Journal of Applied Microbiology 2004, **96,** 1176–1184 doi:10.1111/j.1365-2672.2004.02257.x

Methionine catabolism and production of volatile sulphur compounds by *Œnococcus œni*

L. Pripis-Nicolau, G. de Revel, A. Bertrand and A. Lonvaud-Funel

Faculté d'Œnologie, UMR Œnologie-Ampélologie, INRA/Université Victor Segalen Bordeaux 2, Cours de la Libération, Talence, France

2003/0859: received 29 September 2003, revised 6 January 2004 and accepted 30 January 2004

ABSTRACT

L. PRIPIS-NICOLAU, G. DE REVEL, A. BERTRAND AND A. LONVAUD-FUNEL. 2004.

Aims: During malolactic fermentation (MLF), the secondary metabolisms of lactic acid bacteria (LAB) contribute to the organoleptic modification of wine. To understand the contribution of MLF, we evaluated the capacity of various wine LAB to metabolize methionine.

Methods and Results: Using gas chromatography (GC) coupled either with mass spectrometry (MS) or a flame photometry detector in sulphur mode (FPD), we studied this metabolism in laboratory media and wine. In laboratory media, several LAB isolated from wine were able to metabolize methionine. They formed methanethiol, dimethyl disulphide, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid. These are known to have powerful characteristic odours and play a role in the aromatic complexity of wine. In various red wines, after MLF only the 3-(methylsulphanyl)propionic acid concentration increased significantly, as verified with several commercial starter cultures. This compound, which is characterized by chocolate and roasted odours, could contribute to the aromatic complexity produced by MLF.

Conclusions: This study shows that LAB isolated from wine, especially *Œnococcus œni* strains, the major species in MLF, are able to metabolize methionine to form volatile sulphur compounds.

Significance and Impact of the Study: This is the first study to demonstrate the capacity of wine LAB to metabolize methionine.

Keywords: aroma, lactic acid bacteria, metabolism, methionine, volatile sulphur compounds, wine.

INTRODUCTION

In most cases, winemaking includes two main fermentation processes: yeast ensures alcoholic fermentation (AF) and lactic acid bacteria (LAB) induce malolactic fermentation (MLF). *Œnococcus œni* (heterofermentative, Gram-positive coccus) usually grows spontaneously in wine after AF or commercial starters are added. This species is the major agent of MLF. MLF is necessary for red and white wines. In addition to malic acid degradation, which mainly softens the wine by decreasing its acidity and replacing malic acid by lactic acid, LAB trigger certain transformations that contribute to improving organoleptic quality.

Correspondence to: A. Lonvaud-Funel, Faculté d'Œnologie, UMR Œnologie-Ampélologie, INRA/ Université Victor Segalen Bordeaux 2, 351, Cours de la Libération, F-33405 Talence, France (e-mail: aline.lonvaud@oenologie.u-bordeaux2.fr).

Until now, there has been little consensus on the contribution of MLF. Studies have concerned only the synthesis of compounds with lactic or butter-like odours such as diacetyl or other carbonyl compounds resulting from the metabolism of organic acids (malic and citric) and residual sugars (traces of pentoses and hexoses) (Kunkee 1974; Bertrand *et al.* 1984; Davis *et al.* 1985; Henick-Kling 1995; de Revel *et al.* 1999; Delaquis *et al.* 2000). Interest is growing in *Œ. œni* species for improving their industrial application as malolactic starters. Knowledge in the nature and quality of components associated with LAB activity is becoming increasingly important as various bacterial activities play a role in wine aroma.

Among the wine substrates degraded by LAB, amino acids represent the most important source of nitrogen, carbon and sulphur for sulphurous amino acids. The metabolism of amino acids during MLF and its consequence on wine aroma

are not well understood, with the exception of biogenic amine formation (Coton *et al.* 1998) and the catabolism of arginine (Tonon and Lonvaud-Funel 2000). In the dairy industry, the enzymatic degradation of amino acids, in particular branched-chain and aromatic amino acids, generates aroma compounds and is involved in the complex process of cheese flavour development (Gao *et al.* 1997). Methionine metabolism has been studied in cheese LAB (*Brevibacterium*, *Lactobacillus* and *Lactococcus*) which produce methanethiol, a volatile sulphur compound associated with desirable cheese flavours (Ferchichi *et al.* 1985; Weimer *et al.* 1999). Bonnarme *et al.* (2000) suggest various pathways for production of methanethiol from methionine in cheese-ripening bacteria after detection of several enzymatic activities. Amarita *et al.* (2001) study the enzymatic conversion by *Lactococcus lactis* of methionine to methional another significant component of Cheddar flavour.

In wine, different kinds of volatile sulphurous compounds are known to be responsible for powerful and characteristic odours, in particular those arising from typical grape variety components. Another precursor is methionine whose degradation by yeast during AF leads to methanethiol, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid (Goniak and Noble 1987; Anocibar Beloqui 1998). Like most volatile sulphur compounds, these compounds can contribute to the pleasant or unpleasant flavours of wine according to their nature and concentration.

In addition to deacidification, the sensorial analysis of wine before and after MLF reveals such great differences that it is important to determine which reactions are involved. Moreover, sensorial changes vary according to the predominant *Œ. œni* strain responsible for MLF. Therefore, the most influential metabolic pathways and their control require elucidation. This work is the first to focus on methionine metabolism and MLF by comparing the products formed during cultures of several *Lactobacillus* and *Œnococcus* strains in laboratory media and in wines.

MATERIALS AND METHODS

Chemicals

Methionine, methanethiol, dimethyl disulphide, 3-(methylsulphanyl)propan-1-al, 3-(methylsulphanyl)propan-1-ol, 3-(methylsulphanyl)propionic acide, 2-oxo-4-(methylsulphanyl)butyric acid, 4-(methylsulphanyl)phenol, thiophene and H_3PO_4 were purchased from Sigma-Aldrich Chemicals (St Quentin Fallavier, France). Dichloromethane were obtained from the SDS company (Peypin, France), yeast extract, casamino acids, glucose, fructose, DL-malic acid, KH_2PO_4, KCl, $CaCl_2$, $MnSO_4$ and $MgSO_4$ were purchased from DIFCO (Detroit, MI, USA).

Table 1 Lactic acid bacterial strains used

Genus	Strains
Œnococus œni	IOEB 8406
	IOEB 8413
	IOEB 8908
	ATCC 23279
Lactobacillus brevis	IOEB 8511
	IOEB 8907
	IOEB 9809
	IOEB 9901
Lactobacillus hilgardii	IOEB 7701
	IOEB 8510
	IOEB 9103
Lactobacillus plantarum	IOEB 8512
	IOEB 8605
	IOEB 9113

Lactic acid bacterial strains

The strains used in this study are presented in Table 1. LAB were isolated from wine and belong to the collection of the Faculté d'Œnologie Bordeaux (IOEB) and to the American Type Culture Collection (ATCC). Strains were usually stored at −80°C in glycerol (30% v/v added to exponential growth phase of LAB culture).

Medium and culture conditions

The basal medium contained per litre: yeast extract 5 g, casamino acids 5 g, glucose 2·5 g, fructose 2·5 g, KH_2PO_4 0·55 g, KCl 0·425 g, $CaCl_2$ 0·125 g, $MnSO_4$ 2·5 mg and $MgSO_4$ 0·125 g. It was supplemented or not with L-methionine 1 g and adjusted at pH 5·0 with DL-malic acid. After sterilization by autoclaving for 20 min at 120°C, this medium was inoculated with a preculture in the exponential growth phase and incubated overnight at 25°C. Growth was monitored by measurement of optical density (O.D.) 600 nm on a 932 Uvikon spectrophotometer (Kontron, Rungis, France). For the preculture, we used the same basal medium not supplemented with L-methionine.

Wine trials

MLF in laboratory scale. All the samples used in this experiment came from the same Merlot wine. MLF was carried out in 5 l glass containers by directly adding the various commercial starter cultures (*Œ. œni*) to the wine. We used four commercial starter cultures called Viniflora Œnos (CHR Hansen, Horsholm, Denmark), Maloferm (Littorale Œnologie, Servian, France), EQ 54 (Lallemand S.A., Toulouse, France) and Vitilactic D (Martin Vialatte, Epernay, France).

MLF in cellar scale. Five red wines (Merlot) originating from various wineries in the Bordeaux area were studied. For the five wines, MLF was carried out by adding the Viniflora Œnos (CHR Hansen) starter culture directly.

Sensory evaluation and determination of olfactive perception threshold. For all experiments we used the same wine Merlot (chosen for its low concentration in 3-(methylsulphanyl)propionic acid 108 $\mu g\ l^{-1}$ after AF) from the Graves area, vintage 2000. MLF was carried out by adding the Viniflora Œnos starter culture directly.

Identification of products from methionine metabolism and their quantification in wine

Extraction was performed with 50 ml of centrifuged basal medium or wine added with 50 μl of 4-(methylsulphanyl)phenol at 400 mg l^{-1}, used as internal standard, 100 μl of di-tertbutyl-*p*-cresol at 200 mg l^{-1} and 300 μl of H_3PO_4 1/3 (v/v with water). The mixture was extracted twice by adding 5 ml dichloromethane for 5 min and then repeating the procedure. The extract was dried with 4 g anhydrous sodium sulphate and concentrated under nitrogen to one-fourth their initial volume. Two microlitres of extract were injected in GC/MS. The gas chromatograph (Hewlett-Packard 5890, Agilent Technique, Ramonville, France) was coupled with a HP 5972 mass spectrometer (electronic impact: 70 eV; eMV: 2·7 kV). The column was a BP 21 (SGE, Courtaboeuf, France) (50 m × 0·25 mm, 0·32 μm). The oven temperature was programmed from 40 to 220°C at a rate of 2°C min^{-1}, the final step lasting 20 min. The carrier gas was helium 'Aga 5·6' (Linde Gas S.A., Toulouse, France, 1·5 ml min^{-1}). The injector was a splitless system: the splitless time was 20 s and the split vent 30 ml min^{-1}. The methionine metabolism products were detected using the 'total ion chromatography' method (SCAN). A quantitative determination was carried out in 'selected ion monitoring' (SIM) mode, and selected ions of $m/z = 104$, 106 and 120 were chosen to quantify 3-(methylsulphanyl)propanal, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl) propionic acid, respectively, and ion $m/z = 140$ was used as the internal standard.

To test the capacity of different LAB to metabolize methionine, the above described method was modified to 4 ml centrifuged basal medium, 100 μl of internal standard [4-(methylsulphanyl)phenol at 40 mg l^{-1}], 100 μl H_3PO_4 1/3 (v/v with water) and 4 ml dichloromethane were added. The mixture was extracted by vortex agitation (2000 rev min^{-1} during 2 min). The extract was dried over 2 g anhydrous sodium sulphate and concentrated under nitrogen to one-fourth the initial volume. Two microlitres of extract were injected in GC/MS.

Determination of low boiling point volatile sulphur compounds

They were identified and quantified in wine using 100 ml of sample adjusted with 100 μl of thiophene (internal standard) at 100 mg l^{-1} in a 125-ml bottle. The bottle was hermetically closed by a stopper and was tightened with a metal capsule. After 24 h in the dark at a temperature of 22°C, 1 ml of the gas phase was injected according to the headspace technique.

To test the capacity of different LAB to metabolize methionine, the analysis was performed with 2 ml of sample added with 20 μl of internal standard (thiophene at 10 mg l^{-1}) in a 10-ml bottle. The bottle was hermetically closed by a stopper and was tightened with a metal capsule. After vortex agitation (2000 rev min for 1 min), 1 ml of the gas phase was injected according to the headspace technique. The gas chromatograph (Hewlett-Packard 5890) was coupled with a flame photometric detector (FPD). The column was HP 5 (30 m × 5 μm × 0·53 mm). The oven temperature was kept at 32°C for 1 min and programmed at a rate of 10°C min^{-1} to 100°C and a final rate of 20°C min^{-1} to 180°C. The carrier gas was hydrogen (1·5 ml min^{-1}). Its flow rate in the flame was 65 ml min^{-1} and a mixture of nitrogen/oxygen (80/20) at 80 ml min^{-1} was used. The make-up gas was nitrogen at 45 ml min^{-1}.

Determination of olfactive perception threshold

The 3-(methylsulphanyl)propionic acid odour threshold was determined as the minimum concentration below which 50% of the tasters failed to taste the difference from the control by the triangle test, at five concentrations in red wine. Tasting of the wines placed in AFNOR standard glasses was done by a 20-person jury according to the technique described by Ribéreau-Gayon *et al.* (1975).

Sensory evaluations

Tasting of the wines was carried out by a wine expert's 15-person jury. The wines were tasted at room temperature of 17°C, in AFNOR glasses and were evaluated by sniffing and tasting by quantitative descriptive analysis (NF V 09-016, AFNOR 1995). A card of 12 descriptors, established before, was proposed accompanied by a scale of intensity in eight categories (0–7). The wines were presented at the same time with a code at 3 digits.

Statistical analysis

For the acquisition of the data and the statistical analysis we used the software Fizz (Biosystem, Courtenon, France). The notes were interpreted using the results of a variance

analysis (ANOVA) to two factors (wines/judges) carried out on each of the 12 descriptors.

RESULTS

Methionine metabolism by LAB: identification of products

The reaction products formed after the development of two LAB strains (IOEB 8908 and IOEB 8511) in basal medium supplemented or not with L-methionine were identified using the SCAN method. A control, i.e. the medium supplemented with methionine but not inoculated, was also used. The identified reaction products (NBS 75k.l dated base library and/or commercial products) are shown in Table 2.

The strains tested were able to metabolize methionine to form methanethiol, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid. These compounds were not formed in the noninoculated control. Higher amounts were formed in the basal medium supplemented by methionine in which we also noticed the formation of dimethyl disulphide, and 3-(methylsulphanyl)propanal. These products could have been formed in the basal media without methionine addition because this medium contained 100 mg l^{-1} methionine provided by the casamino acids and yeast extract. Traces of many other compounds were identified, such as hydrogen sulphide, dimethyl sulphide, 2-methylpropionic acid and 3-methylbutanoic acid (results not shown), but their formation was not correlated with methionine metabolism. We found them in the same quantities in basal media supplemented or not with methionine, and some even in the control.

Comparison of several strains belonging to different species

To complement these results we studied the capacity of different *Lactobacillus* species and several strains of *Œ. œni* (Table 1) to produce sulphur compounds. The populations were monitored by measuring absorbance (O.D. 600 nm). The samples were taken when the bacterial cultures reached the end of the exponential growth phase and were repeated in triplicate. Here we present the averages and the standard deviation. All these strains were able to metabolize methionine to form methanethiol and dimethyl disulphide (Fig. 1a). The sum of the concentrations varied from 2·5 to 5·5 μmol l^{-1}. The differences between strains within the species were significant. Roughly, the total concentration was significantly higher for *L. brevis* IOEB 8511, *L. plantarum* IOEB 8605 and for two strains of *Œ. œni*: ATCC 23279 and IOEB 8413.

All the tested strains (Fig. 1b) were also able to form 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl) propionic acid. Compared with methanethiol and dimethyl disulphide, a considerable difference was observed in the capacity of the bacterial genus to form 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid. For the *Œ. œni* strains, higher amounts were produced. For the *Lactobacillus* strains, we did not observe any difference between *L. brevis* and *L. hilgardii*, which formed little 3-(methylsulphanyl)propan-1-ol and only traces of 3-(methylsulphanyl)propionic acid. For *L. plantarum* strains IOEB 9113 and IOEB 8605, the balance between the 3-(methylsulphanyl)propionic acid and 3-(methylsulphanyl)propan-1-ol was in favor of the acid.

Methionine metabolism by LAB in wine

To evaluate the capacity of LAB to metabolize methionine during MLF, we quantified the products before and after MLF of several red wines obtained from the Bordeaux area. Each analysis is repeated in triplicate. We present the averages and the standard deviation (in the majority of cases it is close to the coefficient of variation of the quantification method). To show a difference between the wines with and without MLF, the variation (%) of the concentration of each component was calculated. The difference is considered significant when is variation (%) exceeds the coefficient of

Table 2 Identification of methionine metabolism products after growth of two LAB strains, *Œnococcus œni* (IOEB 8908) and *Lactobacillus brevis* (IOEB 8511), in basal media supplemented or not with L-methionine

	Basal medium		Basal medium supplemented with L-methionine (1 gl^{-1})		
Reaction products	*Œ. œni* (IOEB 8908)	*L. brevis* (IOEB 8511)	Without bacterial strains	*Œ. œni* (IOEB 8908)	*L. brevis* (IOEB 8511)
Methanethiol	**	**	–	****	****
Dimethyl disulphide	–	–	–	***	***
3-(Methylsulphanyl)propan-1-al	–	–	–	*	*
3-(Methylsulphanyl)propan-1-ol	**	**	–	****	**
3-(Methylsulphanyl)propionic acid	*	*	–	***	**

–, Not detected.
*Traces; **weak; ***moderate and ****strong.

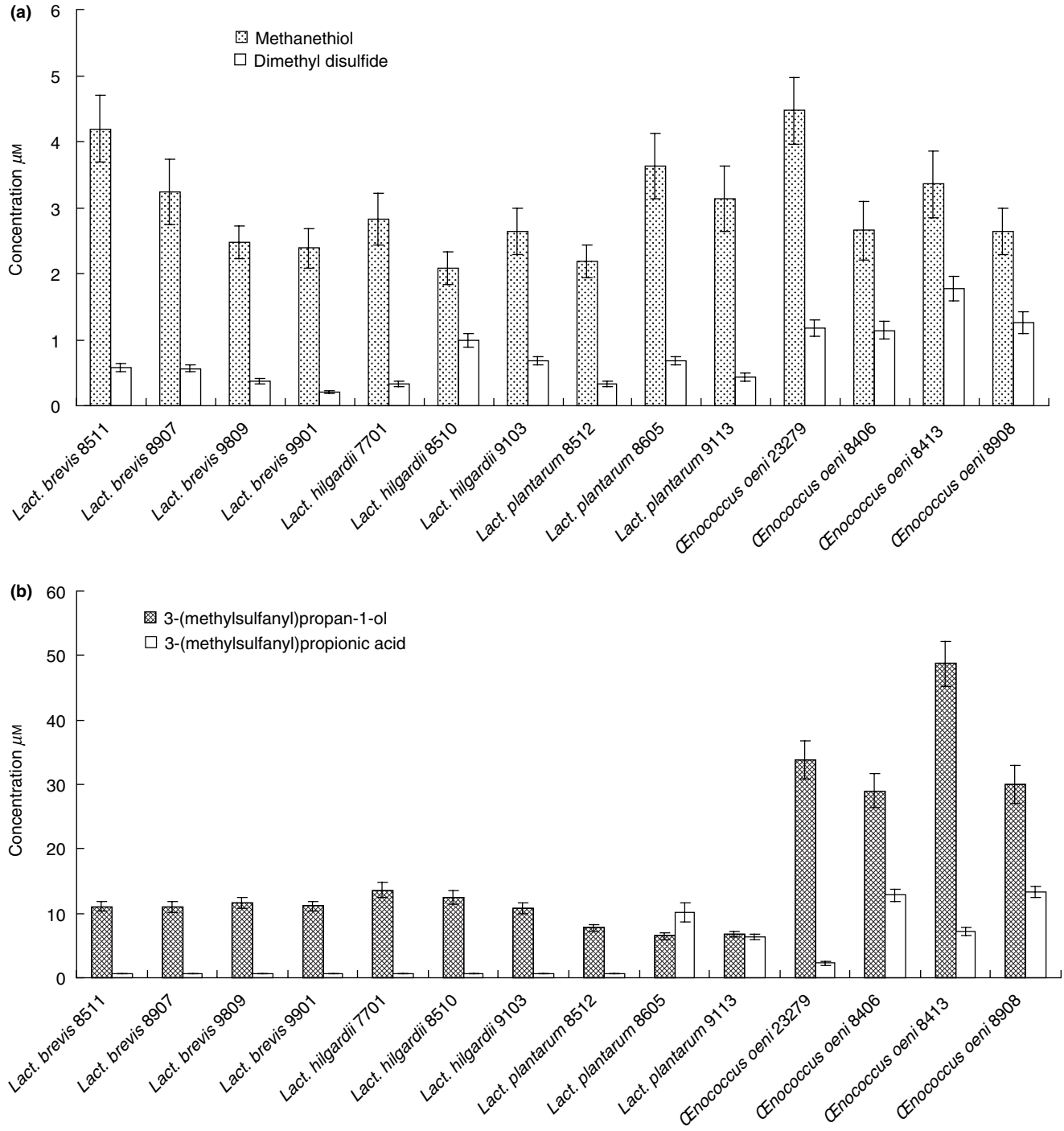

Fig. 1 Production of volatile sulphur compounds by various species and strains of *Lactobacillus* genus and *Œnococcus œni* species. We present the averages (histograms) ± standard deviation (bars) of three determinations. (a) methanethiol and dimethyl disulphide, (b) 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid

variation of the quantification method. First, we carried out a study in the laboratory with a red wine (Merlot) after AF. MLF was induced by using the four commercial starter cultures (B1, B2, B3, B4) and carried out in 5 l glass containers. A control was sulphited with 50 mg l^{-1} SO_2. In this test the heavy volatile sulphur compounds [3-(methylsulphanyl)propanal, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid] were only quantified

Table 3 Concentrations ($\mu g\ l^{-1}$) and variation (%) of the sulphur compounds in a red wine (Merlot) whose MLF was carried out by various commercial preparations

		Without MLF	With MLF			
Methionine metabolism products ($\mu g\ l^{-1}$)	CV %	SO_2 50 mg l^{-1}	B1	B2	B3	B4
3-(Methylsulphanyl)propan-1-ol	3·3	3889	4057 (4%)	4023 (3%)	3843 (−1%)	4125 (6%)
3-(Methylsulphanyl)propionic acid	6·2	262	301 (15%)	307 (17%)	284 (8%)	293 (12%)

CV %, coefficient of variation.
Values within parentheses are variation (%) compared with the sulphited wine (control).

Table 3 shows differences compared with the control as well as differences between the commercial starters used. After AF the concentration of 3-(methylsulphanyl)propionic acid was 262 $\mu g\ l^{-1}$ and it increased by 8–17% after MLF, according to the bacterial preparation. The 3-(methylsulphanyl)propan-1-ol concentration was high after AF (3889 $\mu g\ l^{-1}$), and its formation by LAB was weak, no significant increase occurred in this compound. Only the wine inoculated with B4 presented a significant increase compared with the coefficient of variation of the analytical method (3·3%). Changes in these compounds were the lowest in the wine inoculated by B3.

Secondly, the products were determined in several red wines (Merlot) made in various wineries in the Bordeaux area (Table 4) after spontaneous MLF. The 3-(methylsulphanyl)propionic acid concentration increased from 9 to 17% after MLF, whereas the 3-(methylsulphanyl)propan-1-ol increased significantly only in wine A. For these wines, we also quantified methanethiol and dimethyl disulphide but no increase occurred either during or after MLF.

Sensory consequences of the 3-(methylsulphanyl) propionic acid formation by LAB

First we determined its perception threshold in a red Merlot wine; it was 244 $\mu g\ l^{-1}$. Secondly, a list of 12 descriptors (Table 5) was established to characterize the presence of the 3-(methylsulphanyl)propionic acid in wine. Two samples of the same wine were presented to the jury, one control [3-(methylsulphanyl)propionic acid, 108 $\mu g\ l^{-1}$] and one brought to the concentration of 354 $\mu g\ l^{-1}$. At the later concentration 82% of the answers were positive when the perception threshold was determined. The tasters had to note descriptors which differentiate the two wines. Finally, using the 12 descriptors, we carried out a quantitative descriptive analysis (sensory profile) of the wine with and without addition of the 3-(methylsulphanyl)propionic acid. The wine was brought to the concentration of 262 $\mu g\ l^{-1}$ nearer to the perception threshold. The results of variance analyses carried out on the sensory profile (Table 5) underline a significant judge effect on seven descriptors (rancid butter, cheese, cream/butter, cooked fruits, plum, chocolate and roasted). This reflects a different use of the scale by the tasters. These apparent contradictions of the wine description can be due to the difficulties to simultaneously recognizing and evaluating the intensity of several descriptors, to various sensitivities and preferences for the proposal descriptors, and especially to a lack of training of the jury. The wines could be distinguished significantly on the following descriptors, earthy and red fruits. Moreover for these two descriptors the tasters used the scale in a relatively homogenous manner (no significant judge

Table 4 Concentrations ($\mu g\ l^{-1}$) and variation (%) of the sulphur compounds after MLF in several red wines (Merlot). Wine made in various wineries in the Bordeaux area

Wine	Methionine metabolism products ($\mu g\ l^{-1}$)	Without MLF	With MLF
A	3-(Methylsulphanyl)propan-1-ol	3029 ± 74	3220 ± 106 (6%)
	3-(Methylsulphanyl)propionic acid	407 ± 19	444 ± 15 (9%)
B	3-(Methylsulphanyl)propan-1-ol	2693 ± 52	2759 ± 76 (2%)
	3-(Methylsulphanyl)propionic acid	257 ± 13	288 ± 9 (12%)
C	3-(Methylsulphanyl)propan-1-ol	3437 ± 86	3576 ± 102 (4%)
	3-(Methylsulphanyl)propionic acid	221 ± 9	259 ± 12 (17%)
D	3-(Methylsulphanyl)propan-1-ol	2987 ± 47	3105 ± 63 (3%)
	3-(Methylsulphanyl)propionic acid	273 ± 11	307 ± 15 (12%)
E	3-(Methylsulphanyl)propan-1-ol	3752 ± 82	3804 ± 97 (1%)
	3-(Methylsulphanyl)propionic acid	287 ± 10	332 ± 16 (15%)

Values are mean ± S.D. of three determinations.
Values within parentheses are variation (%) compared with the wine without MLF.

Table 5 Probability study associated with *F*-values of two-factor ANOVA (wines/judge) for the 12 sensory attributes of 3-(methylsulphanyl) propioinc acid and across the two wines and 15 judges

Descriptor	Wines	Judges
Vegetal	0·8687	0·0954
Earthy	0·0453*	0·0583
Rancid butter	0·4649	0·0251*
Cheese	0·8178	0·0003***
Cream/butter	0·1038	0·0061**
Red fruits	0·0342*	0·1879
Plum	0·1442	0·0251*
Cooked fruits	0·4125	0·0038**
Chocolate	0·9999	0·0009***
Roasted	0·4346	0·0357*
Coffee	0·8646	0·1049
Roasted coffee	0·2526	0·0139

Significant to *5%; **1%; ***0·1%.

effect). The wines were not perceived as different for the descriptors, vegetal, rancid butter, cheese, cream/butter, cooked fruits, plum chocolate, roasted, coffee. The averages of the notes for the two wines show that the aromatic effect, for the descriptors earthy and red fruits, was related to the increase of the 3-(methylsulphanyl)propionic acid concentration beyond its perception threshold (Fig. 2).

DISCUSSION

The LAB isolated from wine are able to degrade methionine to form methanethiol, dimethyl disulphide and 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid. In view of the products formed after bacterial development, the methionine metabolic pathway of wine LAB, especially *Œ. œni*, should be similar to the one described in the literature for various micro-organisms (Namba *et al.* 1969; Weimer *et al.* 1999). The formation of the 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid could have a common intermediary, 3-(methylsulphanyl)propan-1-al, which was also identified after bacterial development in the basal media supplemented with methionine (Table 2).

For the species tested, all the strains formed significant quantities of methanethiol and dimethyl disulphide. However, the 3-(methylsulphanyl)propan-1-ol and the 3-(methylsulphanyl)propionic acid were formed in more significant quantities by *Œ. œni* than *Lactobacillus*. The metabolic products were the same for all the species and strains studied, including methanethiol and its oxidation product dimethyl disulphide, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid). The capacity of *Œ. œni* to produce more significant quantities of heavy volatile sulphur compounds was established. This is important

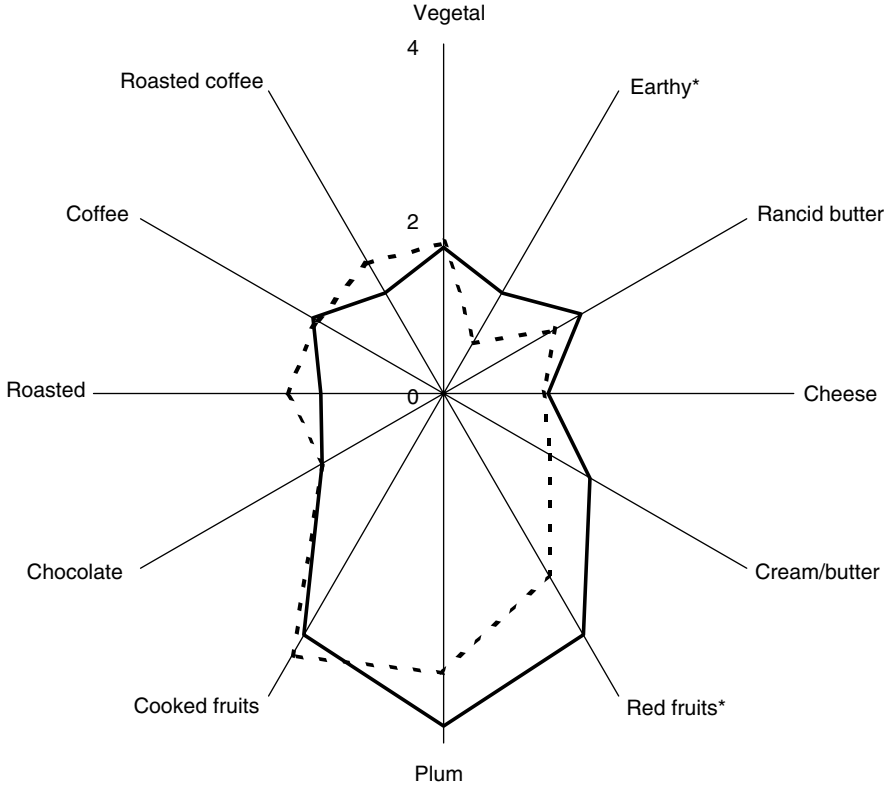

Fig. 2 Presentation of 12 descriptors (average of the note of 15 tasters) selected for the quantitative descriptive analysis (sensory profile) of a red wine (Merlot), with (continuous line) or without (stopped line) addition of the 3-(methylsulphanyl)propionic acid. *Descriptors which made it possible to differentiate the two wines

because *Œ. œni* is the major agent of MLF. The formation of 3-(methylsulphanyl)propionic acid and 3-(methylsulphanyl)propan-1-ol constitutes a difference between wine LAB and cheese LAB, like *Brevibacterium*, *Lactobacillus* and *Lactococcus* (Aston and Douglas 1983; Gao *et al.* 1998). In the latter, these compounds could not be identified from methionine metabolism.

The volatile sulphur compounds studied above are already present in wine (Goniak and Noble 1987; Anocibar Beloqui 1998) and occur during methionine metabolism by yeasts (Barwald and Kliem 1971; Muller *et al.* 1971). Methanethiol and 3-(methylsulphanyl)propan-1-ol are characterized by putrid faecal-like aroma and cooked cabbage descriptors, respectively. Methanethiol has a perception threshold of 0·3 $\mu g\ l^{-1}$ in synthetic solutions (ethanol 12% with water v/v, tartaric acid 5 $g\ l^{-1}$, plugged at pH 3·5 with NaOH 1 N), and its presence in wines is very often related to reduction defects. 3-(methylsulphanyl)propan-1-ol represents quantitatively the most significant volatile sulphur compound in wines and has a perception threshold of 1200 $\mu g\ l^{-1}$ in synthetic solutions. It is known to participate in the aromatic complexity of the wines, but at high concentrations also contributes to the reduction of flavour. Finally, 3-(methylsulphanyl)propionic acid has chocolate and roasted odours with a perception threshold of 50 $\mu g\ l^{-1}$ in synthetic solution.

Considering the importance of these compounds in the wine, it was significant to show that the wine LAB are able to metabolize methionine during MLF. However, in the wines analysed, only 3-(methylsulphanyl)propionic acid systematically produced a significant increase (Tables 3 and 4). To check the involvement of this compound in wine flavour, we determined its perception threshold in a red wine Merlot. As it was 244 $\mu g\ l^{-1}$ it would seem that the formation of 3-(methylsulphanyl)propionic acid during MLF may have an organoleptic consequence. The result after the quantitative descriptive analysis (Fig. 2) showed an aromatic effect related to the increase of 3-(methylsulphanyl)propionic acid as the concentration formed in the wine during AF was above the perception threshold. However, the descriptors which differentiate the wines are not the specific descriptors to the 3-(methylsulphanyl)propionic acid in synthetic solution (chocolate, roasted) but two others, red fruits and earthy odours. This result could show a synergy effect between the 3-(methylsulphanyl)propionic acid and other wine components. This work shows for the first time that LAB isolated from wine are able to degrade methionine to form methanethiol, dimethyl disulphide, 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl) propionic acid. By testing various species of *Lactobacillus* and strains of *Œ. œni*, we show that, the later, the main LAB of MLF, is distinct from the other species in its greater capacity to form 3-(methylsulphanyl)propan-1-ol and 3-(methylsulphanyl)propionic acid. Further work should study the influence of the conditions on the production of volatile sulphur compounds and should determine the various enzymatic pathways involved in methionine metabolism.

ACKNOWLEDGEMENT

Laura Pripis-Nicolau thanks the 'Conseil Interprofessionnel du Vin de Bordeaux' for their financial support.

REFERENCES

AFNOR (1995) Contrôle de la qualité des produits alimentaires. Analyse sensorielle. Recueil des normes françaises. 5° édition. AFNOR éd. Paris.

Amarita F., Fernadez-espla D., Requena T. and Peaez C. (2001) Conversion of methionine to methional by *Lactococcus lactis*. *FEMS Microbiology Letters* **204**, 189–195.

Anocibar Beloqui, A. (1998) Contribution to the study of sulfur compounds of the red wines. *PhD thesis*, no. 611, University Victor Segalen Bordeaux 2, Bordeaux, France.

Aston, J.W. and Douglas, K. (1983) The production of volatile sulfur compounds in Cheddar cheeses during accelerated ripening. *Australian Journal of Dairy Technology* **38**, 66–70.

Barwald, G. and Kliem, D. (1971) Ein Beitrag zum methioninstoffwechsel der hefe (*S. cerevisiae*). *Chemie Mikrobiologie Technologie der Lebensmitttel* **1**, 27–32.

Bertrand, A., Zmirou-Bonnamour, C. and Lonvaud-Funel, A. (1984) Aroma compounds formed in malolactic fermentation. In *Flavour Research of Alcoholic Beverage*; Proceedings Alko Symposium ed. Nykänen ant Lehtonen, pp. 39–49. Helsinki: Foundation for Biotechnical and Industrial Fermentation Research.

Bonnarme P., Psoni L. and Spinnler H.E. (2000) Diversity of L-methionine catabolism pathways in cheese-ripening bacteria. *Applied and Environmental Microbiology* **66**, 5514–5517.

Coton, E., Rollan, G., Bertrand, A. and Lonvaud-Funel A. (1998) Histamine-producing lactic acid bacteria in wines: early detection, frequency, and distribution. *American Journal of Enology and Viticulture* **49**, 199–203.

Davis, C.R., Wibow, D., Eschenbruch, R., Lee, T.H. and Fleet, G.M. (1985) Practical implications of malolactic fermentation: a review. *American Journal of Enology and Viticulture* **36**, 290–300.

Delaquis, P., Cliff, M., King, M., Girard, B., Hall, J. and Reynolds, A. (2000) Effect of two commercial malolactic cultures on the chemical and sensory properties of Chancellor wines vinified with different yeasts and fermentation temperatures. *American Journal of Enology and Viticulture* **51**, 42–48.

Ferchichi, M., Hemme, D., Nardi, M. and Pamboukdjian, N. (1985) Production of methanethiol from methionine by *Brevibacterium linens* CNRZ 918. *Journal of General Microbiology* **131**, 715–723.

Gao, S., Oh, D.H., Broadbent, J.R., Johnson, M.E., Weimer, B.C. and Steele J.L. (1997) Aromatic amino acid catabolism by lactococci. *Lait* **77**, 371–381.

Gao, S., Mooberry, E.S. and Steele, J.L. (1998) Use of ^{13}C nuclear magnetic resonance and gas chromatography to examine methionine

catabolism by lactococci. *Applied and Environmental Microbiology* **64**, 4670–4675.

Goniak, O.J. and Noble A.C. (1987) Sensory study of selected volatile sulfur compounds in white wine. *American Journal of Enology and Viticulture* **38**, 223–227.

Henick-Kling, T. (1995) Control of malo-lactic fermentation in wine: energetics, flavour modification and methods of starter culture preparation. *Journal of Applied Bacteriology Symposium Supplement* **79**, 29S–37S.

Kunkee, R.E. (1974) Malolactic fermentation and winemaking. In *Chemistry of Winemaking*; Advances in Chemistry Series 137 ed. Weeb, A.D. pp. 151–170. Washington, DC: American Chemical Society.

Muller, C.J., Kepner, R.E. and Webb, A.D. (1971) Identification of 3-(metylthio)propanol as an aroma constituent in "Cabernet Sauvignon" and "Ruby Cabernet" wines. *American Journal of Enologie and Viticulture* **22**, 156–160.

Namba, Y., Yoshizawa, K., Ejila, A., Hayashi, T. and Kaneda, T. (1969) Coenzyme A and NAPD branched chain keto-acid dehydrogenase. *Journal of Biological Chemistry* **244**, 4437–4447.

de Revel, G., Martin, N., Pripis-Nicolau, L., Lonvaud-Funel, A. and Bertrand, A. (1999) Contribution to the Knowledge of malolactic fermentation influence on wine aroma. *Journal of Agricultural and Food Chemistry* **47**, 4003–4008.

Ribéreau-Gayon, P., Boidron, J.N. and Terrier, A. (1975) Aroma of Muscat Grape Varieties. *Journal of Agricultural and Food Chemistry* **23**, 1042–1047.

Tonon, T. and Lonvaud-Funel, A. (2000) Metabolism of arginine and its positive effect on growth and revival of *Œ. œni*. *Journal of Applied Microbiology* **89**, 526–531.

Weimer, B., Seefeldt, K. and Dias, B. (1999) Sulfur metabolism in bacteria associated with cheese. *Antonie Van Leeuwenhoek* **76**, 247–261.