malic acid[a]	pH
Before MLF					
new barrel	12.8	7.05	0.29	3.16	3.21
one-year-old barrel	12.8	7.05	0.29	3.16	3.21
stainless-steel tank	12.6	7.05	0.26	3.19	3.20
After MLF					
new barrel control without MLF	12.9	6.97	0.31	2.99	3.29
new barrel, SBY starter-culture	12.9	6.45	0.41	1.85	3.48
new barrel, SBX starter-culture	12.9	6.82	0.34	2.55	3.33
one-year-old barrel, starter-culture	12.8	6.90	0.33	2.54	3.34
stainless-steel tank SBX starter-culture	12.6	6.97	0.30	2.75	3.30

[a] Alcohol, alcohol content by volume (% vol); T.A., total acidity (g/L tartaric acid); V.A., volatile acidity (g/L acetic acid); malic acid in g/L.

Table 3. Percentage of Acids Removed by Lactic Acid Bacteria in the Different Wines

	% malic acid metabolized			% citric acid metabolized		
	after AF[a]	after MLF[a]	total	after AF	after MLF	total
new barrel, control without MLF	9.5	5.3	14.8	1.5	1.5	3.0
new barrel, SBY starter-culture	9.5	41.5	51.0	1.9	23.6	25.5
new barrel, SBX starter-culture	9.5	18.9	28.4	1.9	16.7	18.6
one-year-old barrel, SBX starter-culture	9.5	19.3	28.8	1.5	17.5	19.0
stainless-steel tank, SBX starter-culture	8.6	13.8	22.4	1.9	14.8	16.7

[a] AF = alcoholic fermention, MLF = malolactic fermention.

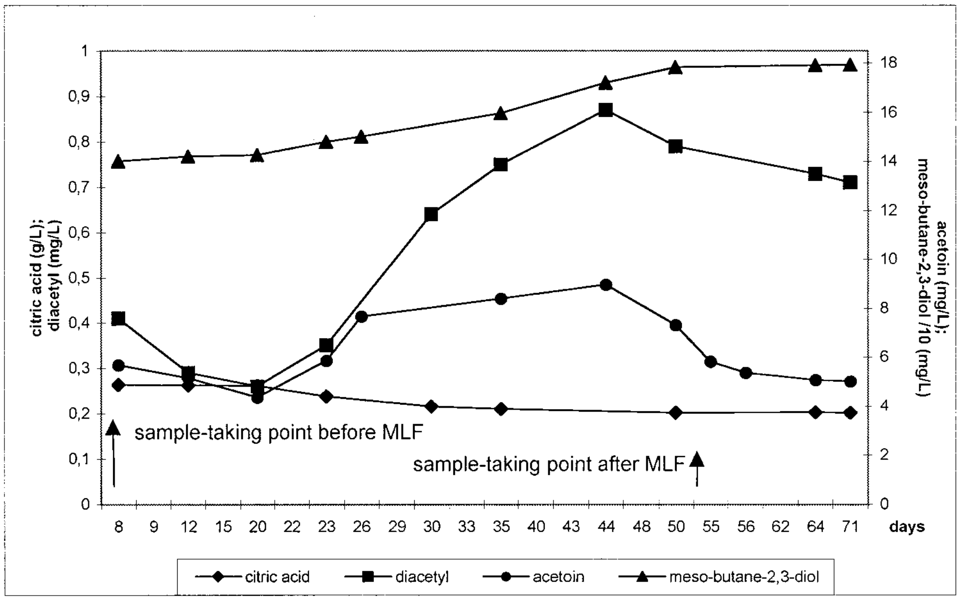

Figure 1. Acid citric degradation and formation of diacetyl, acetoin, and *meso*-butane-2,3-diol during malolactic fermentation occurring in new oak barrel (SBY lactic acid bacteria starter-culture).

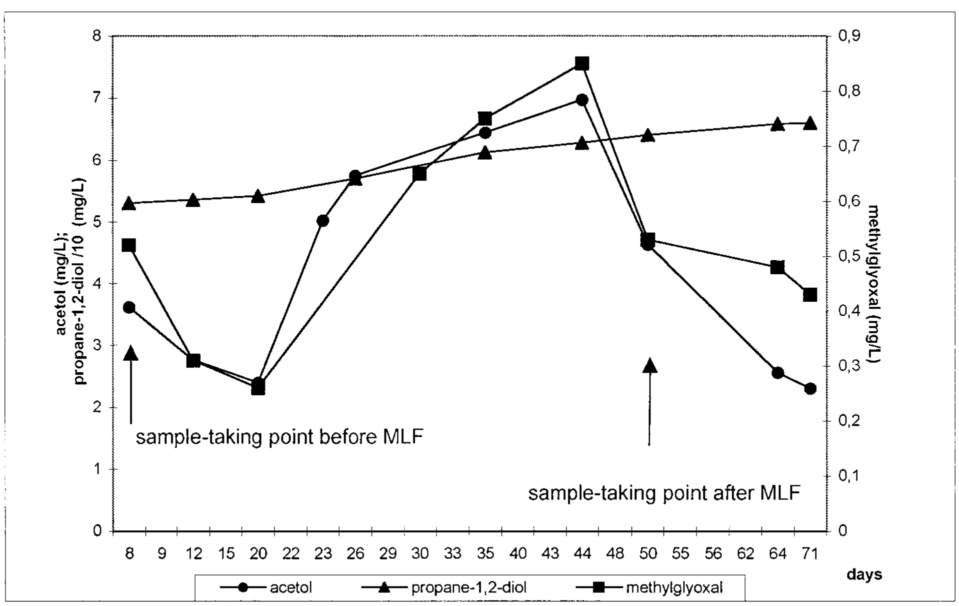

Figure 2. Evolution of methylglyoxal, acetol, and propane-1,2-diol during malolactic fermentation occurring in new oak barrel (SBY lactic acid bacteria starter-culture).

Volatile Compounds. The dicarbonyl compounds, the hydroxy ketones, and the diols were measured regularly during successive fermentations. The evolution of these compounds was very similar in all of the added wines. After a large reduction at the end of alcoholic fermentation, the carbonyl compounds were initially synthesized in variable quantity, lower than 1 mg/L for dicarbonyls, and lower than 10 mg/L for the hydroxy ketones. At the end of malolactic activity, the compounds were reduced into mesobutane-2,3-diol or propane-1,2-diol (Figures 1 and 2). In addition, the concentrations of the carbonyl compounds in the control wine, which was not added with lactic acid bacteria, continued to decrease despite early sulfiting, so the reducing enzymatic activity of yeasts was thus still effective (Tables 4 and 5).

The compounds resulting from the oak wood were measured after alcoholic fermentation and after malo-

Table 4. Methylglyoxal, Acetol, and Propane-1,2-diol Levels (mg/L) after Alcoholic Fermentation and after Malolactic Fermentation in the Different Wines

	methylglyoxal		acetol		propane-1,2-diol	
	after AF[a]	after MLF[a]	after AF	after MLF	after AF	after MLF
new barrel, control without MLF	0.41	0.20	2.98	1.93	40.3	45.6
new barrel, SBY starter-culture	0.31	0.48	2.76	2.56	53.6	65.8
new barrel, SBX starter-culture	0.29	0.31	2.87	0.95	52.0	64.9
one-year-old barrel, SBX starter-culture	0.32	0.35	2.64	0.67	41.0	55.7
stainless-steel tank, SBX starter-culture	0.38	0.20	3.21	0.86	39.0	46.2

[a] AF = alcoholic fermention, MLF = malolactic fermention.

Table 5. Concentrations (mg/L) of Diacetyl, Acetoin, and Butane-2,3-diol Meso Form after Alcoholic and Malolactic Fermentation in the Different Wines

	diacetyl		acetoin		butane-2,3-diol meso form	
	after AF[a]	after MLF[a]	after AF	after MLF	after AF	after MLF
new barrel, control without MLF	0.31	0.23	3.84	2.55	121	123
new barrel, SBY starter-culture	0.29	0.73	5.15	5.06	142	179
new barrel, SBX starter-culture	0.25	0.53	5.09	4.03	132	153
one-year-old barrel, SBX starter-culture	0.29	0.55	3.81	3.97	121	144
stainless-steel tank, SBX starter-culture	0.32	0.31	2.28	2.61	114	131

[a] AF = alcoholic fermention, MLF = malolactic fermention.

Table 6. Volatile Compound Concentration (μg/L) Stemming from Oak Wood Found in the Different Wines

	t-WL[a]	*c*-WL[a]	Eug[a]	isoEug[a]	Van[a]
before malolactic fermentation					
new barrel	22	34	5	8	29
one-year-old barrel	21	32	4	7	28
stainless-steel tank	1	4	3	7	35
after malolactic fermentation					
new barrel, control without MLF	42	115	15	13	89
new barrel, SBY starter-culture	62	118	19	32	180
new barrel, SBX starter-culture	62	112	15	33	163
one-year-old barrel, SBX starter-culture	65	108	13	13	82
stainless-steel tank, SBX starter-culture	6	5	3	15	44
after 8 months in barrel					
new barrel, control	80	214	26	10	197
new barrel, SBY starter-culture	113	234	37	29	298
new barrel, SBX starter-culture	126	195	28	25	272

[a] *t*-WL, *trans*-3-methyloctano-4-lactone; *c*-WL, *cis*-3-methyloctano-4-lactone; Eug, eugenol; isoEug, *trans*-isoeugenol; Van, vanilla.

lactic fermentation, i.e., with 2-month intervals (Table 6). Following alcoholic fermentation, the composition of the wines did not show large differences according to the barrel age. In the stainless steel tank, the wine had extremely low contents of the substances. After 2 months, the control wine placed in the new barrel with no malolactic fermentation had increased concentrations of *trans*-3-methyloctano-4-lactone, *cis*-3-methyloctano-4-lactone, eugenol, *trans*-isoeugenol, and vanillin because of dissolution in the course of time and the possible activity of the yeast lees. In the one-year-old barrel, the wine with partial malolactic fermentation had comparable concentrations to those in the control wine. On the other hand, in the new barrels, malolactic fermentation (even partial) caused a much greater increase in aromatic volatile compounds compared to the control wine: 15% more for isomer trans of the oak lactone, 150% more for isoeugenol, and more than 90% for vanillin (89 μg/L in the control wine and 180 μg/L with SBY). There was little difference with regard to eugenol and cis isomer of oak lactone. After 8 months of aging in new barrels, the differences remained, with a large increase in oak lactone, isoeugenol, and vanillin. Otherwise, with SBX, the concentrations in the wines were, in general, lower than those found with SBY.

In addition, among the volatile compounds of fermentation (results not communicated), various higher alcohols did not have significant variations, since 50 days after alcoholic fermentation, only isoamyl alcohol increased by 9 mg/L, including in the control wine. The ethyl esters of fatty acids had not increased, and higher alcohol acetates had decreased by 40% due to normal chemical hydrolysis. There was an increase in free fatty acids in the course of time, which was not modified by bacterial activity. On the other hand, bacterial activity created a large synthesis of ethyl lactate correlated with the percentage of degradation in malic acid. The increase in ethyl acetate was also great: 25% in the tank and 20% on average in barrels, whatever the starter-cultures (10% of increase in the control without lactic acid bacteria). In addition, SBY which degraded more citric acid, produced more volatile acidity during malolactic fermentation (0.12 g/L in acetic acid), whereas this production was not significant in the other cases (Table 2).

Sensory Analyses. The results of variance analyses carried out on the sensory profile (Table 7) underline a significant judge effect on all of the descriptors. This reflects a different use of the scale by the tasters. However, the wines could be distinguished significantly on the following descriptors: passion fruit, vegetal, grapefruit, buttered, spiced, roasted, vanilla, and smoked. On the other hand, the wines were not perceived as different for the descriptors: lemon, alcohol, roundness, bitter, acid, fresh cream, mild tobacco, and lactic.

The averages of the notes of the judges for the seven wines are presented in Figure 3 for the discriminating descriptors. The wines having undergone malolactic

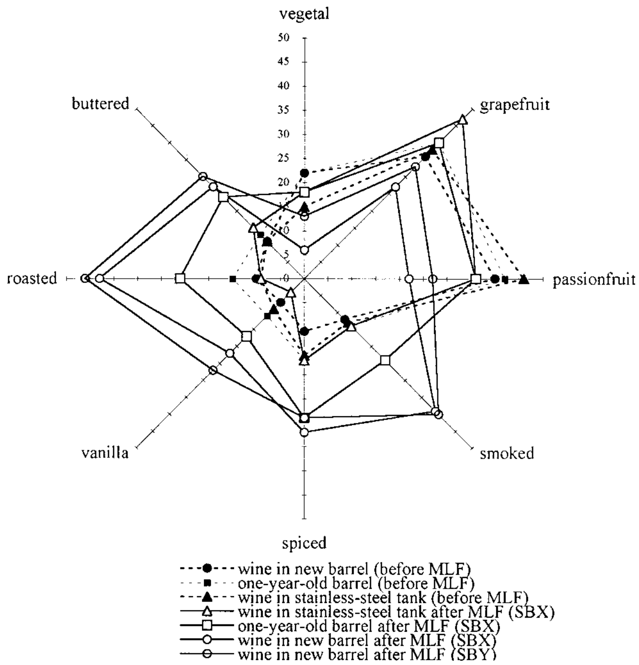

Figure 3. Presentation of 8 descriptors (average of the note of 11 tasters) made it possible to differentiate wines before and after malolactic fermentation (MLF) in different tanks (stainless steel or oak barrels) with two starter-culture preparations (SBX, SBY).

Table 7. Probability Levels (%) Associated with *F* Values of the Two-Factor ANOVA (Wine, Judge) for the 16 Sensory Attributes and across the 7 Wines

descriptor	wines	judges
passion fruit	0.0004	<0.0001
vegetal	0.0001	<0.0001
grapefruit	0.0400	0.0001
buttered	0.0083	0.0010
spiced	0.0004	<0.0001
roasted	<0.0001	0.0021
vanilla	0.0005	0.0001
smoked	<0.0001	<0.0001
lemon	0.3636	<0.0001
alcohol	0.6537	<0.0001
roundness	0.6388	0.0003
bitter	0.8472	0.0032
acid	0.0983	<0.0001
fresh cream	0.2603	0.0002
mild tobacco	0.3904	<0.0001
lactic	0.3802	0.0006

fermentation in the barrel were perceived as more roasted, spiced, smoked, buttered, and vanilla than the wines not having undergone malolactic fermentation or resulting from malolactic fermentation in the tank. The wines in new barrels could be distinguished from those taken from the one-year-old barrel by their more marked roasted and smoked notes. On the other hand, these two wines were less grapefruit, passion fruit, and to a lesser extent vegetable than all of the other wines. In addition, malolactic fermentation in the tank did not generate the development of particular olfactory properties except for a little more marked grapefruit character. The triangle test carried out on these two wines confirmed this result with a nonsignificant difference (9 right answers out of 17). Finally the difference between the two bacterial starter-cultures was not great. Nevertheless, SBY led to slightly stronger intensities on all of the descriptors, except spiced. The triangle test on these two wines gave a significant difference with the threshold of 0.1% (13 right answers out of 17). On the other hand, the test of preference carried out on the wines kept for 8 months in new barrels did not indicate significant preferences either with the students or the professionals ($p > 0.05$ for the analyses carried out for each panel and for both). Nevertheless, there was an identical total classification in all cases (control < SBX < SBY). Moreover, after 8 months, the wines had different odors and flavors which were more or less marked by type of grape variety or by wood odor, in particular for SBY (results not communicated).

DISCUSSION

The halt to malolactic activity before malic acid had been completely fermented did not make it possible to observe the complete influence of bacterial activity on these Sauvignon wines. The possible presence of a large population of phages was checked, but this was lower than 10 phages/mL (Poblet and Lonvaud-Funel, 1995). Poor adaptation of the lactic acid bacteria population in the particularly difficult conditions of fermentation due to the composition of these white wines seems to be the reason for the premature halt in malolactic fermentation. This partial fermentation led to a drop in total acidity, which was high for this particular year, with a low increase in volatile acidity proportional to the degradation of citric acid.

The dicarbonyl and hydroxycarbonyl compounds were at higher concentrations in the wines having carried out their malolactic fermentation, but these levels were close to those obtained after alcoholic fermentation. These compounds have higher levels in the middle of fermentation, as described previously (de Revel et al., 1995). Here, the final contents were low and well below the threshold of tasting, which is near to 5 mg/L for diacetyl in a white wine (Bertrand et al., 1984). Starter-culture SBY seems to produce more dicarbonyl compounds, but this remains to be confirmed in complete malolactic fermentations. Small volumes such as the barrel have the advantage of allowing the fast reduction of carbonyl compounds by greater contact of the wine with the lees (yeasts or bacteria). This reduction is also faster at the high temperatures maintained for the development of the bacteria. Malolactic fermentation in a barrel thus leads to a discrete lactic flavor (often rejected by tasters if it is too marked) and which harmonizes well with the flavors of wood.

With regard to the products extracted from the oak wood, after malolactic fermentation in new barrels, very high concentrations are noted which could be due to intervention of the bacteria on the wood substances. These results are interesting, because they show that there is a difference between wines: on one hand in connection with the nature of the commercial starter-cultures, on the other hand with malolactic activity. If malolactic fermentation occurs in a wine placed in an one-year-old barrel, the oak lactone, eugenol, and vanillin contents are comparable with those of the same wine kept in a new barrel but not having undergone malolactic fermentation. These results point to a direct action of the bacteria on the contents of this type of molecule during malolactic fermentation in a barrel. Early filling of a cask at "high" temperature, leading to the dissolution of oak compounds, is not the only reason for the large concentrations generally found during malolactic fermentation in barrels.

The differences in concentration observed, in particular regarding the substances extracted from wood, are for certain molecules high enough to be perceived by the tasters. This is the case for the oak lactone and vanilla in connection with their organoleptic threshold defined by Boidron et al. (1988), 120 μg/L and 400 μg/L, respectively. On the other hand, eugenol, whose threshold for the white wines is 100 μg/L, is found in lower concentrations. However, the profiles of these wines (Figure 3) seem to indicate differences in certain perceptions such as roasted and smoked caused in particular by eugenol or isoeugenol.

Here, the results of the sensory profile also show that wines are not very different before or after partial malolactic fermentation in a tank (starter-culture SBX), unlike the situation in new barrels (starter-culture SBX). In the case of wines in the tank, the intensities for each descriptor were very similar before and after malolactic fermentation. These results are confirmed by those of the triangle test. In new barrels, the bacterial activity and the contact with new wood increased the perceptions buttered, roasted, vanilla, spiced, smoked and decreased the grapefruit, passion fruit, and vegetal notes. These descriptors are regarded as typical of grape variety. Their perception strongly decreases, either by the increase in the intensity of the other descriptors or by the intervention of the bacteria on the substances responsible for these flavors.

As shown in the sensory profile, the wines fermented with SBY appear slightly more aromatic than those fermented with SBX. They were significantly discriminated in the triangle test. Malolactic fermentation and 8 months of aging in new barrels strongly marked the wines by decreasing the typicity of the grape variety. The perception of wood was variable according to the starter-culture. Wines having undergone malolactic fermentation were not rejected by the tasters who appreciated their complexity, although no significant preference could be established.

CONCLUSION

Tests were carried out to specify the organoleptic modifications which Sauvignon white wine undergoes during malolactic fermentation. The influence of various fermentation conditions (commercial starter-cultures of bacteria and use or not of barrel) was examined using chemical and sensory analyses. Despite incomplete malolactic fermentation in all of the batches, interesting results were observed suggesting new paths of research.

From an aromatic point of view, the consequence of malolactic fermentation in barrel was described by the terms smoked, roasted, and to a lesser extent by the descriptors vanilla, spiced, and buttered. Malolactic fermentation in new barrels accentuated the intensity of these descriptors. Differences according to starter-cultures were noted and the chemical analyses confirmed these differences for some compounds: vanillin, oak lactone, and eugenol. On the other hand, with regard to the carbonyl compounds responsible for lactic notes, the differences were less pronounced.

A first approach to the organoleptic consequences of the use of commercial starter-cultures of lactic acid bacteria has therefore been made. These studies should be continued on other recent starter-cultures.

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