Amino acids and volatile compounds in wines from *Cabernet Sauvignon* and *Tempranillo* varieties subjected to malolactic fermentation in barrels

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Article

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Amino acids and volatile compounds in wines from *Cabernet Sauvignon* and *Tempranillo* varieties subjected to malolactic fermentation in barrels

P Hernández-Orte[1], A Peña[1], I Pardo[2], J Cacho[1] and V Ferreira[1]

Abstract

The aim of the present paper is to compare the behaviour of industrial lactic bacteria and indigenous bacteria of the cellar when malolactic fermentation was carried out in barrels. The effects of these bacteria on the concentration of metabolised amino acids during malolactic fermentation and on the composition of volatile compounds both before and after malolactic fermentation are studied. The experiment was performed with wines of the *Tempranillo* and *Cabernet Sauvignon* varieties. An analysis has been made of the easily extractable volatile compounds of the wood and the compounds from the grapes, and the action of the yeasts during the alcoholic fermentation. Acetoin and diacetyl decreased during the malolactic fermentation in barrels and the concentrations of furfural and its derivatives were up to 100 times higher in wines not subjected to malolactic fermentation. Most of the volatile phenols increased during the malolactic fermentation in wines of the *Tempranillo* variety, while only guaiacol ($p < 0.05$) and t-isoeugenol increased in the *Cabernet Sauvignon* wines. The decrease in amino acids during the malolactic fermentation depends much more on the variety than on the bacterial strain which carries out the malolactic fermentation.

Keywords

Amino acids, malolactic fermentation, oak barrels, aroma compounds, wine, Cabernet Sauvignon, Tempranillo

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INTRODUCTION

Extensive research has been carried out recently on the role placed by lactic bacteria (LAB) in the formation of compounds that have an adverse effect on the health, mainly ethyl carbamate and biogenic amines (Costantini et al., 2006; Izquierdo Cañas et al., 2009; Vincenzini et al., 2009). One of the factors which may increase the amount of amines is the abundance of their precursor amino acids. In the elaboration of wine, the LAB which carry out the malolactic fermentation (MLF) are mainly *Oenococcus*, *Lactobacillus* and *Pediococcus* (Lonvaud-Funel, 1999). *Oenococcus oeni* is the best adapted to the conditions of wine (low pH, high ethanol content and few nutrients) and is used almost exclusively for the induction of the MLF in red wines. Malic acid is converted into lactic acid in this type of fermentation and there is an increase in wine stability and in the complexity of its aroma.

The flavour modifications caused by MLF are complex, often involving changes in the fruit notes and floral notes and a decrease in vegetable and herbaceous aromas (Bartowsky and Henschke, 1995; Henick-Kling, 1995). Sauvageot and Vivier (1997) reported a

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very low impact of MLF on the tasting of wines of the Chardonnay and Pinot Noir varieties. Some authors (Delaquis et al., 2000; Maicas et al., 1999) have observed certain esters increase due to the action of the LAB, while others consider that they decrease during the MLF process (Du Plessis et al., 2002; Gambaro et al., 2001). Indeed, the increase or decrease depends on the bacteria used (Maicas et al., 1999; Ugliano and Moio, 2005). Several researchers have reported an increase in the concentration of ethyl esters during the MLF, including ethyl acetate, ethyl lactate, hexanoate and ethyl octanoate, and a decrease in other esters (de Revel et al., 1999; Delaquis et al., 2000; Gambaro et al., 2001). These variations in concentration seem to indicate that esterases are involved in both the synthesis and the hydrolysis of esters. Different changes in the concentration of esters during the MLF may degrade wine or enhance its quality.

Pozo Bayon et al. (2005) pointed out important role played by amino acids in the evolution of the MLF and the requirements of different bacterial species of this important nitrogenated fraction.

Among all the substrates of the wine metabolised by LAB, amino acids are the main source of nitrogen, carbon and sulphur (Swiegers et al., 2005). Soufleros et al. (1998) reported that the concentration of amino acids decreases during the MLF carried out by indigenous bacteria, whereas the concentration of biogenic amines increases. These authors found that most of the indigenous amines were negatively correlated with their respective precursors but there was also a positive correlation between the total concentration of amino acids and the biogenic amine content, indicating that the abundance of amino acids affects biogenic amine formation. Apart from that, certain amino acids like lysine and ornithine may be converted into potent off-flavours (Costello et al., 2001).

The aim of the present paper is to study the influence of the bacteria used on the amino acids and different aromatic compounds related not only with the alcoholic fermentation and MLF but also with compounds from the grapes and wood when the MLF is carried out in oak barrels (an increasingly widespread practice in wine cellars to obtain quality wines).

MATERIAL AND METHODS

Reagents and standards

The pure reference compounds used in the quantitative analysis of amino acids and volatile components were purchased from Aldrich (Gillingham, UK), Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland), Poly Sciences (Niles, IL, USA), Lancaster (Strasbourg, France) and Chemservice (West Chester, PA, USA). Dichloromethane and methanol (LiChrosolv-quality) were purchased from Fisher Chemicals (Loughborough, UK); pentane from Fluka; absolute ethanol, ammonium sulphate and sodium hydroxide were supplied by Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, USA). LiChrolut EN resins were purchased from Merck (Darmstadt, Germany). Semi-automated solid-phase extraction was carried out with a Vac Elut 20 station from Varian (Walnut Creek, USA).

Methods

Industrial winemaking. The wine used in all the experiments was from the Denomination of Origin Somontano. Tempranillo wine was added together with 50 mg/L of sulphur dioxide (SO_2) and inoculated at a concentration of 20 g/hL with a commercial yeast strain (UCLMS 377, Bio Springer Maisons-Alfort, France). Fermentation took place in a week; when the wine reached 13% (v/v) it was racked and then divided into three lots: (1) one lot was placed in two French oak barrels (Seguin Monreau, France) to perform MLF with indigenous microbiota; (2) another lot was treated with 20 mg/L of lysozyme and 20 mg/L of SO_2 and then inoculated with Viniflora Oenos (Viniflora Oenos, chr Hansen, Hoersholm, DK) at 5.8 mg/L. The inoculated wine was used to fill two French oak barrels, where MLF would take place; (3) the third lot was added together with 500 mg/L of lysozyme and 50 mg/L of SO_2 in order to prevent MLF and stored in two French oak barrels (control wine).

A total of 50 mg/L of SO_2 were added to the Cabernet must and inoculated at a concentration of 20 g/hL with a commercial yeast strain (ICVD254, Lallemand, Blagnac, France). After alcoholic fermentation (an alcoholic degree of 13.5% [v/v] was reached), the wine was racked as Tempranillo wines. The inoculation of Viniflora Oenos was carried out at 4.6 mg/L.

General enological parameters. Methods of analysis for general parameters (acidity, % alcohol, pH, residual sugars) were as by the Office International de la Vigne et du vin (1990).

Quantification of amino acids by HPLC

α-Amino acids were quantified by high-performance liquid chromatography (HPLC), following the procedure described by Hernandez-Orte et al. (2003).

Lab quantification

Samples of 0.1 mL of decimal dilutions of wine samples were spread onto Man, Rogosa and Sharpe medium (MRS) (Scharlab, Barcelona) or Leuconostoc oenos

Table 1. Analytical parameters of the Tempranillo and *Cabernet Sauvignon* wines before and after malolactic fermentation

Wine	Bacteria	Malic acid (g/L)	Lactic acid (g/L)	Alcohol % (v/v)	Total acidity	pH	Volatile acidity	Residual sugars (g/L)
Tempranillo wine								
Before MLF		1.7	nd	13	4.08	3.54	0.24	2.53
	W[a]	0.06	1.1	13.3	3.49	3.64	0.37	2.35
	S[b]	0.08	1.08	13.2	3.51	3.64	0.36	2.22
After MLF	NMLF[c]	1.7	nd	13.2	3.98	3.54	0.26	2.09
Cabernet Sauvignon wine								
Before MLF		1.11	nd	14.2	4.51	3.56	0.28	2.16
	W[a]	0.24	1.01	14.4	3.91	3.62	0.41	2.16
	S[b]	0.16	1.08	14.4	3.94	3.61	0.41	2.09
After MLF	NMLF[c]	1.15	0.16	14.0	4.23	3.52	0.3	1.87

MLF: malolactic fermentation.
[a]Wines which underwent MLF with indigenous bacteria (W).
[b]Wines which underwent MLF with selected bacteria (S).
[c]Wines which did not undergo MLF (NMLF).

medium (MLO) plates (Maicas et al., 2000) together with 0.15 mg/L of natamycin to avoid yeast and mould growth. The plates were incubated at 28 °C for 7 days in anaerobic jars. The colonies formed were counted and various isolates of the different morphologies isolated on MRS or MLO plates were retained. These isolates were stored at −20 °C in glycerol 15%, and their identification was performed by 16 S-amplified rDNA restriction analysis (ARDRA) analysis (Rodas et al., 2003). Molecular characterization at strain level was achieved by ramdom amplification of polimorphic DNA (RAPD), using primer and amplification conditions described previously (Zapparoli et al., 2000).

Major compounds (microextraction and gas chromatographic-flame ionisation detection analysis). Quantitative analysis of major compounds was carried out using the method proposed and validated by Ortega et al. (2001).

Minor compounds (*SPE* and *GC*-ion trap-*MS* analysis). This analysis was carried out using the method proposed and validated by Lopez et al. (2002).

Statistical analysis. The concentration of amino acids and aromatic compounds after MLF was analyzed by one-way analysis of variance (ANOVA), in which the bacteria that carried out the MLF are applied as a factor. All analyses were carried out using Stat View (SAS Institute, Cary, NC, USA) for Windows, version 5.0.

RESULTS AND DISCUSSION

Wine chemistry: effect of the MLF

After MLF, the wines of the Tempranillo variety had less than 0.1 g/L of malic acid, and about 1.1 g/L of lactic acid was formed (Table 1). Total acidity decreased 0.5–0.6 units and the pH increased by just 0.1 unit. There was also a slight increase in volatile acidity (0.12–0.13 g/L). The results obtained were similar for the Cabernet Sauvignon wines: malic acid had a slightly higher concentration (0.24–0.16 g/L), lactic acid had similar values and the total acidity dropped by about 0.6 g/L, while the pH increased by 0.06 units. Volatile acidity increased about the same as for the Tempranillo wines (0.13 g/L). The parameters had similar values to the initial ones for the wines which did not undergo MLF (control wines) and remained the same period of time in barrels. Studies were made of the bacterial strains which carried out the MLF, and it was observed that in the deposits inoculated with *O. oeni*, the fermentation was principally carried out by the inoculated strain. However, smaller amounts of other *O. oeni* strains were also found. In the barrels in which the MLF was carried out by the indigenous bacterial strains, large populations of other *O. oeni* strains were found, as occurred in the inoculated barrels.

Amino acid variation during MLF

A one-way ANOVA was carried out to obtain information about the amino acids which varied significantly ($p < 0.05$). Wines which were not subjected to MLF (control wine) were compared with those that were inoculated with *O. oeni* and those that underwent MLF with indigenous bacterial strains.

There was a slight decrease in most of the amino acids in the Tempranillo wines subjected to MLF compared with the control wines (Table 2). Only glutamine

Table 2. Amino acid concentration of Tempranillo and *Cabernet Sauvignon* wines. Wines subjected to malolactic fermentation (MLF) with indigenous bacteria (W). Wines subjected to MLF with selected bacteria (S). Wines not subjected to MLF (NMLF)

Amino acids (mg/L)	Tempranillo wine			*Cabernet Sauvignon* wine		
	NMLF	S	W	NMLF	S	W
ASP	8.61 ± 0.57	8.84 ± 0.62	8.16 ± 0.35	8.33 ± 0.30	8.10 ± 0.6	8.00 ± 0.43
ASN	16.9 ± 1.33	13.5 ± 4.3	14.0 ± 3.9	–	–	–
SER	12.2 ± 0.11	12.1 ± 1.43	12.3 ± 0.88	17.4 ± 0.34	13.1 ± 2.6	14.9 ± 2.4
GLU	24.0 ± 3.04	26.1 ± 6.36	23.6 ± 2.77	–	–	–
HIS	14.0 ± 0.92	11.2 ± 3.57	14.0 ± 0.28	16.0 ± 0.09	15.6 ± 0.96	15.5 ± 0.23
GLN	14.3 ± 0.43	16.3 ± 3.09	16.1 ± 0.48	15.0 ± 0.13	14.7 ± 0.88	14.9 ± 1.1
GLY	16.2 ± 2.45	15.2 ± 0.87	14.9 ± 0.66	14.1 ± 0.04	13.8 ± 1.02	13.3 ± 0.38
ARG	27.0 ± 1.57	25.3 ± 2.22	26.4 ± 1.12	23.3 ± 0.12	22.8 ± 1.61	22.1 ± 0.85
THR	96.3 ± 7.56	94.0 ± 3.43	95.1 ± 2.16	90.3 ± 3.29	83.8 ± 5.3	85.1 ± 4.7
ALA	25.5 ± 1.52	23.9 ± 2.08	24.8 ± 0.55	28.1 ± 0.45	27.7 ± 1.85	26.1 ± 0.51
GABA	14.2 ± 0.60	13.7 ± 0.57	13.9 ± 0.64	11.0 ± 0.60	11.2 ± 0.78	10.6 ± 0.44
CYS	1.44 ± 0.19	1.39 ± 0.25	0.94 ± 0.43	1.85 ± 0.11	1.27 ± 0.49	1.6 ± 0.58
TYR	8.39 ± 1.08	7.44 ± 0.67	6.89 ± 0.30	7.81 ± 0.07 a	6.95 ± 0.45 b	7.14 ± 0.25
VAL	8.51 ± 0.25	7.86 ± 0.33	7.77 ± 0.42	7.72 ± 0.18	7.76 ± 0.43	7.49 ± 0.18
MET	7.07 ± 2.1	8.79 ± 0.19	10.2 ± 0.53	6.52 ± 0.24	6.39 ± 0.54	6.42 ± 0.25
ORN	6.59 ± 1.9	5.10 ± 3.8	5.00 ± 3.09	4.79 ± 0.75 a	3.41 ± 0.80 b	3.42 ± 0.76 b
LYS	13.4 ± 0.36	12.5 ± 0.91	12.1 ± 0.53	13.9 ± 0.53	13.6 ± 1.57	13.2 ± 0.52
ILE	3.30 ± 0.90	2.93 ± 0.51	2.90 ± 0.15	2.61 ± 0.01	2.54 ± 0.69	2.67 ± 0.09
LEU	8.67 ± 0.83	6.91 ± 1.58	6.70 ± 1.16	7.74 ± 1.74	6.63 ± 0.92	7.99 ± 1.16
PHE	10.5 ± 0.45	10.3 ± 0.40	10.5 ± 0.06	10.0 ± 0.04	10.5 ± 0.78	9.12 ± 1.50

Data (mean SD) followed by different letters indicate significant differences ($p < 0.05$).

and methionine increased slightly in comparison to the control wine. The increase in some of the amino acids may be due to the production of peptidase or extracellular protease of some *O. oeni* strains, as previously reported by Manca de Nadra et al. (1999). No significant differences were found for any amino acids. The highest decreases in concentration for these amino acids were found in the wines which underwent MLF with indigenous bacterial strains.

The evolution of the amino acids in the *Cabernet Sauvignon* wines was similar to that of the Tempranillo wines (Table 2). Nevertheless, there is a slight decrease in the concentration of most of the amino acids if MLF was carried out, with significant differences in TYR and ORN.

Volatile compounds

Volatile compounds were determined both before and after the MLF (Table 3). The concentrations of the acetates of the higher alcohols are similar to the initial concentrations, experimenting slight increases or decreases depending on the bacteria which carried out the MLF and the grape variety used. The formation and hydrolysis of the esters during the MLF were probably due to the action of esterases produced by the LAB or by the yeasts, and acid hydrolysis caused by the low pH of the wine cannot be excluded. The esters ethyl lactate, diethyl succinate and ethyl acetate are the only ones that showed significant differences ($p < 0.05$) in the wines of both varieties and an increase in all the wines subjected to MLF, which was even greater if the MLF was carried out by indigenous bacteria. According to Soufleros et al. (1998), diethyl succinate is a characteristic product of the MLF in young wines and, together with ethyl lactate, indicates that the fermentation process has taken place. The increase in ethyl acetate was high in Tempranillo variety (40% on average).

The fatty acids decrease slightly during the MLF process, which was about 20 days for all the wines. Butyric acid, isobutyric acid, isovaleric acid and acetic acid are indicative of spoilage of the wine and usually indicate bacterial activity with the indigenous flora.

Table 3. Concentrations of the fermentative compounds of *Cabernet Sauvignon* and Tempranillo wines before and after malolactic fermentation (MLF) with inoculated bacteria (S) or with indigenous bacteria (W) and the same wines not subjected to MLF (NMLF)

	Tempranillo wine				*Cabernet Sauvignon* Wine					
		After MLF				After MLF				
Compound	Before MLF	S	W	NMLF	Before MLF	S	W	NMLF	Odour threshold (μg/L)	Aroma
Ethyl isobutyrate (μg/L)	30.6	11.6 ± 0.95 a	28.9 ± 0.75 b	7.83 ± 2.90 a	50.6	51.1 ± 0.92	17.1 ± 2.26	56.7 ± 4.82	15 (Ferreira et al., 2000)	Fruity
Ethyl isovalerate (μg/L)	7.38	5.39 ± 1.27	6.03 ± 0.05	2.04 ± 0.98	8.15	5.76 ± 1.10	3.50 ± 0.64	6.64 ± 0.54	3 (Ferreira et al., 2000)	Fruity, anise
Ethyl 2-methylbutyrate (μg/L)	1.21	1.12 ± 0.09 a	1.57 ± 0.05 b	0.81 ± 0.13 a	2.21	3.25 ± 0.18	2.85 ± 0.42	3.48 ± 0.40	18 (Ferreira et al., 2000)	Fruity
3-Hydroxy-ethylbutyrate (mg/L)	0.51	0.55 ± 0.09	0.52 ± 0.01	0.71 ± 0.08	0.45	0.49 ± 0.05	0.57 ± 0.02	0.55 ± 0.00	20,000 (Escudero et al., 2007)	Strawberry
Ethyl lactate (mg/L)	72.4	105 ± 16.7 a	134 ± 1.36 a	29.2 ± 6.43 b	67.4	89.4 ± 15.3 a	97.8 ± 3.59 a	17.9 ± 1.37 b	15,400 (Etievant, 1991)	Synthetic
Diethyl succinate (mg/L)	0.82	1.56 ± 0.38 a	1.80 ± 0.06 b	0.76 ± 0.26 c	1.69	2.75 ± 0.26 a	2.64 ± 0.03 a	0.91 ± 0.12 b	200,000 (Etievant, 1991)	Fruit, wine
Ethyl acetate(mg/L)	23.1	34.5 ± 0.01 a	35.2 ± 0.57 a	17.1 ± 3.30 b	28.3	50.5 ± 3.68 a	7.65 ± 2.66 b	29.0 ± 0.82 c	12,300 (Escudero et al., 2004)	Solvent
Butyl acetate (μg/L)	7.02	6.81 ± 0.58	5.21 ± 0.15	3.43 ± 0.60	5.90	3.20 ± 0.11	5.15 ± 0.80 a	2.32 ± 0.18 b	1800 (Etievant, 1991)	Herbaceous
Isobutyl acetate (μg/L)	46.6	62.5 ± 1.07	70.9 ± 0.68	41.8 ± 2.39	57.7	64.4 ± 11.1	44.2 ± 10.6	51.9 ± 6.25	1600 (Ferreira et al., 2002)	Fruity
Isoamyl acetate (mg/L)	0.91	0.93 ± 0.15	1.08 ± 0.07	1.09 ± 0.06	1.17	1.21 ± 0.10	0.83 ± 0.12	1.02 ± 0.03	30 (Guth, 1997)	Banana
Hexyl acetate (mg/L)	nd	nd	nd	nd	nd	nd	0.03 ± 0.00	0.09 ± 0.01	1500 (Etievant, 1991)	Banana
Phenylethyl acetate (μg/L)	100	105 ± 3.90 a	93.5 ± 2.54 b	48.9 ± 1.80 c	109	57.4 ± 4.79	40.3 ± 8.44	51.1 ± 4.79	250 (Guth, 1997)	Roses
Ethyl butyrate (mg/L)	0.17	0.15 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.18	0.18 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	125 (Laboratory LAAE)	Strawberry
Ethyl hexanoate (mg/L)	0.22	0.20 ± 0.02	0.24 ± 0.01	0.23 ± 0.02	0.27	0.28 ± 0.03	0.25 ± 0.02	0.23 ± 0.01	62 (Laboratory LAAE)	Fruity
Ethyl octanoate (mg/L)	0.12	0.14 ± 0.01	0.14 ± 0.03	0.15 ± 0.00	0.14	0.16 ± 0.03	0.14 ± 0.01	0.12 ± 0.00	580 (Etievant, 1991)	Fruity
Ethyl decanoate (μg/L)	11.1	12.0 ± 1.90	10.9 ± 0.65	9.46 ± 1.03	12.7	30.0 ± 2.14	24.3 ± 5.30	20.4 ± 0.58	200 (Ferreira et al., 2000)	Grape
Propanoic acid (μg/L)	2.86	2.96 ± 0.19	3.95 ± 0.43 a	2.33 ± 0.05 b	4.32	2.82 ± 0.14	7.04 ± 2.57	2.58 ± 0.22	8100 (Etievant, 1991)	Rancid, sweat
Isobutyric acid (mg/L)	1.35	1.12 ± 0.10	1.36 ± 0.32	1.02 ± 0.26	2.32	2.16 ± 0.07	2.09 ± 0.19	1.53 ± 0.45	50 (Gemert, 1997)	Cheese
2-Methylbutyric acid (mg/L)	153	116 ± 3.39 a	116 ± 3.88 a	91.6 ± 2.15 b	198	104 ± 3.45	122 ± 2.14	110 ± 11.1	33 (Ferreira et al., 2000)	Cheese
Isovalerianic acid (mg/L)	1.41	1.37 ± 0.29	1.39 ± 0.02	1.78 ± 0.11	2.12	2.50 ± 0.04	2.59 ± 0.06	2.38 ± 0.14	33 (Ferreira et al., 2000)	Feet, cheese
Butyric acid (mg/L)	1.03	1.05 ± 0.04	0.90 ± 0.01	0.93 ± 0.03	1.65	1.35 ± 0.27	1.33 ± 0.14	1.20 ± 0.22	173 (Ferreira et al., 2000)	Vomit, cheese
Hexanoic acid (mg/L)	2.32	2.10 ± 0.20	2.07 ± 0.01	2.09 ± 0.02	2.21	2.32 ± 0.16	2.41 ± 0.04	2.07 ± 0.05	420 (Ferreira et al., 2000)	Cheese
Octanoic acid (mg/L)	1.62	1.58 ± 0.30	1.57 ± 0.08	1.57 ± 0.00	1.60	1.64 ± 0.15a	1.66 ± 0.02 a	1.22 ± 0.02b	500 (Ferreira et al., 2000)	Rancid
Decanoic acid (mg/L)	2.50	2.07 ± 0.25	2.07 ± 0.48	1.92 ± 0.28	2.47	2.01 ± 0.26	2.06 ± 0.25	1.17 ± 0.18	1000 (Ferreira et al., 2000)	Rancid
1-Butanol (mg/L)	2.62	2.57 ± 0.19	2.52 ± 0.01	2.75 ± 0.20	2.49	2.43 ± 0.18	2.59 ± 0.04	2.59 ± 0.04	150,000 (Etievant, 1991)	Medicine
Isobutanol (mg/L)	61.3	58.0 ± 1.75	56.3 ± 1.71	67.2 ± 8.40	85.1	80.7 ± 10.6	90.3 ± 3.30	90.9 ± 2.07	40,000 (Guth, 1997)	Bitter
Isoamylic alcohol (mg/L)	255	267 ± 7.70 a	218 ± 4.92 b	290 ± 14.7 c	305	319 ± 30.8	391 ± 26.1	399 ± 35.8	30,000 (Guth, 1997)	Feet, solvent
β-Phenylethanol (mg/L)	53.6	45.7 ± 0.34	45.3 ± 1.53	45.3 ± 0.84	70.5	73.5 ± 4.93	74.1 ± 4.58	71.8 ± 0.96	14,000 (Ferreira et al., 2000)	Roses
1-Hexanol (mg/L)	1.45	1.36 ± 0.22	1.36 ± 0.05	1.40 ± 0.00	1.61	1.56 ± 0.04	1.72 ± 0.09	1.50 ± 0.02	8000 (Gemmert, 2003)	Leaves
Z-3-Hexenol (μg/L)	0.18	0.17 ± 0.02	0.19 ± 0.02	0.18 ± 0.00	0.06	0.06 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	400 (Guth, 1997)	Grass
Methionol (mg/L)	2.04	1.89 ± 0.08	2.04 ± 0.09	2.05 ± 0.03	3.08	3.10 ± 0.40	2.79 ± 0.01	2.90 ± 0.11	1000 (Ferreira et al., 2000)	Backed potato
Acetoin (mg/L)	16.5	10.4 ± 0.04	11.4 ± 0.25	7.93 ± 2.96	6.13	7.34 ± 1.17a	6.24 ± 1.24a	0.82 ± 0.02 b	150,000 (Ferreira et al., 2000)	Lactic
Diacetyl (mg/L)	1.34	0.42 ± 0.07a	0.22 ± 0.16 a	3.55 ± 0.61b	0.91	1.01 ± 0.70	2.31 ± 0.16	2.34 ± 0.42	100 (Guth, 1997)	Butter

Data expressed as mean ± SD. Values within the same row followed by different letters are significantly different ($p < 0.05$).

Table 4. Volatile compounds concentrations of *Cabernet Sauvignon* and Tempranillo wines before and after malolactic fermentation (MLF) with inoculated bacteria (S) or with indigenous bacteria (W) and the same wines not subjected to MLF (NMLF)

	Tempranillo wine				*Cabernet Sauvignon* Wine					
		After MLF				After MLF				
Compound	Before MLF	S	W	NMLF	Before MLF	S	W	NMLF	Odour threshold (μg/L)	Aroma
Furfural (μg/L)	22.6	31.5 ± 2.66 a	31.5 ± 2.48 a	111 ± 2.80 b	26.4	16.2 ± 1.59 a	15.4 ± 0.45 a	240 ± 6.03 b	14,100 (Ferreira et al., 2000)	Dried fruit
5-Methylfurfural (μg/L)	6.22	nd	nd	145 ± 0.10	nd	nd	nd	134 ± 13.0	20,000 (Etievant, 1991)	Wood
5-Hydroxy-methylfurfural (μg/L)	nd	17.1 ± 1.42 a	15.8 ± 1.30 a	27.6 ± 3.14 b	nd	12.3 ± 1.92	12.0 ± 1.64	10.9 ± 4.12	100,000 (Gemert, 1997)	Aldehyde, caramel
Vanillin (μg/L)	12.3	46.2 ± 2.44 a	43.7 ± 1.94 a	58.0 ± 3.84 b	10.3	43.7 ± 1.32 a	39.1 ± 3.03 a	20.8 ± 8.87 b	995 (Escudero et al., 2007)	Vanillin
Acetovanillone (μg/L)	109	116 ± 0.68 a	111 ± 0.88 a	68.6 ± 0.38 b	65.0	42.9 ± 0.19	40.1 ± 6.37	41.7 ± 6.01	1000 (Lopez et al., 2002)	Vanillin
Syringaldehyde (μg/L)	5.96	20.8 ± 2.32	17.9 ± 2.08	16.2 ± 2.67	4.45	23.5 ± 0.45	13.1 ± 2.54	7.18 ± 4.58	5000[4]	Medicinal
Ethyl vanillate (μg/L)	68.6	48.5 ± 1.75 a	45.7 ± 1.25 a	29.2 ± 0.14 b	52.1	29.9 ± 2.10	27.1 ± 4.65	28.8 ± 3.79	3000 (Lopez et al., 2002)	Vanillin
Methyl vanillate (μg/L)	4.36	4.25 ± 0.18 a	3.75 ± 2.14 a	2.14 ± 0.00 b	19.5	11.5 ± 1.60	10.2 ± 1.47	10.3 ± 0.90	990 (Escudero et al., 2007)	Vanillin
Z-Whisky lactone (μg/L)	nd	nd	nd	35.0 ± 4.91	nd	22.0 ± 7.12	34.5 ± 4.05	35.1 ± 4.21	67 (Etievant, 1991)	Coconut
E-Whisky lactone (μg/L)	nd	nd	nd	nd	nd	nd	nd	nd	790 (Etievant, 1991)	Coconut, wood
δ-Octalactone (μg/L)	5.32	4.75 ± 0.01	nd	0.99 ± 2.09	3.40	4.92 ± 0.26 a	1.69 ± 0.66 b	3.67 ± 0.06 a	400 (Gemert, 1997)	Coconut
δ-Decalactone (μg/L)	12.0	14.0 ± 0.75 a	10.3 ± 0.25 a	7.49 ± 1.68 b	11.5	3.10 ± 2.20	9.07 ± 1.37	6.64 ± 0.99	38 (Lopez et al., 2002)	Peach
γ-Butyrolactone (μg/L)	6.52	7.89 ± 0.54	8.85 ± 0.07	7.31 ± 0.35	26.0	24.9 ± 6.59	21.7 ± 4.19	13.8 ± 0.82	35,000 (Escudero et al., 2007)	Caramel
γ-Nonalactone (μg/L)	24.8	24.8 ± 0.55 a	23.7 ± 2.68 a	12.6 ± 0.87 b	26.1	14.9 ± 1.77	14.3 ± 0.72	13.8 ± 0.06	25 (Gemmert, 2003)	Peach
γ-Decalactone (μg/L)	3.08	2.79 ± 0.47	2.72 ± 0.51	1.65 ± 0.31	3.35	2.28 ± 0.32	1.95 ± 0.22	2.02 ± 0.18	0.7 (Gemmert, 2003)	Spicy
Guaiacol (μg/L)	3.54	5.95 ± 0.39 a	6.79 ± 0.15 b	3.75 ± 0.13 c	2.71	3.11 ± 0.02 a	3.08 ± 0.10 a	2.58 ± 0.15 b	9.5 (Ferreira et al., 2000)	Medicinal
Eugenol (μg/L)	7.18	16.2 ± 0.48 a	20 ± 0.70 b	6.23 ± 0.22 c	7.79	6.50 ± 0.21	7.76 ± 1.16	7.21 ± 0.98	6 (Ferreira et al., 2000)	Clove, spicy
t-Isoeugenol (μg/L)	4.42	15.0 ± 0.15 a	15.0 ± 0.07 a	5.14 ± 0.56 b	6.04	7.74 ± 1.37	7.15 ± 1.03	6.49 ± 1.20	6 (Escudero et al., 2007)	Clove, floral
4-Ethylphenol (μg/L)	2.80	18.2 ± 0.93 a	5.43 ± 0.40 b	6.76 ± 0.54 b	nd	nd	nd	nd	35 Laboratory LAAE	Leather, phenolic
4-Ethylguaiacol (μg/L)	1.41	6.11 ± 0.31 a	3.70 ± 0.21 b	3.02 ± 0.58 b	1.29	0.93 ± 0.27	0.97 ± 0.02	0.79 ± 0.14	33 (Ferreira et al., 2000)	Clove
4-Vinylphenol (μg/L)	8.05	13.6 ± 1.16 a	7.72 ± 0.20 b	nd	3.54	nd	nd	nd	180 (Boidron et al., 1988)	Cypress, vanillin
4-Vinylguaiacol (μg/L)	12.1	30.4 ± 0.68 a	27.6 ± 0.44 b	nd	13.1	1.40 ± 0.39	1.40 ± 0.00	nd	40 (Guth, 1997)	Clove, curry
4-Propylguaiacol (μg/L)	0.63	1.38 ± 0.27 a	1.25 ± 0.18 a	2.91 ± 0.40 b	0.26	nd	0.34 ± 0.01	0.31 ± 0.01	10 (Escudero et al., 2007)	Clove
2,6-Dimethoxyphenol (μg/L)	22.7	37.8 ± 0.77 a	33.6 ± 1.18 b	21.2 ± 0.65 c	28.1	21.4 ± 3.26	20.0 ± 2.28	18.1 ± 0.10	570 (Lopez et al., 2002)	Phenolic, dirty
4-Allyl-2,6-dimethoxyphenol (μg/L)	7.37	14.7 ± 0.97 a	16.6 ± 1.19 a	9.11 ± 0.00 b	5.17	6.48 ± 1.88	6.53 ± 0.47	7.44 ± 0.46	1200 (Gemert, 1997)	Smoky, phenolic
m-Cresol (μg/L)	3.14	3.38 ± 0.19 a	2.99 ± 0.01 b	1.67 ± 0.08 c	5.81	0.65 ± 1.52	3.16 ± 0.20	0.65 ± 0.00	68 (Ferreira et al., 2009)	Chlorine
o-Cresol (μg/L)	1.88	2.53 ± 0.37 a	3.49 ± 0.35 a		1.98	nd	nd	nd	31 (Etievant, 1991)	Phenolic
Ethyl cinnamate (μg/L)	1.37	1.09 ± 0.06 a	1.23 ± 0.16 a	0.40 ± 0.03 b	1.43	0.62 ± 0.11	0.76 ± 0.05a	0.43 ± 0.00 b	1.1 (Ferreira et al., 2000)	Floral
Ethyl furoate (μg/L)	3.89	5.06 ± 1.42	5.10 ± 1.48	3.00 ± 0.01	4.76	3.65 ± 0.38	2.89 ± 0.68	3.27 ± 0.30	16,000 (Ferreira et al., 2000)	Smoke, mushroom

(continued)

Table 4. Continued

Compound	Tempranillo wine				Cabernet *Sauvignon* Wine					
	Before MLF	After MLF			Before MLF	After MLF				
		S	W	NMLF		S	W	NMLF	Odour threshold (μg/L)	Aroma
Phenylacetic acid (μg/L)	42.9	42.3 ± 1.25 a	41.4 ± 1.05 a	19.6 ± 0.06 b	55.5	48.8 ± 4.94	44.6 ± 3.99	46.4 ± 2.94	1000 (Maga, 1973)	Honey
Benzoic acid (μg/L)	4.03	2.69 ± 0.49	2.24 ± 0.17	nd	18.9	7.66 ± 1.66	5.23 ± 5.44	5.48 ± 0.50	1000 (Escudero et al., 2007)	Urine
Benzyl alcohol (mg/L)	1.43	1.15 ± 0.32	1.42 ± 0.02	0.99 ± 0.19	0.83	0.78 ± 0.03	0.02 ± 0.93	0.63 ± 0.22	200,000 (Escudero et al., 2007)	Sweat, floral
β-damascenone (μg/L)	3.46	3.26 ± 0.18	2.35 ± 0.25	2.02 ± 0.44	3.77	0.20 ± 0.54 a	2.03 ± 0.20 b	nd	0.05 (Guth, 1997)	Apple
α – Ionone (μg/L)	nd	nd	nd	0.08 ± 0.18	nd	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	2.6 (Etievant, 1991)	Wood, violet
β-Ionone (μg/L)	nd	nd	nd	0.31 ± 0.04	nd	nd	0.37 ± 0.06	0.16 ± 0.01	0.09 (Ferreira et al., 2000)	Violet
α – Terpineol (μg/L)	1.60	1.69 ± 0.10	1.86 ± 0.10	nd	2.16	1.85 ± 0.18	1.92 ± 0.21	1.58 ± 0.00	250 (Ferreira et al., 2000)	Anise
Linalool (μg/L)	2.86	3.19 ± 0.13 a	3.70 ± 0.21 a	2.66 ± 0.47 b	1.67	2.40 ± 0.01 a	2.57 ± 0.09 a	1.90 ± 0.10 b	25 (Ferreira et al., 2000)	Floral, citrus
Geraniol (μg/L)	nd	nd	nd	1.10 ± 0.26	nd	0.73 ± 0.01 a	1.09 ± 0.09 a	0.73 ± 0.01 b	20 (Escudero et al., 2007)	Floral, citrus
β-Citronellol (μg/L)	3.41	4.94 ± 0.66	4.47 ± 0.33	5.13 ± 0.09	2.51	3.75 ± 0.26 a	4.51 ± 0.19 a	2.60 ± 0.29 a	100 (Etievant, 1991)	Fruity, floral

MLF: malolactic fermentation.
[a]Data expressed as mean ± SD. Values within the same row followed by different letters are significantly different ($p < 0.05$).

In our case, the indigenous bacteria did not produce higher concentrations of these compounds than the inoculated bacteria.

Most of the higher alcohols did not undergo significant changes. Isoamylic alcohol increased in both varieties, as well as for control wines. These results are in good agreement with those reported by other authors (Pozo Bayon et al., 2005; Soufleros et al., 1998). The considerable increase in ethyl decanoate in the *Cabernet Sauvignon* wines should be noted. Its concentration was doubled and even more than twice the original concentration was achieved with the selected bacteria strains. However, this compound decreased in the Tempranillo wines. In Table 3, we can see that only seven esters for Tempranillo wines and four for *Cabernet Sauvignon* wines show significant differences depending on the strain of bacteria that carry out the MLF.

Acetoin decreased when the MLF is completed and that the decrease was most significant in the *Cabernet Sauvignon* control wines. This might be due to the fact that acetoin is generated during the MLF, and simultaneously, the enzymes produced by the yeast, which have not been deactivated because of the SO_2 added to the wines, degrade it (de Revel et al., 1999). Finally, the concentration of diacetyl mainly depends on the variety of grape used. In Tempranillo grapes, the concentration diminishes when FML is produced, while in the *Cabernet Sauvignon* variety, the concentration increases (>50%), especially when indigenous bacteria are used. One advantage of carrying out the MLF in small volumes (225 L barrels) is the faster reduction of carbonylic compounds due to the greater contact of the wine with the lees of the yeast and the bacteria themselves.

Furfural and 5-methylfurfural (Table 4) were only present at high concentrations in the control wines ($p < 0.05$). These results seemed to indicate microbial activity in extractable compounds of the wood, as they decrease significantly during the MLF process. 5-Methylfurfural was not detected in wines which underwent MLF, whereas concentrations of around 140 μg/L were obtained in the control wines of both varieties.

The wines obtained after the alcoholic fermentation had very low concentrations of the extractable components of the wood (Table 4). However, after MLF took place in barrels for a period of about 20 days, some compounds showed a 2–4 fold increase of their initial concentrations in all the wines, even the control ones. In the case of vanilla and syringaldehyde, the increase may be due to their dissolution with time and the action of the yeast sediment. Acetovanillone, ethyl vanillate and methyl vanillate decrease to half their initial concentrations ($p < 0.05$ for the Tempranillo wines), regardless of the bacteria used to carry out the MLF.

In wines of the Tempranillo variety, Z-whisky lactone was only detected in the control wine, whereas in the *Cabernet Sauvignon* wines up to 35 μg/L of the compound were extracted.

The concentrations of the other lactones usually decrease more in the control wines and the vast majority has lower concentrations after the MLF. Only γ-butyrolactone increased in the Tempranillo wines which underwent MLF, the increase being even greater if the fermentation was carried out using indigenous bacteria.

With regard to the volatile phenols, concentration is totally dependent on the action of the bacteria (Table 4). A comparison of the values before and after MLF indicates that the amount of most of the compounds increases in the two varieties after MLF. However, it can be observed that the increase in the control wine, that has remained in barrels for the same period of time, i was greater when there is bacterial activity. This is especially remarkable ($p < 0.05$) in Tempranillo wines, in which all the phenols, except for m-cresol, double or triple their concentrations in the wines which underwent MLF in barrels, whereas only a very slight increase occurred in the control wines (around 10%). For the *Cabernet Sauvignon* wines, the increase was around 10% (in case there was one at all) but the concentrations of several phenols like eugenol, 4-ethylguaiacol, 4-vinylphenol, 2,6-dimethoxyphenol, m- and o-cresol decrease after the MLF or after 20 days in barrels. De Revel et al. (1999) reported an increase of volatile phenols during the MLF process in Sauvignon Blanc wines. Regarding the results obtained, it can be concluded that the variations of volatile phenols depends mainly on the grape variety.

Linalool ($p < 0.05$) and β-citronellol increased during the MLF with the two bacterial strains in wines of both varieties. The β-ionone has higher concentrations in the wines which did not undergo MLF, while β-damascenone decreases in all the wines after 20 days (Table 4).

Finally, most of the analysed compounds show concentrations below their olfaction threshold (Tables 3 and 4). Notwithstanding, there are esters, acids, alcohols, norisoprenoids, lactones and phenols which are above their olfaction threshold, and hence they participate in the aroma of the wines of both varieties.

CONCLUSIONS

The results obtained in this work suggest that there is a slight decrease in the amino acids during the MLF, regardless of whether the bacteria which carries out the fermentation is inoculated or indigenous. This decrease depends on the grape variety used to obtain the different wines. There are very few significant differences between the wines which underwent MLF and the control wine. The fermentative aromas, acids, esters and higher alcohols all undergo slight changes. Only acetate, succinate and ethyl lactate increase significantly after the MLF. The concentration of furfural and its derivatives depends to a great extent on whether MLF was carried out or not. They are present at much higher concentrations in the control wines, indicating some kind of bacterial activity. The increase in the volatile phenols during the MLF depends a lot on the grape variety. All the analysed volatile phenols, except m-cresol, increase in the Tempranillo wines ($p < 0.05$), while in the *Cabernet Sauvignon* wines only guaiacol ($p < 0.05$) and t-isoeugenol have higher concentrations after MLF than in the control wines, regardless of the bacteria used to carry out the fermentation.

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