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Changes in Red Wine Soluble Polysaccharide Composition Induced by Malolactic Fermentation

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The polysaccharide content of wine is generally assumed to originate from grapes and yeasts, independent of bacterial metabolism, except for the action of certain spoilage species. This study shows that malolactic fermentation (MLF) significantly modifies the soluble polysaccharide (SP) concentration of various red Bordeaux wines. Wines with the highest initial SP concentration go on to present decreased SP concentration, whereas those with the lowest initial SP concentration rather go on to have a higher SP concentration after MLF. These tendencies were observed whatever the *Oenococcus oeni* strain (indigenous or starter) used for MLF. Neutral and charged SPs were affected, but to a degree that depended on the microorganisms driving the MLF. The SP modifications were directly linked to bacterial development, because non MLF controls did not present any significant change of SP concentration.

KEYWORDS: Soluble polysaccharides; *Oenococcus oeni*; malolactic fermentation; wine

INTRODUCTION

Polysaccharides constitute the most complex high molecular weight molecules in wine. The difficulty in separating and purifying them has been stressed in several studies (*1–5*). As a result, polysaccharides have been studied less than polyphenolic compounds, the other group of macromolecules present in wine.

Wine polysaccharides are mainly soluble (*6*) and can be classified into two distinct groups: those originating from grapes (arabinans, A; arabinogalactans, AG; arabinogalactan proteins, AGP; rhamnogalacturonans, RG I and RGII; and homogalacturonan oligomers, HG) and those liberated by microorganisms [glucans produced by *Botrytis* on infected grapes or mannoproteins (MP) released by yeasts during alcoholic fermentation or aging on lees (*3*, *7*)]. The various polysaccharides can interact together, but also with other wine molecules. Hence, they can significantly modify the self-aggregation of tannins or disrupt protein–tannin aggregates, thus decreasing the astringency of wine and increasing the sensation of fullness on the palate (*8–11*). Other polysaccharides also increase color stability (*12*), prevent protein haze (*13*), or affect tartrate salt crystallization (*14*). As a result, polysaccharides are involved in several colloidal phenomena during winemaking such as precipitation, color stabilization, and filter blocking (*3*).

Malolactic fermentation (MLF) follows alcoholic fermentation. The main change observed during MLF (in terms of quantity) is the transformation of malic acid into lactic acid. It occurs after significant development of a lactic acid bacteria population (10^6 CFU/mL), essentially *Oenococcus oeni* (*15*). Many other known and, until recently, unknown metabolic transformations occur during MLF. These undoubtedly have a major effect on wine quality. Nowadays, MLF is recommended for most red wines (and sometimes for white ones), especially when they are meant to age in oak barrels or even in bottles (*15*).

The evolution of polysaccharide content during MLF is considered to be relatively stable and thus not worthy of further study (*3*). However, lactic acid bacteria produce significant glycosidase activity likely to liberate aromas or other aglycons from the heterosidic molecules present in wine (*16–18*). This strongly suggests that bacteria driving MLF also hydrolyze the osidic bonds present in polysaccharidic material.

On the other hand, certain strains of *O. oeni* isolated in our laboratory were shown to produce a β-glucan (*19*), supporting the view that polysaccharide concentration increases during MLF. Moreover, preliminary results showed that, in a model medium, certain *O. oeni* strains, such as starter Lactoenos SB3, present outstanding soluble polysaccharide (SP) production capacities together with highly efficient glycolytic activities (*20*). In addition, *O. oeni* genome analysis reveals the presence of various glycosidase and glycosyltransferase genes (*21*).

The aim of the present work was to answer the question of whether or not wine polysaccharide composition changed during MLF and to determine if a specific group of polysaccharides is

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Table 1. Evolution of Estimated SP Concentration during MLF, after Inoculation of Red Wines with Lactoenos SB3, in Different Cellars of the Bordeaux Region and Three Consecutive Years

wine	variety	year	MLF in[a]	initial pH of the wine	duration of MLF (days)	[SP] i[b] (mg/L)	[SP] f (mg/L) in non MLF controls[c]	[SP] f[d] (mg/L)
A	Merlot	2005	barrels	3.61	8	763 ± 31	751 ± 45	430* ± 26
B	Cabernet Sauvignon	2004	barrels	3.68	28	514 ± 23	521 ± 8	397* ± 25
C	Cabernet Sauvignon	2004	bottles	3.62	11	480 ± 24	492 ± 15 *495 ± 24*	289* ± 22
D	Cabernet Sauvignon	2004	barrels	3.45	16	481 ± 21	510 ± 33	403* ± 20
E	Merlot	2004	barrels	3.52	38	425 ± 22	433 ± 31	452 ± 18
F	Merlot	2006	barrels	3.48	17	368 ± 7	ND	505* ± 11
G	Merlot	2006	barrels	3.65	19	350 ± 33	ND	511* ± 14
H	Cabernet Sauvignon	2005	bottles	3.72	16	338 ± 20	353 ± 28	328 ± 21
I	Merlot	2005	barrels	3.53	28	331 ± 25	326 ± 21	397* ± 18
J	Cabernet Sauvignon	2006	barrels	3.65	22	327 ± 9	ND	284* ± 21
K	Merlot	2006	bottles	3.51	19	322 ± 17	331 ± 21	358 ± 24
L	Merlot	2004	bottles	3.51	11	308 ± 11	297 ± 17	281 ± 19
M	Merlot	2006	bottles	3.48	22	301 ± 25	308 ± 7 *317 ± 22*	279 ± 30
N	Merlot	2006	bottles	3.75	15	297 ± 11	312 ± 22 *299 ± 17*	479* ± 10
O	Merlot	2005	barrels	3.76	16	254 ± 17	270 ± 31	350* ± 13
P	unknown red	2004	bottles	3.60	33	252 ± 21	251 ± 26 *256 ± 13*	388* ± 7
Q	Merlot	2004	bottles	3.56	29	216 ± 14	229 ± 21 *218 ± 15*	293* ± 3
R	Merlot	2004	barrels	3.79	45	215 ± 16	204 ± 17	361* ± 11
S	Cabernet Sauvignon	2004	bottles	3.51	18	204 ± 12	215 ± 12	243* ± 5
T	Merlot	2005	bottles	3.63	27	203 ± 10	222 ± 26	356* ± 15

[a] MLF trials were conducted in barrels in the winery cellars and in bottles in the laboratory. [b] SP concentration at the end of alcoholic fermentation (initial), $n = 3$ independent measurements. [c] Non MLF controls: samples kept at 10 °C or (*values in italic*) at 20 °C, $n = 3$ independent measurements. ND, not done. [d] SP concentration after MLF started with Lactoenos SB3 (final), $n = 6$ or more (at least two independent MLF trials and three measurements for each). An asterisk indicates significant change between initial and final SP concentration.

affected by spontaneous or induced MLF. We also compared the changes we observed to those in other macromolecules such as tannins.

MATERIALS AND METHODS

Bacterial Strains. Lactoenos SB3, 450 Preac, B1 (Laffort Oenologie, France), and VF (Martin Vialatte Oenologie, France) were used as *O. oeni* malolactic starters.

MLF Trials. After completion of alcoholic fermentation in the winery cellar, red wines were collected, analyzed for SP concentration, and then distributed into bottles (10 L) for laboratory trials or into barrels for winery trials (**Table 1**). For each wine, we made one spontaneous MLF trial, two or more MLF trials using starter SB3, and one trial with each of the other starters. **Figure 1** presents the various trials and controls made with wine C. The wines were inoculated separately with 10 mg/L of each commercial bacterial preparation (Lactoenos SB3, 450 Preac, B1, and VF), previously re-hydrated in sterile distilled water as specified by the manufacturers. Malolactic fermentation was carried out in the absence of lees, at 20 °C for laboratory scale assays, and at the wine cellar temperature for winery trials. Winemaking was carried out without the need for extracting enzymes.

After starter addition, the inoculation rate was checked by enumerating the lactic acid bacteria in the wine. Initial populations of 10^6 CFU/mL were routinely achieved. Viable cell counts were done by plating diluted wine on solid MRS broth (agar 2% w/w) containing 50 mg/L Delvocid (DSM) to inhibit yeast growth. Plates were incubated under anaerobic conditions for 10 days.

For each wine, we also analyzed samples from control barrels that were not inoculated, in which MLF was therefore conducted by indigenous flora. Non MLF controls were done using either (i) red wine samples stabilized by sulfur dioxide (50 mg/L) addition that were kept at 10 °C for 2 months or (ii) red wine samples sterilized by filtration (0.2 μm membrane cutoff) that were kept at 20 °C for 2 months.

Residual malic acid concentration was measured using an enzymatic in vitro test (Roche, no. 10 139 068 035). The absence of malic acid degradation was checked in non MLF controls. When half of the total malic acid was degraded in the inoculated wines, an implantation control of starters was performed using a PFGE technique according to a previously described method (*22*). PFGE profile comparison enabled verification that the predominant *O. oeni* strain in the wine was the inoculated starter. The strains of bacteria conducting spontaneous MLF were unknown, and no PFGE control was done. When MLF was completed (malic acid exhaustion), wine was stabilized by adding sulfur dioxide (50 mg/L). Conventional analyses (pH, alcohol content) were carried out according to the methods recommended by the International Organization of the Vine and Wine (OIV). Residual glucose and fructose concentrations were measured using an enzymatic in vitro test (Roche, no. 10 139°106°035).

Soluble Polysaccharide (SP) Analysis. The wine was centrifuged (10000*g*, 5 min, 20 °C) to eliminate insoluble matter. SP content was then analyzed without preliminary concentration, unless otherwise stated in the text. In this instance, 15 mL of wine was put into Amicon Ultra tubes (Millipore) with a 5 kDa membrane cutoff and centrifuged (5000*g*, 4 °C) for as long as it took for the retentate to reduce to half the volume of the original sample. It was then analyzed like the other samples.

Three volumes of 96% ethanol containing 5% 1 M HCl was added to the supernatant to precipitate the soluble polysaccharides. The tubes were left to stand for 24 h at 4 °C. They were then centrifuged (18000*g*, 5 min, 4 °C), and the pellet was washed with 80:20 (v/v) ethanol/water, centrifuged again, dried for 20 min at 65 °C, and dissolved in distilled water. The amount of total polysaccharides was determined by the phenol–sulfuric acid method, with glucose as the standard (*23*). The determination was done in triplicate. The result was considered as an estimated concentration because wine SPs contain glucose but also many other monosaccharides.

Samples for polysaccharide analysis were taken at the end of alcoholic fermentation just before inoculation with malolactic starters (initial estimated SP concentration) and at the stage of malic acid exhaustion (final estimated SP concentration). Delta SP is the difference between final and initial SP concentrations.

Size Exclusion Chromatography. Wine samples (100 mL) were centrifuged (10000*g*, 20 min, 4 °C), and macromolecules from the

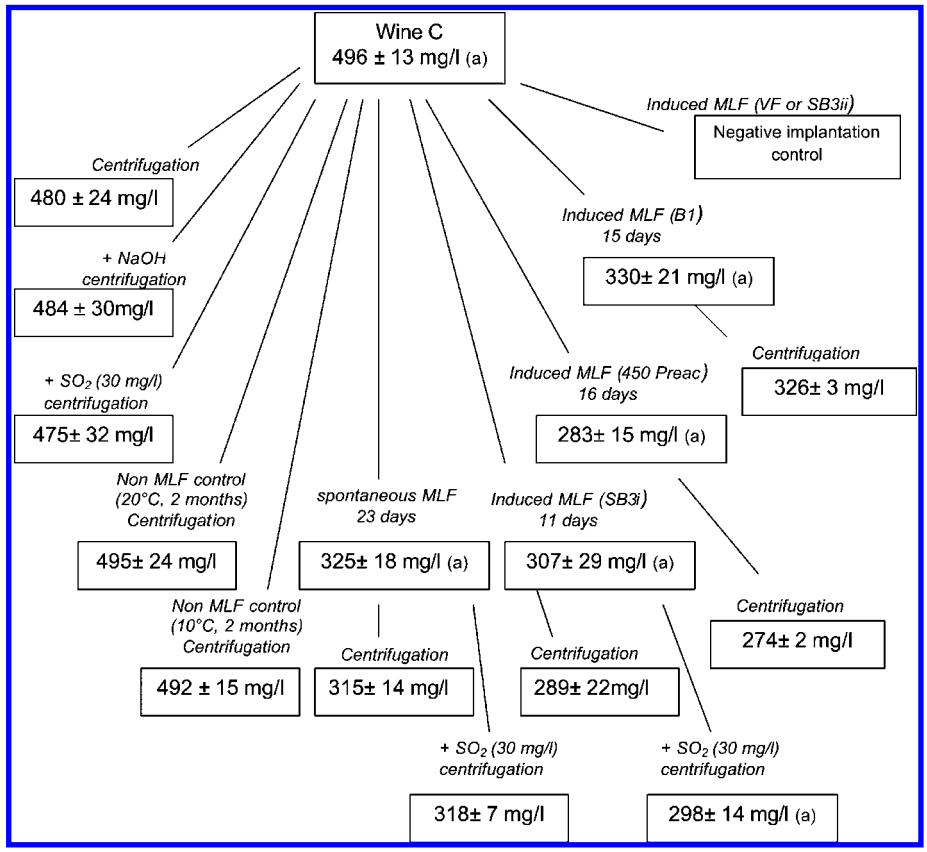

Figure 1. Overview of the MLF trials and controls made with wine C. SP concentrations were measured on supernatant after centrifugation (10000g, 5 min, 20 °C) except indicated (a), where SP concentrations were measured on whole wine. Two MLF trials were made using starter SB3 (i and ii).

supernatant were precipitated with 3 volumes of 95% ethanol containing 5% 1 N HCl. After 24 h at 4 °C, the pellet was recovered by centrifugation (18000*g*, 20 min, 4 °C), washed with 80% ethanol, resuspended in water, and freeze-dried.

The freeze-dried powder (30 mg) was resuspended in 3 mL of elution buffer (50 mM K_2HPO_4, 150 mM NaCl, pH 7.0) and analyzed using a Sephacryl S400 HR column (Amersham Biosciences, 1.6 × 83 cm), eluted at a 1.2 mL/min flow rate with a Waters 515 pump. Detection was done with a Waters 2414 refractometer. Column calibration was carried out with commercial dextrans having a molecular mass ranging from 15000 to 2×10^6 Da.

Monomer Composition Analysis. Monomer composition of the SPs contained in the freeze-dried powder (see above) was determined after acidic hydrolysis (2 N H_2SO_4, 6 h, 100 °C).

Neutral monomer composition was determined by gas–liquid chromatography of alditol acetate derivatives using inositol as the internal standard. Sugar analysis was performed with an Agilent 6850 series GC system equipped with an ESP2380 macrobore column (25 m × 0.53 mm).

Uronic acid titration was carried out according to two different techniques. In the first one, uronic acid content was measured using the Blumenkrantz method (*24*). In the second, analysis of uronic acids was done by high-performance anion exchange liquid chromatography (HPAEC) with pulsed amperometric detection (PAD) using a CarboPak PA10 (4 × 250 mm) column from Dionex, eluted with 60 mM NaOH and 10 mM sodium acetate at 1 mL/min at a temperature of 35 °C. The system was calibrated with glucuronic and galacturonic acids from Sigma. Both methods produced similar results.

Tannins–Polyphenols. Tannin concentration was determined according to the Ribereau-Gayon and Stonestreet method (*25*). The optical density at 280 nm of a 100-fold diluted wine sample made it possible to estimate the total polyphenol index (TPI).

Gelatin Index. The reactivity of tannins toward salivary proteins is responsible for wine astringency. The gelatin index reflects tannin reactivity toward gelatin and is supposed to mimic the phenomena occurring in mouth.

Five milliliters of a gelatin solution (70 g/L, Gecoll, Laffort Oenologie, France) was added to 50 mL of wine. This wine and a control (the same wine without gelatin) were gently shaken at 4 °C for 72 h. The samples were then centrifuged (10000*g*, 4 °C, 20 min), and the tannin concentration in the supernatant was measured. The gelatin index (GI) is defined as

$$GI = ([\text{tannins}]_{\text{control}} - [\text{tannins}]_{\text{gelatin}})/[\text{tannins}]_{\text{control}}$$

Color Analysis. The wine color intensity was defined as the sum of the optical densities measured with a 1 mm cuvette at 420, 520, and 620 nm. The OD_{420}/OD_{520} ratio enabled us to analyze the tone. This ratio was proposed by Glories (*26*) to analyze the color change from blue to yellow upon aging.

Data Analysis. The statistical significance of differences between means was calculated by analysis of variance (ANOVA) followed by Tukey post hoc comparisons ($P < 0.05$).

RESULTS

Total SP Analysis. The analysis of SP concentration of 20 red wines from various cellars in the Bordeaux region was carried out for three consecutive years: 2004, 2005, and 2006 (**Table 1**). The various wines differed in terms of not only grape variety, vintage, and extraction and maceration methods but also yeast strains responsible for alcoholic fermentation. They all displayed an alcohol degree of between 11.5 and 13° and a residual glucose and fructose concentration below 1 g/L.

To avoid heating the wine samples or losing material on microfilters, we analyzed the SP concentration after alcoholic precipitation without any preliminary concentration (filtration, evaporation), even though several authors recommended this (*6*, *27*). We nevertheless obtained highly reproducible SP concentrations with a standard deviation lower than 10% with all of the assays (**Table 1**). This standard deviation was similar to that currently obtained using nonchromatographic quantifica-

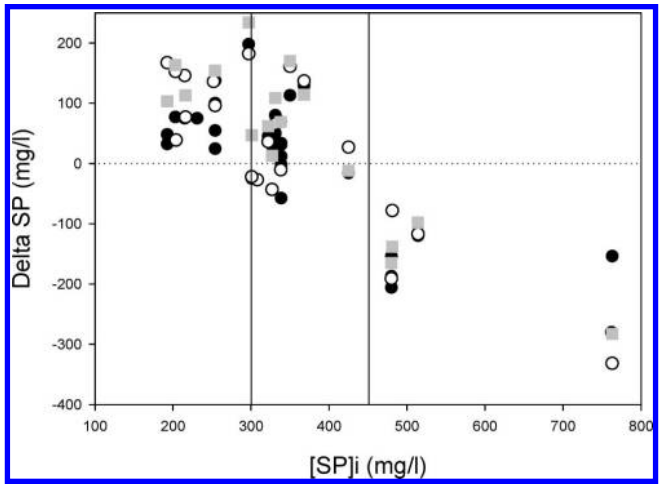

Figure 2. Variation of the estimated SP concentration (Delta SP, see Materials and Methods) during MLF as a function of the initial SP concentration $[SP]_i$: (gray squares) spontaneous MLF conducted by the indigenous microflora; (open circles) MLF induced by starter SB3; (black circles) MLF induced by other starters (450 Preac, B1, or VF). Each point represents delta SP (mean of three measurements) for one MLF trial where the malic acid concentration significantly decreased within 2 months and where the inoculated starter did actually settle (implantation control positive).

tion methods after alcoholic precipitation of polysaccharides (*6*, *27*). Moreover, wines M and N, as well as a third wine with an initial SP concentration of 240 g/L, were concentrated 2-fold by ultrafiltration before and after MLF. At this concentration level, we did not observe any improvement in SP precipitation. Unfortunately, this method did not enable us to concentrate our wine samples any further.

The estimated SP concentration before MLF varied from 200 mg/L to nearly 800 mg/L. All of the wines challenged MLF spontaneously or after inoculation with the different *O. oeni* starters selected for the study (B1, SB3, 450 Preac, or VF). The duration of MLF for the same wine varied greatly depending on the bacterial strain used for inoculation (**Figure 1**). Spontaneous MLF was sometimes delayed or even stopped. Moreover, after starter addition, MLF sometimes remained uncompleted or the implantation control was negative (wrong PFGE profile). These trials were not analyzed for SP content (**Figure 1**). **Table 1** shows the results obtained in 20 wines inoculated with strain SB3. The significant degradation of malic acid and the positive implantation control proved that strain SB3 did actually settle and drive MLF in all 20 wines. In 15 of the 20 wines, estimated SP concentration was significantly modified after MLF with strain SB3. **Table 1** can be divided into three categories of wines. The first consists of wines A–D, with high initial estimated SP concentration (>450 mg/L); this decreased significantly after MLF with strain SB3. The third consists of wines N–T, with a low initial estimated SP concentration (<300 mg/L); this increased significantly after MLF with strain SB3. The second (wines E–M), intermediate, group combines various tendencies (positive, negative, or not significant). As shown in **Table 1**, the initial estimated SP concentration and the variation in total SP content had no influence on MLF duration after the addition of starter SB3.

Figure 2 shows that each wine exhibited a similar variation in SP concentration (accumulation or loss) after MLF whether initiated by starters such as SB3, 450 Preac, B1, or VF or spontaneous. The wines with the highest initial estimated SP concentration underwent a decrease after MLF. In contrast, the wines with the lowest SP concentration always displayed an increase after MLF.

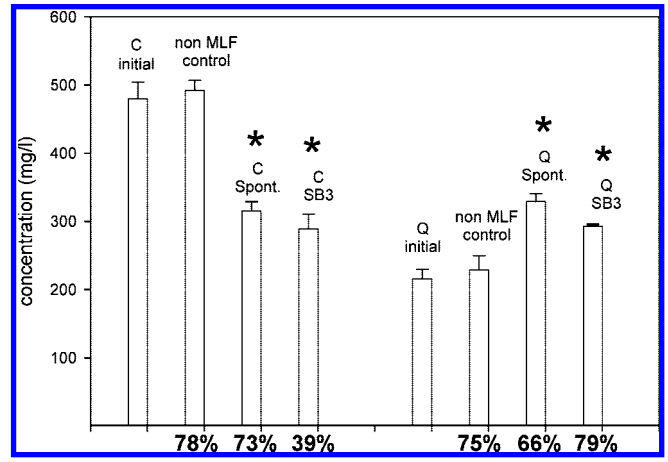

Figure 3. Estimated SP concentration at the beginning of MLF (initial), in non MLF controls, and at the end of spontaneous MLF (Spont.) or inoculated (SB3) ($n = 3$ for ANOVA; *, significantly different from initial); for each bar, the proportion of neutral monosaccharides in the SP is indicated as a percentage.

We wished to prove that the variation in SP concentration was linked to bacterial development during MLF. To do so, wines C, M, N, P, and Q (with initial estimated SP concentrations of 480, 301, 297, 252, and 216 mg/L, respectively) were sterilized by filtration after alcoholic fermentation and incubated for 2 months at 20 °C. Moreover, wine samples were left for 2 months at 10 °C. No bacterial development, no malic acid consumption, and no significant change in estimated SP concentration were observed (**Table 1** and **Figure 1**).

All wines contained suspended particles both before and after MLF. These were eliminated by centrifugation before SP concentration measurements. We decided to see whether or not SP concentrations were identical when measurements were taken in wines containing particles (experiments carried out on wines C, F, G, J, K, M, N, and Q). In fact, the difference was nonsignificant except in the case of wine G, in which insoluble material accounted for 5% of the total estimated SP content after MLF initiated with Lactoenos SB3 or 450 Preac (data not shown). However, most of the wines did not contain suspended insoluble polysaccharides either before or after MLF (example of wine C in **Figure 1**).

Soluble Polysaccharide Composition of Two Different Wines. Two wines, C and Q, representing wines with decreased or enhanced SP production after MLF, were chosen for further analysis. Both wines came from the same cellar and were made in a similar way. They differed only in terms of grape variety (C, Cabernet Sauvignon; and Q, Merlot). During MLF, both wines presented a significant variation in SP concentration during spontaneous or induced MLF (**Figure 3**).

Wine C had an initial pH of 3.62 and wine Q an initial pH of 3.56. During MLF, pH increased by 0.2 unit in both wines. Similar changes of pH were artificially induced by adding NaOH (10 M) in these two wines. The SP contents were 484 ± 30 mg/L for wine C (initially 480 ± 24 mg/L) and 230 ± 6 mg/L for wine Q (initially 216 ± 14 mg/L). The pH increase was not responsible for changes in SP content during MLF. Furthermore, we made sure that the addition of sulfur dioxide (30 mg/L) at the end of MLF did not modify SP concentration (**Figure 1**).

Neutral and acidic monomers in the alcohol-precipitated SP were analyzed both in non MLF controls kept at 10 °C and after induced or spontaneous MLF (**Figure 3**). The indigenous

Table 2. Monomer Composition (Mass Percent) of the SP of Wines C and Q in Non MLF Controls Kept at 10 °C and at the End of Spontaneous MLF or Induced MLF (SB3)

	wine C			wine Q		
	non MLF	after MLF		non MLF	after MLF	
	control	spontaneous	SB3	control	spontaneous	SB3
mannose	31.4	29.2	13.7	25.6	28.5	31.5
galactose	18.1	13.8	6.8	18.8	13.4	14.7
arabinose	9.0	8.9	9.6	11.6	8.1	10.4
rhamnose	9.5	10.6	4.1	9.2	9.1	12.1
glucose	6.2	5.7	4.1	6.0	4.8	5.2
fucose	1.2	1.1	0.3	1.2	1.1	1.3
xylose	1.2	1.6	1.4	1.2	1.1	1.7
2-*O*-Me-Fucose	1.2	1.1	0.4	0.8	0.5	1.7
2-*O*-Me-glucose	0.8	1.1	0.1	0.8	0.5	1.1
glucuronic acid	18.6	24.6	52.6	21.7	28.6	17.2
glucuronic acid	2.8	2.2	6.9	3.1	4.2	3.1
GalA/glcA	6.7	11.3	7.6	6.9	6.9	5.5

microflora maintained the proportion between acidic and neutral monomers in the alcohol-precipitable SP better than starter SB3. As a result, during spontaneous MLF, the change (increase or decrease) of estimated SP concentration seemed to result from simultaneous modification of neutral and acidic polysaccharide content. In contrast, after induced MLF in wine C, the decrease in total estimated SP concentration seemed to be mainly due to a disappearance of neutral monomers, whereas the acidic monomer content strongly increased. In wine Q, the increase of apparent SP concentration was also mainly due to increased neutral monomer content after induced MLF.

The detailed monomer composition of the precipitated SP is presented in **Table 2**. Galacturonic acid and mannose were the most abundant monomers in polysaccharides precipitated from wines examined both in non MLF control and after MLF. This indicated the predominance of MP, as well as the pectic polysaccharides containing rhamnose and uronic acids (HG oligomers and RGI). The lack of apiose indicated the absence of RGII (*2*) in the precipitated polysaccharides, whereas the relative abundance of arabinose and galactose indicated the presence of AG and AGP (*1*). The glucosyl residues may have originated either from condensed anthocyanins or from polysaccharides produced by microbial species such as *Botrytis* or yeasts on grapes and musts (*3, 7*). Concentrations of fucose, xylose, and methylated forms of these monomers were very low (almost undetectable), indicating the low level of hemicellulose in the wines studied (*3*).

The wine SP monomer composition changed after MLF (**Table 2**). In wine C, in which the estimated SP concentration decreased during MLF, galactose proportion decreased after spontaneous and induced MLF, whereas the galacturonic and glucuronic acid proportions both increased and that of arabinose was little modified. Apart from these common features, certain changes in monomer composition of wine C varied depending on whether MLF was spontaneous or induced. The mannose, rhamnose, and glucose contents of precipitated polysaccharides strongly decreased after induced MLF and was little modified after spontaneous MLF. The ratio between galacturonic and glucuronic acid strongly increased after spontaneous MLF, whereas it was little modified after induced MLF. However, the inoculation of wine C with the malolactic starter SB3 caused monomer composition changes greater than spontaneous MLF. In wine Q, the concentration increased during MLF. The proportion of mannose increased while those of galactose, arabinose, and glucose decreased after spontaneous and induced MLF. In contrast, the glucose and uronic acid contents evolved differently depending on whether MLF was spontaneous or induced. Indeed, the monomer distribution analysis was unable to isolate monomers and thus to identify a family of SP that preferentially disappeared by the bacteria during MLF. Moreover, we did not observe a specific monomer belonging to a SP systematically synthesized by bacteria during MLF.

We analyzed the sizes of the various SP in wines C and Q (**Table 3**). Examples of chromatograms (wine C) are shown in **Figure 4**. In non MLF controls, the SPs were eluted in four (wine Q, Merlot) or five (wine C, Cabernet Sauvignon) peaks corresponding to molecular mass ranging from 20 to 550 kDa, resulting in a mean molecular mass of approximately 144 kDa in both wines. MLF significantly modified the SP size distribution: all of the chromatographic peaks changed size, moved to a higher retention time (corresponding to lower molecular mass), and tailed (**Figure 4**). The bacterial development resulted in a significant decrease of the mean size of the SP in both wines. This reduction in polymer size was more important in the wine with decreased SP concentration (C). In this wine, the concentration of low molecular mass SP increased, whereas the greatest SP (molecular mass > 100 kDa) partly disappeared. On the other hand, in wine Q, the increase of total SP concentration was due to the formation, during MLF, of SP with molecular mass lower than those initially present in the wine (mainly molecular mass < 25 kDa). In both wines (C and Q), the occurrence of peak tailing together with the decrease of the average molecular mass suggested the occurrence of partial degradation of the SP during MLF (**Figure 4** and **Table 3**).

Relationship between SP and Color Compounds. The colloidal equilibrium of wine is often considered as a key parameter in the sensory properties of wine tannins and in color stability (*9, 11, 12, 26*). We therefore analyzed the color and tannin content of wines and compared their evolution to that of SP. For all 20 wines studied, no significant color change (change of color intensity > 15%) was observed, whatever the MLF conditions (induced or spontaneous, data not shown). The results obtained for wines Q and C are presented in **Table 4**. For each trial, the color intensity decreased by <15%. Indeed, the change was not visible to the naked eye. On the contrary, the tone evolved, with a decrease of blue and an increase of yellow nuances according to the effects generally described for MLF

Table 3. Molecular Size Analysis of the SP of Wines C and Q in Non MLF Controls Kept at 10 °C and at the End of Spontaneous MLF or Induced MLF (SB3)

	wine C			wine Q		
	non MLF	after MLF		non MLF	after MLF	
	control	spontaneous	SB3	control	spontaneous	SB3
mean molecular mass (kDa)	145	73	82	144	125	119
polymers with molecular mass > 100 kDa (mg/L)	288	43	18	94	99	92
polymers with molecular mass between 25 and 100 kDa (mg/L)	155	183	172	113	138	100
polymers with molecular mass < 25kDa (mg/L)	49	89	99	23	96	101

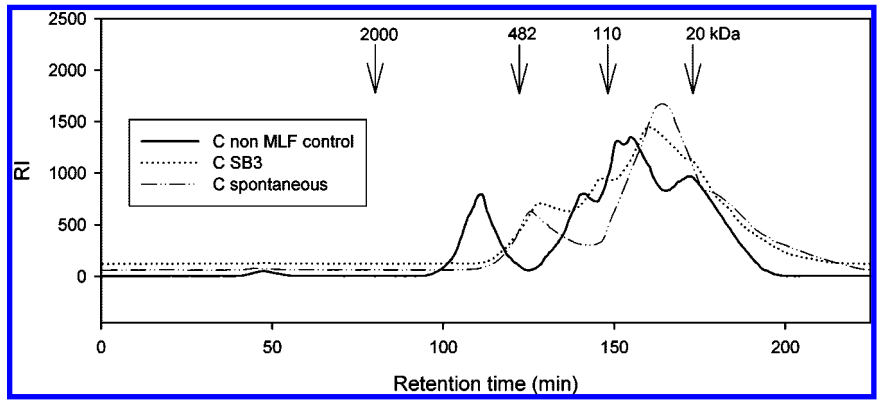

Figure 4. SP size distribution analysis in wine C in non MLF controls kept at 10 °C for 2 months and at the end of spontaneous MLF or induced MLF (SB3).

Table 4. Evolution of Tannins and Polyphenolic Compound Concentrations and Colorimetric Parameters in Wines C and Q in Non MLF Controls Kept at 10 °C and at the End of Spontaneous MLF or Induced MLF (SB3)

wine			tannins (g/L)	TPI	GI[a]	color intensity[b]	tone
C	non MLF control	2.1 ± 0.1	0.079 ± 0.004	52 ± 1	1.15 (−5%)	0.541	
	ater MLF	spontaneous	2.4 ± 0.1	0.087 ± 0.010	NM	1.06 (−6%)	0.869
	SB3	2.4 ± 0.0	0.085 ± 0.006	49 ± 1	1.09 (−4%)	0.871	
Q	nn MLF control	2.4 ± 0.1	0.085 ± 0.009	55 ± 1	1.44 (−6%)	0.473	
	ater MLF	spontaneous	2.8 ± 0.1	0.089 ± 0.004	NM	1.23 (−8%)	0.814
	SB3	2.6 ± 0.1	0.101 ± 0.012	48 ± 1	1.33 (−7%)	0.827	

[a] NM, not measured. [b] Figures in parentheses indicate the color intensity lost after sulfur dioxide addition (total SO_2 = 50 mg/L).

and aging (*26*). These modifications in color were similar in wines C and Q, although SP concentration evolved in an opposite way during MLF. The color loss induced by adding sulfur dioxide was also similar, indicating that the interaction between anthocyanins and tannins was similar in both wines, in non MLF controls and after MLF (*26*).

In wines Q and C, the concentrations of polyphenolic compounds and tannins were changed very little by MLF. Moreover, the gelatin index (GI) decreased in both wines, indicating a slight decrease of tannin reactivity to gelatin, regardless of the evolution of SP concentration.

DISCUSSION

The analysis of various red wines from the Bordeaux region for three consecutive years showed that MLF correlates to significant changes in total estimated SP concentration. These changes were due to bacterial development. They do not occur in sterile wine at 20 °C or in sulfured wine at 10 °C, nor are they induced by sulfuring or by artificially altering pH to mimic the pH increase encountered during MLF. These changes are not due to insoluble polymer formation or solubilization because none of the wines we studied contained significant levels of insoluble polysaccharides. The relative absence of insoluble polysaccharides was previously noticed by Doco et al. (*6*) in red wines from the Carignan grape. Indeed, we have shown that the changes in SP content are caused by bacterial activity during MLF. This is the first such report to our knowledge.

Many reactions may be responsible for the changes observed in total estimated SP concentration. First, with regard to the charged SP, the modification of polymer susceptibility to ethanol precipitation through bacterial metabolism may be partially responsible. Actually, several authors have shown that the precipitation of low molecular mass SP containing uronic acids (molecular mass < 10 kDa) such as RGII or HG oligomers was difficult, but improved by introducing a preliminary concentration step (*6*, *27*). The direct precipitation of polysaccharide from wine without a preliminary concentration step may lead to underestimation of the RGII and HG oligomer content and could explain the absence of RGII in the wines analyzed. Nevertheless, the RGII structure was not sufficiently affected by MLF to modify its ethanol solubility, because no apiose was detected in the SP precipitated from wine after MLF (**Table 2**). In contrast, the precipitation of neutral SP with a high molecular mass (AG, AGP, and MP) was shown to be independent of the preliminary concentration step (*6*). Their decreased concentration should thus result from the action of glycosidases excreted by malolactic microflora: the presence of such activities was demonstrated for various *O. oeni* strains using synthetic *p*-nitrophenyl substrates (*16*–*18*, *20*). Moreover, *O. oeni* Psu1 genome reveals the presence of several genes encoding potential endo- and exoglycanase activities: for mannan hydrolysis, galactan hydrolysis, stachyose, raffinose, cellobiose, and arbutin hydrolysis (accession no. CP000411). The evolution of SP monomer composition suggests that the combined action of the glycosidases excreted by *O. oeni* enables the degradation of numerous SP structures (differing by osidic bonds and monomer content) as already shown for wine heterosidic structures (*18*). Moreover, our results suggest the occurrence of partial degradation leading to a decrease of the SP molecular mass. This polymer degradation does not seem to provide bacteria with sufficient carbon and energy to enable more rapid malic acid degradation in wines, where SP concentration decreases. This contrasts with the results of Guilloux-Benatier et al. (*28*). However, limited nutrients are not the only growth inhibition factor prevailing in wine (*15*). On the other hand, increased estimated SP concentration may result from bacterial synthesis of exopolysaccharides (*20*, *29*, *30*). Alternately, it may also follow bacterial lysis, which leads to the liberation of intracellular macromolecules. The SPs liberated by bacteria during MLF displayed quite low molecular mass (20–100 kDa) and are expected to contained mannose, galactose, arabinose, rhamnose, glucose, and galacturonic acids. Galactose,

rhamnose, mannose, glucose, and glucuronic acid are commonly found in heteropolysaccharides produced by mesophilic lactic acid bacteria ((*29*, *30*)). Arabinose and galacturonic acids may be present in the exopolysaccharides produced by *O. oeni* in wine. Indeed, these monomers, initially present in wine, are metabolized by *O. oeni* (*15*), and certain strains possess the genes necessary to polymerize them (*21*). *O. oeni* Psu1 genome analysis indicates that this strain possesses several genes for UDP-galacturonate and UDP-glucuronate formation and genes for polymerization of these residues. It also possesses genes for UDP-xylose, UDP-mannose, and UDP-rhamnose formation and for β-glycoside formation (accession no. CP000411). This bacterium seems to be equipped to produce complex polysaccharides containing uronic acids and others from the corresponding nucleotide sugars. In addition, it is possible that the malolactic microflora liberates polysaccharides tightly interacting with HG oligomer, thus improving their precipitation. Such strong interactions [between AGP(s) and RGI oligomers] were demonstrated by Pellerin et al. (*1*).

Whatever the metabolic pathways leading to SP formation or degradation, they are active enough to bring noticeable changes in wine SP composition. In the studied wines with the highest initial estimated SP concentration, phenomena leading to SP disappearance prevail, whereas for the studied wines with the lowest initial estimated SP concentration, SP formation prevails. This takes place whether MLF is spontaneous or induced, although different strains do not cause the same changes in SP monomer distribution. Spontaneous MLF was shown to generate fewer changes in SP monomer composition than induced ones. This may be due to the fact that, during spontaneous MLF, several bacterial species can grow simultaneously and thus display a wider range of glycosidase or glycosyltransferase activities than a predominant strain of bacteria does during induced MLF (*22*). However, the size and monomer composition of the newly formed SP in our results do not enable us to distinguish them from the initially present SP. Neither do they allow us to identify a SP preferentially consumed by bacteria during MLF. This may explain why MLF is commonly assumed to maintain wine SP content unchanged (*3*).

Indeed, significant changes in estimated SP concentration such as those presented in this study certainly induce changes in colloidal equilibrium (*3*, *6*, *27*). In principle, the decrease of the polysaccharide molecular mass is in favor of colloidal stability (*3*). In the wines studied, bacterial development modifies wine color and tannin association, but this seems to be independent of SP metabolism. In fact, the opposite changes in SP content did not lead to an opposite evolution of red wine color or tannin stability.

The results of wine studies are often difficult to interpret because of the complexity of the medium. Nevertheless, they are essential to investigate the natural occurrence of certain phenomenon and to underscore the interest of subsequent studies in model media on a laboratory scale. At this time, we do not know whether or not winemakers should encourage the presence of polysaccharides in wine, nor which polysaccharides are useful or desirable for color stability, precipitate formation, and taste, but we have proved that MLF modifies the wine SP content. If certain efficient *O. oeni* starters also carry out beneficial changes to wine SP content, then the presence of genes dedicated to SP metabolism (production of degradation) could be a good selection basis for malolactic starters. Further work is now of great interest (i) to characterize the polymer degraded or adsorbed during MLF and those produced by bacteria in order to (ii) assess the respective role of polymer production and polymer degradation and (iii) analyze the consequences of SP modification on wine colloidal stability and taste modification during MLF.

ABBREVIATIONS USED

SP, soluble polysaccharide; MLF, malolactic fermentation; A, arabinan; AG, arabinogalactan; AGP, arabinogalactan protein; RG, rhamnogalacturonan; HG, homogalacturonan; MP, mannoprotein.

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