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Changes in carbonyl compounds in Chardonnay and Cabernet Sauvignon wines as a consequence of malolactic fermentation

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Summary

To study changes in carbonyl compounds in Chardonnay and Cabernet Sauvignon wines as a consequence of malolactic fermentation (MLF), wines were fermented by inoculation of commercial strains of *Oenococcus oeni*, and compared with unfermented (control) wines. Carbonyl compounds were determined by GC/MS analysis on the basis of their *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine derivatives after sample preparation on an ion exchange column to remove pyruvic acid. With MLF, marked changes were revealed, particularly with regard to diacetyl, acetoin and aliphatic saturated aldehydes; the presence of unsaturated aldehydes was also revealed. A significant increase in glycoladehyde was observed, which is presumed to be part of a reduction system with glyoxal. Higher acetoin/diacetyl ratios were found in Chardonnay and higher glycolaldehyde/glyoxal ratios in Cabernet Sauvignon.

K e y w o r d s : carbonyl compounds, glycolaldehyde, malolactic fermentation, wine, PFBOA (*O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine), GC/MS.

Introduction

During malolactic fermentation (MLF) also carbonyl compounds undergo profound changes, by which the organoleptic characteristics of the wine can be improved (Sauvageot and Vivier 1997). Changes in the herbaceous odor of wine were observed with MLF, associated with methoxypyrazines, some C6 alcohols, and some aliphatic aldehydes such as hexanal, (*E*)-2-hexanal, (*E*)-2-eptenal, octanal, (*E*)-2-octanal (de Revel and Bertrand 1993 a, Allen 1995). Changes in decanal and (*E*)-2-nonenal contents, associated with 'sawdust' or 'plank' odor (Chatonnet and Dubourdieu 1996, 1998), may also occur.

During MLF, the butter-like 'fat' note increases, due to 2,3-butanedione (diacetyl) and its reduced form, 3-hydroxy-2-butanone (acetoin). The second reduced form, 2,3-butanediol, is linked to the 'cooked' note (Davis *et al*. 1985, de Revel and Bertrand 1993 a, Laurent *et al*. 1994).

Carbonyl compounds glyoxal, methylglyoxal and hydroxypropandial are also present in wine. They are produced by microorganisms such as *Saccharomyces cerevisiae* and *Leuconostoc oenos*, and by *Botrytis cinerea* attack on berries. The three above mentioned compounds are probably very important for wine flavor, because of their very low odor threshold. Moreover, dicarbonyl compounds are associated with browning processes due to their reactions with amino acids; they are more toxic than their reduction products (de Revel and Bertrand 1993 a, Guillou *et al*. 1997).

In this work, we studied changes in carbonyl compounds as a consequence of MLF in Chardonnay and Cabernet Sauvignon wines. We performed fermentation by inoculation of two commercial *Oenococcus oeni* strains; the carbonyl compounds of the wines were compared with those of unfermented controls. Compounds were determined by GC/MS analysis of their *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA) derivatives (de Revel and Bertrand 1993 b).

Material and Methods

W i n e s : Cabernet Sauvignon and Chardonnay wines were produced from grapes cultivated in Conegliano (Veneto, Italy) and harvested in 2000. Must was fermented at the Istituto Sperimentale per la Viticoltura of Conegliano. At the end of fermentation, sugar residues in wine were less than 2 $g \cdot l^{-1}$. In order to limit carbonyl/bisulfite adduct formation and to eliminate the basic hydrolysis step in sample preparation, no SO_2 was added during or after alcoholic fermentation. This also favored bacterial growth (Nielsen and Richelieu 1999).

M a l o l a c t i c f e r m e n t a t i o n : MLF was performed by membrane-resistant (MBR) Uvaferm ALPHA (strain A) and MLD (strain B) (Esseco spa, Trecate, Italy) according to the manufacturer's guidelines. Lyophilized bacteria (50 mg) were hydrated in 2 ml of distilled water at 25 °C for 15 min. The bacterial suspension was added to 200 ml of wine, stirred for 1 h and kept at 25 °C for about 10 d. Fermentation was followed by organic acid screening (until the disappearance of malic acid) by HPLC analysis (Flamini and Dalla Vedova 1999). Organic acids, pH and titratable acidity of samples before and at the end of MLF are listed in Tab. 1.

The HPLC system consisted of a chromatograph Varian 9010 (Varian Instrument, Walnut Creek, CA, USA) equipped with a 20 µl loop, connected to a Varian 2550 UV-vis detector (wavelength 210 nm). Column: LiChrospher 100 RP-18 (250 mm x 4.6 mm i.d., 5 µm) with LiChrocart guard column

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Table 1

Organic acids, titratable acidity and pH of wines before MLF (control) and after MLF by two *Oenococcus oeni* strains

	Cabernet Sauvignon			Chardonnay		
	control	strain A	strain B	control	strain A	strain B
malic acid ($g \cdot l^{-1}$)	1.81	trace	trace	2.53	trace	trace
lactic acid ($g \cdot l^{-1}$)	0.54	1.94	1.94	0.10	1.71	1.72
pyruvic acid ($g \cdot l^{-1}$)	0.14	0.01	0.01	0.08	0.05	0.05
acetic acid ($g \cdot l^{-1}$)	0.25	0.43	0.47	0.44	0.47	0.48
titratable acidity ($g \cdot l^{-1}$)	7.90	6.80	6.80	7.60	5.15	5.15
pH	3.55	3.70	3.70	3.24	3.50	3.50

(4 mm x 4 mm, 5 µm) (E. Merck, Darmstadt, Germany). A Varian 4400 integrator connected with WOW Chemstation software (Thermo Separation Products, Riviera Beach, FL, USA) was used. Fermentations were carried out in duplicate.

Reagents and standards: *O*-(2,3,4,5,6-penta-fluorobenzyl)-hydroxylamine hydrochloride (PFBOA), decanal, (*E*)-2-hexenal, (*E*)-2-nonenal, nonanal, diacetyl, (*E,E*)-2,6-nonadienal, (*E,Z*)-2,6-nonadienal, (*E*)-2-pentenal, (*E*)-2-octenal, (*E*)-2-heptenal and glycoladehyde were purchased from Sigma-Aldrich (Milan, Italy). Vanillin, acetoin, pentanal and glyoxal were purchased from Fluka (Milan, Italy); (*E*)-crotonaldehyde and heptanal from Carlo Erba Reagenti (Milan, Italy); hexanal from Merck (Milan, Italy) and acetaldehyde from BDH.

Sample preparation for carbonyl compound analysis: The ion exchange column was prepared with 10 g of Amberlite IRA 400 resin (Fluka, Milan, Italy), preconditioned with 20 ml of HCl 1 % (v/v), 100 ml NaF 0.5 mol and rinsed with water. The pH of 50 ml of wine was raised to 7.5 by the addition of 1 ml NaOH (4 mol). This solution was passed through the column. The stationary phase was washed with 10 ml ethanol and both fractions passed through the column were combined. After the addition of 200 µl *o*-chlorobenzaldehyde (0.105 mmol) as internal standard, the solution was adjusted to pH 3.0; after the addition of 20 mg PFBOA, it was stirred for 1 h at room temperature. *O*-pentafluorobenzyl oximes (*O*-PFB-oximes) were extracted with ethyl ether/hexane 1:1 v/v (3x3 ml) and stirred for 5 min; the organic phases were combined and dried over Na_2SO_4. The volume was reduced to 0.4 ml under a stream of nitrogen before GC/MS analysis in the SIM (Selected Ion Monitoring) mode.

Determination of *O*-PFB-oxime retention times on GC column: Solutions of each carbonyl compound were prepared by dissolving 10 mg of standard in 50 ml ethanol; 2 ml of HCl (pH 3) solution were added with 0.1 ml standard solution, 200 µl internal standard *o*-chlorobenzaldehyde and 5 mg PFBOA. After reaction, the solution was extracted with ethyl ether/hexane 1:1 v/v (3x1 ml) and analyzed as reported above.

Determination of compound recovery from ion exchange column: Two different samples were prepared from the standard solution obtained by dissolving about 10 mg of each carbonyl compound in 50 ml ethanol. The first was prepared by diluting 0.5 ml of standard solution in 50 ml ethanol/water (12 % v/v, pH 3), followed by addition of 10 ml ethanol and 200 µl internal standard. Reaction and analysis were performed as reported above. The second sample was prepared by diluting 0.5 ml of standard solution in 50 ml ethanol/water (12 % v/v, pH 7.5) and then passing it through a preconditioned ion exchange column (Amberlite IRA 400 10 g). The column was washed with 10 ml ethanol, the organic phase combined with the aqueous one, and pH was adjusted to 3. Internal standard was added to the solution; reaction and analysis were performed as reported above. The recovery of each compound from the column was estimated on the basis of the difference between peak areas in the chromatograms of the two samples. Experiments were repeated three times.

Sample preparation for 2,3-butanediol analysis: Samples were prepared with Isolute ENV(+) 500 mg cartridges (International Sorbent Technology Ltd., UK), preconditioned by the passage of 10 ml methyl chloride and 10 ml methanol, rinsed with 20 ml water. 15 ml of wine was adjusted to 45 ml with water, and 250 µl of a 1-heptanol (1.53 mmol) solution were added as internal standard. The solution was loaded on the cartridge, the stationary phase raised with 20 ml water, and the organic fraction eluted with 8 ml of methyl chloride. The organic phase was dried over Na_2SO_4 and filtered. The volume was reduced to 0.4 ml before performing GC/MS analysis (SCAN mode). The content of 2,3-butanediol in wine was estimated on the basis of the recovery coefficient from the cartridge of acetoin, calculated as 9.47.

GC/MS analysis: Analyses were performed on a HP 5890 gas chromatograph, coupled with a HP 5971A mass spectrometer, equipped with an HP Innowax fused silica capillary column (30 m x 0.25 mm i.d.; df= 0.25 µm).

Conditions for *O*-PFB-oxime analysis: Injector temperature 250 °C; splitless injection; volume injected: 0.5 µl; carrier gas: He. Programmed oven temperature: 5 min at 60 °C, 3 $C \cdot min^{-1}$ to 210 °C, 5 min at 210 °C. Identification mode and retention times of *syn* and *anti* oximes for each compound are listed in Tab. 2, with corresponding peak numbers of chromatograms shown in the Figure.

Conditions for 2,3-butanediol analysis: Injector temperature 200 °C; splitless injection; volume injected: 0.5 µl; carrier gas: He. Programmed oven tempera-

Table 2

Identification mode and retention times on column of *syn* and *anti* PFBOA-oximes of carbonyl compounds in wine. Left: peak numbers of chromatogram in the Figure. RT = retention time; m/z 181, 239, 250: chromatogram registered in SIM mode; MS/EI = mass fragmentation spectra; lit. = data reported in literature

Peak number	Compound	Identification mode	RT (min)
1	acetaldehyde	MS/EI;RT	13.70,13.93
2	butyraldehyde	m/z 181,239;RT	18.68,19.25
3	isovaleraldehyde+ 2-methylbutyraldehyde	m/z 181,239;RT	19.79,20.63
4	hexanal	m/z 181,239;RT	25.67,26.20
5	heptanal	m/z 181,239;RT	29.12,29.45
6	(*E*)-2-hexenal	m/z 181,250;RT	30.99,31.47
7	octanal	m/z 181,239;RT	32.43,32.69
8	nonanal	m/z 181,239;RT	35.77,35.95
9	3-hydroxy-2-butanone	MS/EI;RT	37.24,39.00
10	decanal	m/z 181,239;RT	38.94,39.09
11	(*E*)-2-nonenal	m/z 181,250;RT	41.04
12	glycolaldehyde	MS/EI;RT	41.86,43.20
13	(*E,Z*)-2,6-nonadienal	m/z 181,250;RT	42.06
14	2,3-butanedione	MS/EI;RT	43.29,45.04,47.75
15	*o*-chlorobenzaldehyde (I.S.)	MS/EI;RT	47.24,47.98
16	methylglyoxal	lit.	48.53
17	glyoxal	MS/EI;RT	49.41,49.70

ture: 1 min at 32 °C, 3 °C min^{-1} up to 180 °C, 5 min at 180 °C, 15 °C min^{-1} up to 230 °C, 5 min at 230 °C.

Results and Discussion

Study of carbonyl compound *O*-PFB-oximes by GC/MS: To determine carbonyl compounds at the low levels occurring in wine, mass detector signals were recorded in SIM mode. Carbonyl compounds react with PFBOA to form oxime derivatives, characterized as highly volatile, and by the loss of the m/z 181 fragment as base peak in mass spectra, corresponding to the pentafluorobenzyl ion.

For each carbonyl compound, the two geometrical isomers, *syn* and *anti*, were formed (except for formaldehyde) and the diacetyl shows three peaks corresponding to the (*Z,Z*), (*E,E*) and (*Z,E*)+(*E,Z*) isomers.

To distinguish the aliphatic saturated aldehydes from the unsaturated ones, signals at m/z 239 and 250 were also recorded. The first one is typical of non-α-substituted compounds, and probably corresponds to the *N*-vinyl pentafluorobenzyl oxime cation formed by 1-vinyl neutral aliphatic chain loss. The second is typical of α,ß unsaturated compounds, corresponding to the probable loss of the aliphatic radical chain and the formation of the isoxazoline ring. Chromatograms recorded at m/z 181, 239 and 250 are shown in the Figure.

Determination of carbonyl compound recovery from ion exchange column: Before the reaction with PFBOA, samples had to be prepared by ion exchange solid phase extraction, in order to remove the large amount of pyruvic acid in wine. PFBOA–pyruvate oxime derivatives leave the capillary column as one broad peak covering a large part of the chromatogram, and their formation subtracts a considerable amount of PFBOA from reaction with aldehydes.

Recoveries from the ion exchange column were estimated by the passage of three standard aldehyde solutions. Average recovery percentages are listed in Tab. 3, together with the Variation Percent Coefficients calculated for each compound (100xSD/av.). The ratios of *syn*/*anti* oximes are not constant, which induces an error in calculating recoveries for compounds which could only be estimated on the basis of some of the peaks because of overlap with others. This explains why recovery values exceeding 100 % were found for acetoin, glycolaldehyde and diacetyl. As may be seen (Tab. 3), except for hexanal, the longer the compound chain, the lower the recovery from the column.

Effect of MLF on carbonyl compounds: Fermentation tests were carried out in duplicate, and average contents of carbonyl compounds identified are listed in Tab. 4 with Variation Percent Coefficients (SDx100/av.). They are quantified on the basis of recoveries and expressed as μgl^{-1} and mgl^{-1} of *o*-chlorobenzaldehyde; the 2,3-butanediol is expressed as mgl^{-1} of 1-heptanol.

Diacetyl and acetoin contents generally increase in MLF, as confirmed in Tab. 4 (Arlete Mascarenhas 1984, Davis *et al*. 1985, Martineau and Henick-Kling 1995, Martineau *et al*. 1995 b, Nielsen and Richelieu 1999).

Cabernet Sauvignon is characterized by higher diacetyl and lower acetoin contents as compared to Chardonnay, the

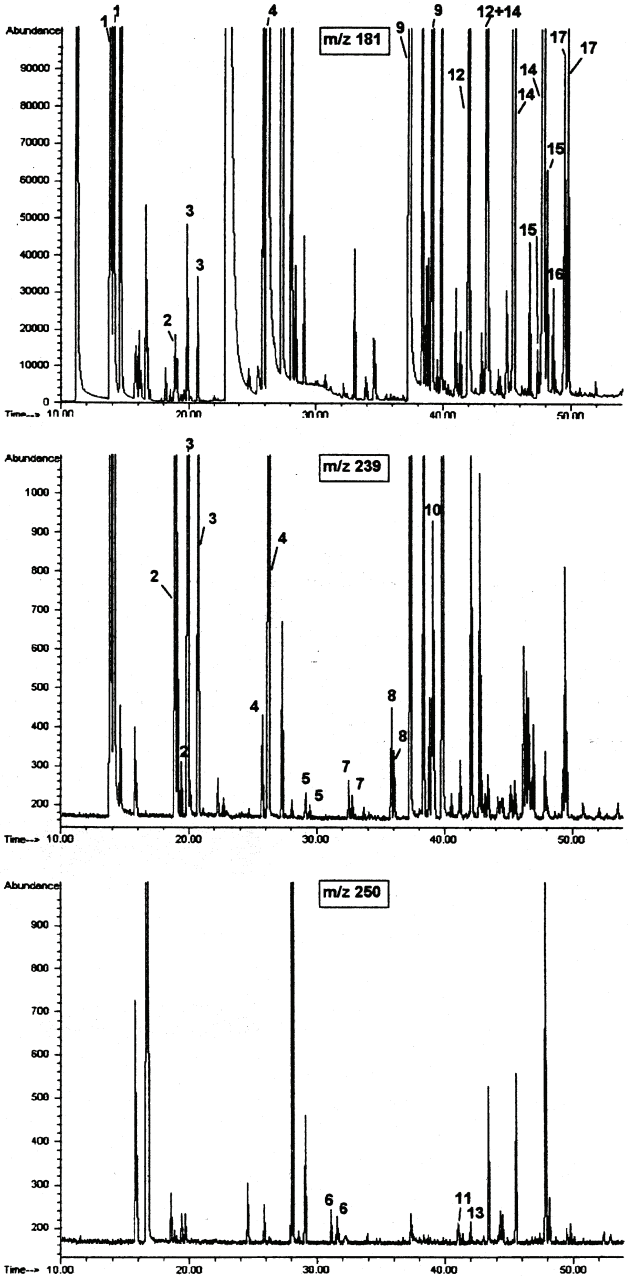

Figure: Chromatograms of Cabernet Sauvignon wine after MLF obtained by recording signals at m/z 181, 239 and 250 in SIM mode. Spectra for m/z 181 show signals of all PFBOA-oximes; m/z 239 allows signals of saturated aldehyde derivatives to be distinguished from the others; m/z 250 shows signals of α,β unsaturated aldehyde derivatives. Compounds corresponding to numbers in chromatograms are listed in Tab. 2.

latter is consequently characterized by higher acetoin/diacetyl ratios. The sensory thresholds of diacetyl in Chardonnay and Cabernet Sauvignon were 0.2 and 2.8 mgl^{-1} respectively (MARTINEAU *et al*. 1995 a). The average diacetyl content in Chardonnay exceeds the sensory threshold (0.94 mgl^{-1}), indicating that the compound confers its particular 'buttery' and 'fat' note to the wine. The increase in diacetyl was also accompanied by a proportional increase in 2,3-butanediol.

Table 3

Average recovery percentages of carbonyl compounds from ion exchange column, calculated on three standard solutions

Compound	Recovery, %	(SD/av.)x100
isovaleraldehyde	87	5.6
(*E*)-crotonaldehyde	101	1.7
hexanal	78	13.2
heptanal	84	4.5
(*E*)-2-hexenal	94	2.5
octanal	74	0.8
nonanal	64	2.2
3-hydroxy-2-butanone*	122	4.7
decanal	55	4.3
(*E*)-2-nonenal	75	0.5
glycolaldehyde*	110	3.2
(*E*,*E*)-2,6-nonadienal	79	1.0
2,3-butanedione**	136	23.4
glyoxal	105	15.7
vanillin	75	15.2

* Recoveries calculated on basis of 1 of 2 *syn*/*anti* oxime peaks.

** Recoveries calculated on basis of 2 of 3 *syn*, *anti* and *anti*+*anti* oxime peaks.

In MLF, glyoxal and methylglyoxal contents also generally increase. Methylglyoxal was identified by data reported in the literature and, as it was determined on the basis of one of the two *syn/anti* isomer peaks, its amounts were estimated in default (DE REVEL and BERTRAND 1993 b). Higher methylglyoxal contents were found in Chardonnay, with a relevant increase after MLF. No significant differences in glyoxal contents were found between the two wines.

After MLF, the chromatograms, particularly those for Cabernet Sauvignon, revealed a dramatic increase in the peak between those of acetoin and diacetyl, at a retention time of 43.2 min. The compound was identified by spectral fragmentation as glycolaldehyde. Glycolaldehyde contents were estimated in only one of the two oxime peaks, because of the overlap of the other with a diacetyl, so that it was quantified in default. It has been reported (HOFMANN and SCHIEBERLE 1998, HOFMANN *et al*. 1999) that glycolaldehyde may form after heating of a mixture of L-alanine and glucose by a radical mechanism of glyoxal reduction, and that it is a carbohydrate degradation product. Our results confirmed this, since we found that the higher the glyoxal contents, the higher those of glycolaldehyde. Cabernet wines were characterized by higher glycolaldehyde/glyoxal ratios.

In MLF, the total contents of saturated aldehydes generally increase in both wines. In particular, after MLF, the average content of decanal is 4 times higher in Cabernet and 2 times higher in Chardonnay with respect to control wines. Decanal is correlated to the 'sawdust' note, and may be induced by the formation of decanoic acid during MLF (EDWARDS *et al*. 1990). Significant increases in 2-methyl butanal+3-methyl butanal (isovaleraldehyde) and hexanal

Table 4

Carbonyl compounds identified in wines before MLF (control) and after MLF by two *Oenococcus oeni* strains. In brackets: Variation Percent Coefficients of each value (100xSD/av.) calculated for two replicated fermentations. n.f. = not found.

Compound ($\mu g \cdot l^{-1}$)*	Cabernet Sauvignon control		Cabernet Sauvignon strain A		Cabernet Sauvignon strain B		Chardonnay control		Chardonnay strain A		Chardonnay strain B	
butyraldehyde	12.36	(40)	16.73	(51)	23.24	(107)	8.48	(17)	8.36	(24)	1.81	(54)
isovaleraldehyde+ 2-methylbutyraldehyde	25.69	(41)	33.50	(50)	57.53	(38)	45.85	(29)	20.63	(20)	28.29	(7)
hexanal	94.63	(42)	19.87	(17)	163.48	(81)	10.79	(20)	35.23	(6)	144.65	(13)
heptanal	3.36	(9)	0.53	(31)	0.60	(35)	0.35	(38)	3.50	(29)	0.48	(49)
(*E*)-2-hexenal	3.43	(41)	0.45	(24)	0.71	(10)	0.82	(7)	1.50	(7)	0.67	(20)
octanal	0.82	(94)	0.92	(24)	0.62	(33)	0.39	(42)	0.96	(30)	0.65	(12)
nonanal	1.30	(42)	1.85	(2)	1.84	(28)	1.05	(21)	3.91	(18)	1.93	(31)
decanal	0.34	(12)	0.41	(114)	2.72	(29)	1.84	(35)	2.91	(27)	5.36	(41)
(*E*)-2-nonenal	trace	(35)	0.16	(7)	0.20	(13)	0.18	(35)	0.34	(17)	0.24	(99)
glycolaldehyde**	48.43	(7)	322.70	(84)	460.50	(35)	38.51	(41)	86.20	(1)	135.54	(15)
(*E*,*Z*)-2.6-nonadienal	13.78	(94)	0.16	(7)	0.39	(14)	n.f.		n.f.		n.f.	
methylglyoxal**	10.96	(21)	11.10	(34)	17.64	(17)	34.73	(5)	61.83	(50)	81.27	(8)
glyoxal	41.33	(24)	103.14	(39)	108.87	(60)	49.11	(46)	117.09	(29)	74.75	(17)
tot. aliphatic aldehydes	138		74		250		69		75		183	
glycoladehyde/glyoxal	1.20		3.10		4.20		0.80		0.70		1.80	
acetaldehyde	52.01	(33)	3.39	(30)	6.65	(55)	46.54	(29)	91.20	(20)	42.87	(14)
acetoin	1.62	(9)	3.35	(19)	5.77	(55)	5.03	(3)	13.25	(4)	8.57	(29)
diacetyl	0.25	(51)	0.48	(9)	5.15	(34)	0.05	(27)	0.25	(38)	1.63	(4)
2.3-butanediol***	8.97		13.11		28.35		17.25		21.99		21.52	
acetoin/diacetyl	6.60		6.90		1.10		98.70		52.70		5.20	

* Amounts expressed as *o*-chlorobenzaldehyde (I.S.).
** Quantified on basis of 1 of 2 *syn*/*anti* oxime peaks.
*** Amounts expressed as 1-heptanol (I.S.).

in Cabernet and of nonanal in Chardonnay were found. Small amounts (<1 $\mu g l^{-1}$) of unsaturated aldehydes (*E*)-2-hexenal and (*E*)-2-nonenal were determined in all samples – not fitting the results of Hashizume and Takashi (1997).

The chromatograms of red wines show signals at m/z 181 and 250, with a similar column retention time for (*E,Z*)-2,6-nonadienal. Increased contents of this compound were observed during storage of samples after derivatization with PFBOA.

The acetaldehyde content also changes with MLF, and a dramatic decrease was noted in red wine (Bartowsky and Henschke 1995). Isolation of anthocyanins from samples after MLF by a previously reported solid phase extraction procedure, and hydrolysis of the extract at pH 12 at room temperature showed considerable release of acetaldehyde, which was not observed after hydrolysis in the acid media at pH 2 (Flamini and Tomasi 2000). This suggests that the disappearance of acetaldehyde is due to the formation of an adduct with the bisulfite ion, added during grape pressing or produced during alcoholic fermentation by the yeast. The negative ion forms salt with the cationic form of anthocyanin, and this salt is retained on the ion exchange column during sample preparation. Because of the low bisulfite level in the wine, adduct formation requires a considerable period of time, and was not observed in controls, which were immediately frozen after alcoholic fermentation. The formation of adducts is confirmed by the known ability of bisulfite to release carbonyl compounds in basic media.

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