Food Chemistry 116 (2009) 208–213

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of alcoholic and malolactic starter cultures on bioactive amines in Merlot wines ☆

Luciano Manfroi [a], Paulo H.A. Silva [b], Luiz A. Rizzon [c], Priscila S. Sabaini [d], M. Beatriz A. Glória [d,*]

[a] Centro Federal de Educação Tecnológica de Petrolina, Br 407 km 08, s/n, Jd. São Paulo, Petrolina, PE 56300-000, Brazil
[b] Depto Tecnologia de Alimentos, Universidade Federal de Viçosa – UFV, Viçosa, MG 36570 000, Brazil
[c] Embrapa Uva e Vinho, Rua Livarmento, 515. CP 130, Bento Gonçalves, RS 95.700-000, Brazil
[d] LBqA – Laboratório de Bioquímica de Alimentos, FAFAR, Universidade Federal de Minas Gerais, Av. Antonio Carlos 6627, Belo Horizonte, MG 31270 901, Brazil

ARTICLE INFO

Article history:
Received 29 January 2008
Received in revised form 17 January 2009
Accepted 16 February 2009

Keywords:
Biogenic amines
Malolactic fermentation
Alcoholic fermentation
Wine

ABSTRACT

The influence of alcoholic and malolactic fermentations on the levels of amines in Merlot wines was investigated. *Saccharomyces bayanus*, *S. cerevisiae*, *Lactobacillus plantarum*, *Oenococcus oeni* (DSM 7008 and 12923) and spontaneous fermentations were used. Four of the 10 amines investigated were detected: spermidine, serotonin, putrescine and cadaverine. When considering the factors independently, the malolactic bacteria significantly affected the levels of serotonin and total amines, whereas the fermentation yeasts significantly affected the levels of spermidine (two way Kruskal–Wallis, $p \leqslant 0.05$). Spermidine levels were significantly higher in wines produced with *S. cerevisiae*. Significantly higher serotonin levels were found in wines made with *L. plantarum*. Putrescine and cadaverine were not detected in wines produced by spontaneous alcoholic fermentation or by *L. plantarum*. There were significant differences in alcohol content, total and volatile acidity, sulphite levels and taste quality among wines (Tukey test, $p \leqslant 0.05$).

1. Introduction

According to Vidal-Carou, Codony-Salcedo, and Mariné-Font (1990), the first report on the levels of histamine in wines was available in 1965, due to histamine poisoning associated with wine samples. In the 1980s interest was extended to other amines, among them, tyramine, putrescine and cadaverine, due to technological and toxicological aspects. From a technological point of view, high levels of these amines are associated with low quality products or with defective winemaking practices, indicating poor hygienic conditions during processing. The toxicological interest is based on health problems, such as 'histamine poisoning' and migraine headache, caused by histamine and tyramine, respectively. Furthermore, putrescine and cadaverine were observed to potentiate the toxic effect of histamine and tyramine (Gloria and Vieira, 2007; Vidal-Carou et al., 1990). Some amines are also significant to wines in terms of aroma and flavour (González-Marco & Ancín-Azpilicueta, 2006). In general, a weakening of the flavour impression is attributed to amines, whereby an unpleasant bitter aftertaste has been described in wines with high amine levels (Gloria, 2005). Furthermore, putrescine and cadaverine can negatively affect the sensory quality of wines (García-Villar, Hernández-Cassou, & Saurina, 2007).

Recently, other amines are attracting attention: spermidine, spermine, tryptamine, phenylethylamine, agmatine and serotonin (Gloria, Watson, Simon-Sarkadi, & Daeschel, 1998; mo Dugo, Vilase, la Torre, & Pellicanò, 2006; Soufleros, Bouloumpasi, Zotou, & Loukou, 2007; Souza, Theodoro, Souza, Motta, & Glória, 2005; Yildirim, Üren, & Yücel, 2007). The polyamines, spermidine and spermine, play important roles in cell growth and differentiation. They are implicated in plant response to environmental challenges (Bouchereau, Aziz, Larher, & Martin-Tanguy, 1999; Darrieumerlou, Geny, Broquedis, & Doneche, 2001; Soleas, Carey, & Goldberg, 1999). The importance of polyamines to human health has also been described (Moinard, Cynober, & de Bandt, 2005). They are essential in the maintenance of cells and have antioxidant activity. Agmatine is a precursor of polyamines. Tryptamine and phenylethylamine are associated with migraine headache (Gloria, 2005).

Some amines are normal constituents of grapes, the amounts varying with grape variety, soil type and composition, fertilisation and climatic conditions during growth and degree of maturation. Spermidine is usually abundant in grapes, whereas putrescine, agmatine, cadaverine, spermine, histamine, tyramine and phenylethylamine have been found in small amounts (García-Villar et al., 2007; Glória et al., 1998; Hajós, Sass-Kiss, Szerdahelyi, & Bardócz, 2000; Soleas et al., 1999; Souza et al., 2005).

☆ This work was part of the doctorate thesis in Food Science and Technology of Lucaino Manfroi at UFV, Viçosa, MG.

* Corresponding author. Tel.: +55 31 34982419; fax: +55 31 34096911.
E-mail address: mbeatriz@ufmg.br (M. Beatriz A. Glória).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.02.034

Beside the amines already present in grapes, several can be formed and accumulate during winemaking. The formation of amines depends on the pH of the wine, addition of sulphur dioxide, use of clarification agents, ageing with or without lees and, length of barrel aging (Alcaide-Hidalgo, Moreno-Arribas, Martín-Álvarez, & Pólo, 2007; Gloria & Vieira, 2007; Marques, Leitão, & San Romão, 2008; Soleas et al., 1999). However, reports are contradictory. According to Vidal-Carou et al. (1990), there was formation of tyramine and histamine during alcoholic fermentation. However, in this study, there was no control of the microbial population present in the must; therefore, the formation of amines could not be attributed solely to yeasts.

Evidence of amine formation during malolactic fermentation has been described. According to Soufleros et al. (2007), the concentration of histidine decreases while that of histamine increases. Most researchers attribute the formation of amines, especially of tyramine and histamine, to the action of bacteria involved in malolactic fermentation (Soufleros et al., 2007; Vidal-Carou et al., 1990; Zhijun, Yongning, Gong, Yunfeng, & Changhu, 2007). However, there are reports indicating the possibility that histamine can be formed in wines by the action of contaminant microorganisms, for example enteric bacteria (Kiss, Korbáz, & Sass-Kiss, 2006; Soufleros et al., 2007). Studies are needed to determine the role of winemaking practices on a broader spectrum of amines.

The objective of this study was to investigate the influence of different species and strains of alcoholic and malolactic microorganisms on the profile and levels of 10 bioactive amines in Merlot wines.

2. Materials and methods

2.1. Samples and reagents

Merlot grapes from Bento Gonçalves, Rio Grande do Sul, Brazil, from the 2001 vintage, were used. The wines were obtained from microvinification at Embrapa Uva e Vinho, Bento Gonçalves, RS, Brazil. Pure active dry wine yeasts, *Saccharomyces cerevisiae* and *S. bayanus* (Maurivin™, AB Brasil, São Paulo, SP, Brazil) and pure active dry malolactic bacteria *Oenococcus oeni* DSM 7008, *O. oeni* DSM 12923, *Lactobacillus plantarum* DSM 4361, (Viniflora, Chr. Hansen, Hørsholm, Denmark), as well as spontaneous fermentation, e.g. indigenous yeast and bacteria, were used.

Ten bioactive amine standards (99% pure) were purchased from Sigma Chemical Co. (Saint Louis, MO, USA): putrescine (PUT) dihydrochloride, spermidine (SPD) trihydrochloride, spermine (SPM) tetrahydrochloride, agmatine (AGM) sulphate, cadaverine (CAD) dihydrochloride, serotonin (SRT) hydrochloride, histamine (HIM) dihydrochloride, tyramine (TYM), tryptamine (TRM) and 2-phenylethylamine (PHM) dihydrochloride. The reagents used in the analysis (trichoroacetic acid, acetic acid, potassium hydroxide and boric acid) were of analytical grade, except the HPLC solvent, acetonitrile, which was of LC grade. Ultrapure water was obtained from Milli-Q. Sulphur dioxide used in the wine was of commercial grade.

2.2. Winemaking

The wine was obtained as follows: immediately after harvest, the grapes (16 kg for each treatment, performed in triplicate) were destemmed, crushed and placed with the skins in 20 l glass containers, along with sulphur dioxide (50 mg/l). For the alcoholic fermentation, the yeasts (*S. bayanus* and *S. cerevisiae*) were incorporated at concentrations of 250 mg/l. The yeasts were hydrated with water at 36 °C/15 min and inoculated into the must (~10 million cells/ml). Spontaneous alcoholic fermentation was achieved by naturally present indigenous yeasts. The containers were closed with Muller valves and kept at 25 °C. After 15 days of maceration, the skins and other solid matter, including lees, were removed by pressing (racking).

For the malolactic fermentation, commercial pure strains cultures of *O. oeni* DSM 7008, *O. oeni* DSM 12923 and *L. plantarum*, and also naturally present indigenous bacteria, were used. The dried *L. plantarum* (200 mg/l) was incorporated 2 h after the addition of the yeast; however, dried *O. oeni* was added (6.0 mg/l) after alcoholic fermentation had been completed and the lees removed. The jars were kept at 25 °C until malolactic fermentation ended, as determined by paper chromatography (20–40 days). After conclusion of malolactic fermentation, the wines were transferred to 2 l bottles and kept at 0 °C for 15 days to allow precipitation of tartaric acid. The wine was transferred to 750 ml bottles, closed with corks and stored horizontally at 18 °C.

Overall, 12 batches of wine were prepared under exactly the same conditions in triplicate. Fifty days after bottling, the wines were analysed for bioactive amines, and some physicochemical and sensory characteristics.

2.3. Determination of bioactive amines

The wine samples were homogenised, centrifuged at 10,000*g* at 4 °C for 20 min, and filtered through HAWP membranes (Millipore, Barueri, SP, Brazil). The amines were separated by ion-pair reverse-phase HPLC and quantified by fluorescence at 340 nm excitation and 445 nm emission, after post-column derivatisation with *o*-phthalaldehyde (Souza et al., 2005). The amines were identified by comparing retention times with those of standards and also by addition of the suspected amine to the sample. Quantification was possible by interpolation in analytical curves.

2.4. Determination of the physicochemical characteristics

Most of the physicochemical characteristics were determined according to the methods described by the Office International de la Vigne et du Vin (OIV, 1990). The pH was determined using a digital pH meter (model 125 Corning, New York, USA). Total and volatile titratable acidity were determined by titration with 0.1 *N* NaOH in the presence of bromothymol and phenolphthalein, respectively. Total sulphur dioxide was determined by titration with 0.02 *N* I_2. Dry extract was determined by dehydration. The alcoholic degree was determined with a Zeiss refractometer (Carl Zeiss, São Paulo, SP, Brazil) with # 1 prism and the reducing sugars were determined with Fehlings' solution (Meyer & Leygue-Alba, 1991).

2.5. Sensory evaluation

The wines were evaluated by seven panellists, trained for descriptive analysis, from the Sensory Evaluation Laboratory at Embrapa Uva e Vinho, Bento Gonçalves, RS, Brazil. Different sensory components of the wines were evaluated using a blind test. The parameters evaluated included appearance, odour, aroma, flavour and varietal characteristics. Visual appearance was investigated with regard to clarity, intensity and colour; the odour (olfactory sensation felt by the nose) was evaluated for intensity, balance, quality and undesirable odours; the taste (olfactory sensation upon mastication) was investigated with regard to intensity, body, astringency, acidity, balance, quality and undesirable tastes; the olfactory-taste examination focused on balance and persistence; finally, varietal characteristics were evaluated. Each of these aspects was evaluated according to a hedonic scale from 1 to 7 for each parameter, as indicated in the score sheet. The overall quality of the wine was also evaluated on a 20 point scale (defective = 1–2; below average = 3–7; average = 8–13; above average = 14–18;

high = 19–20). The analysis was performed in three sections when four different wines were evaluated with three repetitions a day (Amerine & Roessler, 1983).

2.6. Statistical analysis

Every experiment was performed in triplicate and the analyses were in duplicate. The experimental design used is presented in Table 1. Normality and homogeneity of the variance were studied with the Lilliefors and Bartlett tests, respectively, at 5% probability. Parametric data were submitted to two-way analysis of variance and the means were compared by the Tukey test at 5% probability. Non-parametric data were submitted to the Kruskal–Wallis two-way test. Pearson correlation at 1% probability was used to investigate correlation among physicochemical characteristics, sensory quality and bioactive amines. All data were evaluated using SAEG 9.0 Statistical software.

3. Results and discussion

3.1. Types of amines detected in the Merlot wines produced

Among the 10 amines investigated, only four (spermidine, putrescine, cadaverine and serotonin) were detected in the samples. According to Shiozaki, Ogata, and Horiuchi (2000), these four amines can be naturally present in grapes and, therefore, in wines. The polyamine spermidine is involved in several physiological processes relevant to plant development. Putrescine is an obligate intermediate in the synthesis of spermidine. Cadaverine plays an important role in cell elongation and serotonin can have a protective role in deterring predators (Gloria, 2005; Gloria, Tavares-Neto, Labanca, and Carvalho, 2006). However, putrescine and cadaverine can also be formed by intentionally added (starter cultures) or contaminant microorganisms (Alcaide-Hidalgo et al., 2007; Gloria & Vieira, 2007; Kiss et al., 2006).

Histamine, tyramine, tryptamine, phenylethylamine, spermine and agmatine were not found in the wines produced in the present study. The presence of the first four amines was reported in the literature for wines and their concentration was observed to be affected by cultivation practices (soil fertilisation, climatic conditions), grape microbiota and also by microbial contamination during wine making (Gloria & Vieira, 2007). Therefore, it can be hypothesised that the cultivation practices and winemaking conditions prevalent in this study prevented the formation of these amines. Furthermore, the wines investigated in this study were only 50 days old, and, therefore, much younger than the samples analysed in other studies (Souza et al., 2005). Such an age difference could significantly affect amine profiles and levels.

Table 1
Types of alcoholic and malolactic fermentation cultures used during Merlot wine making under standardised conditions.

Treatments[a]	Alcoholic fermentation	Malolactic fermentation
T1	Spontaneous[b]	Spontaneous[b]
T2	Spontaneous[b]	*Oenococcus oeni* DSM 7008
T3	Spontaneous[b]	*Oenococcus oeni* DSM 12923
T4	Spontaneous[b]	*Lactobacillus plantarum* DSM 4361
T5	*Saccharomyces bayanus*	Spontaneous[b]
T6	*Saccharomyces bayanus*	*Oenococcus oeni* DSM 7008
T7	*Saccharomyces bayanus*	*Oenococcus oeni* DSM 12923
T8	*Saccharomyces bayanus*	*Lactobacillus plantarum* DSM 4361
T9	*Saccharomyces cerevisiae*	Spontaneous[b]
T10	*Saccharomyces cerevisiae*	*Oenococcus oeni* DSM 7008
T11	*Saccharomyces cerevisiae*	*Oenococcus oeni* DSM 12923
T12	*Saccharomyces cerevisiae*	*Lactobacillus plantarum* DSM 4361

[a] $n = 3$.
[b] Spontaneous fermentation.

3.2. Influence of starter culture on the quality of Merlot wines

Analysis of normality and homogeneity of variance indicated that the amine data did not follow a normal distribution; therefore, the two-way Kruskal–Wallis test, at 5% probability, was used for the comparison of means. Data for the physicochemical and sensory characteristics followed a normal distribution and had variance homogeneity; therefore, two way Anova and Tukey tests, at 5% probability, were used.

Statistical analysis indicated that, when considering the factors independently, the malolactic bacteria significantly affected the levels of serotonin and total amines, whereas the alcoholic fermentation yeasts significantly affected the levels of spermidine. Significant interaction between yeast and malolactic bacteria was not observed for any amine.

The mean total levels of amines present in the wines produced with the different microorganisms varied from non-detected (<0.40 mg/l) to 24.2 mg/l, as indicated on Table 2. Significantly higher total amine levels were obtained when *L. plantarum* was used during malolatic fermentation. Similar results were obtained for serotonin. Significantly higher serotonin levels were observed when *L. plantarum* was used. The influence of alcoholic and malolactic fermentation microorganisms on the formation of serotonin was investigated for the first time.

The type of yeast used significantly affected the levels of spermidine in the wine, with significantly higher levels detected when *S. cerevisiae* was used. It is generally accepted that yeasts are unable to liberate polyamines and diamines in significant amounts. According to Bover-Cid, Izquierdo-Pulido, Mariné-Font, and Vidal-Carou (2006), the levels of spermidine and spermine usually decrease during alcoholic fermentation as these amines can be used by the alcoholic fermentative yeasts as an energy source. The results obtained in this study suggest that spontaneous fermenting yeasts and *S. bayanus* were able to use spermidine, which is a natural polyamine from the grape, whereas *S. cerevisiae* could not.

With regard to the biogenic amines, no significant difference was observed for putrescine and cadaverine; however, when spontaneous fermenting yeasts were used and *L. plantarum* was used for malolactic fermentation, no putrescine was detected. For cadaverine, the use of spontaneous fermentation, along with *O. oeni* DSM 12923 or *L. plantarum*, prevented the accumulation of cadaverine.

Landete, Ferrer, and Pardo (2007) observed no formation of biogenic amines during alcoholic fermentation using different strains of several species of yeasts, including *S. cerevisiae* and *S. bayanus*. However, Garde-Cerdán and Ancín-Azpilicueta (2007) observed the formation of putrescine during spontaneous alcoholic fermentation of *Parellada* grapes. In sterilised musts of the same grape inoculated with *S. cerevisiae* subsp. *cerevisiae* (Na33), phenylethylamine was detected and putrescine and spermidine were produced at higher levels than with spontaneous fermentation. According to Caruso et al. (2002), low levels of putrescine and cadaverine were produced by different strains of *S. cerevisiae*. However, high cadaverine levels are usually associated with decarboxylase activity of contaminant enterobacteria. Therefore, the presence of low or non-detected levels of cadaverine, which is a good indicator of the degree of spoilage (Bover-Cid et al., 2006), reinforces the good hygienic conditions used during wine making.

The addition of starter culture during malolactic fermentation compared to spontaneous fermentation, provided better quality wine as it avoided the accumulation of putrescine (*L. plantarum* DSM 4361) and cadaverine (*L. plantarum* DSM 4361 or *O. oeni*

Table 2
Levels of spermidine, serotonin, putrescine, cadaverine and total amines produced during Merlot wine making with different alcoholic and malolactic cultures.

Alcoholic fermentation	Amine levels (mg/l)/malolactic fermentation				
	Spontaneous	*O. oeni* DSM 7008	*O. oeni* DSM 12923	*L. plantarum* DSM 4361	Yeast
Spermidine					
Spontaneous	17.3 ± 1.63	15.8 ± 0.14	14.7 ± 0.64	15.6 ± 3.18	*15.8 ± 1.70*^{y}
S. bayanus	14.1 ± 1.27	14.9 ± 1.63	17.7 ± 0.35	20.0 ± 0.78	*16.6 ± 0.78*^{xy}
S. cerevisiae	18.9 ± 0.71	20.8 ± 1.06	16.7 ± 2.4	14.7 ± 0.14	*17.8 ± 2.64*^{x}
(Bacteria)	*16.8 ± 2.39*	*17.1 ± 2.96*	*16.3 ± 1.77*	*16.7 ± 2.92*	
Serotonin					
Spontaneous	0.00	1.93 ± 0.043	15.5 ± 1.62	14.3 ± 0.76	*7.94 ± 7.54*
S. bayanus	11.4 ± 0.33	5.75 ± 0.64	5.98 ± 1.13	22.9 ± 1.14	*11.5 ± 7.47*
S. cerevisiae	4.37 ± 0.69	6.64 ± 1.28	4.69 ± 0.85	13.6 ± 0.21	*7.33 ± 4.04*
(Bacteria)	*5.25 ± 5.15*^{ab}	*4.77 ± 2.33*^{b}	*8.72 ± 5.36*^{ab}	*17.0 ± 4.67*^{a}	
Putrescine					
Spontaneous	0.00	0.00	0.00	0.00	*0.00*
S. bayanus	1.01 ± 1.43	1.22 ± 1.72	1.72 ± 0.33	0.00	*0.99 ± 1.08*
S. cerevisiae	0.77 ± 1.10	0.84 ± 1.18	0.59 ± 0.83	0.00	*0.55 ± 0.77*
(Bacteria)	*0.59 ± 0.93*	*0.68 ± 1.09*	*0.77 ± 0.88*	*0.00*	
Cadaverine					
Spontaneous	0.00	0.00	0.00	0.00	*0.00*
S. bayanus	0.00	0.43 ± 0.60	0.00	0.00	*0.11 ± 0.30*
S. cerevisiae	0.89 ± 1.26	0.76 ± 1.07	0.00	0.00	*0.41 ± 0.77*
(Bacteria)	*0.30 ± 0.73*	*0.40 ± 0.65*	*0.00*	*0.00*	
Total amines					
Spontaneous	0.00	1.93 ± 0.03	15.5 ± 1.62	14.3 ± 0.61	*7.94 ± 7.54*
S. bayanus	12.4 ± 1.10	7.40 ± 1.75	7.70 ± 1.46	24.1 ± 0.93	*12.9 ± 7.31*
S. cerevisiae	6.88 ± 0.95	9.08 ± 1.41	6.23 ± 0.00	14.6 ± 0.26	*9.20 ± 3.59*
(Bacteria)	*6.43 ± 5.59*^{ab}	*6.13 ± 3.49*^{b}	*9.81 ± 4.55*^{ab}	*17.7 ± 5.01*^{a}	

Mean levels (zero was used for non-detected levels, nd < 0.40 mg/l) for each amine with different letters (xy in the columns and ab in the lines) are significantly different (Kruskal–Wallis two-way test, $p < 0.05$).

DSM 12923), which could impart a putrid flavour to the wine. Similar results were obtained by Pillate (1998) who observed low putrescine levels when using *O. oeni* during malolactic fermentation of Merlot must. Guerrini, Mangani, Granchi, and Vincenzini (2002) also observed that several *O. oeni* strains were able to form both putrescine and cadaverine to different extents and in variable relative proportions. However, according to Pramateftaki, Metafa, Kallithraka, and Lanaridis (2006), and Alcaide-Hidalgo et al. (2007), certain strains of *Oenococcus* can produce low levels of putrescine, cadaverine, histamine, tyramine and phenylethylamine. Furthermore, Landete et al. (2007) observed that *L. plantarum* cells were prolific producers of histamine, but did not produce tyramine and phenylethylamine.

The absence of putrescine and cadaverine formation when *L. plantarum* was used is in accordance with studies by Arena and Manca de Nadra (2001), who observed that this microorganism was a poor producer of putrescine.

Even though there are reports of the formation of biogenic amines, especially tyramine and histamine, during malolactic fermentation, this type of fermentation does not necessarily result in the formation of these amines (Marcobal et al., 2006; Pramateftaki et al., 2006; Soufleros et al., 2007). In fact, in this study, there

Table 3
Alcohol content, pH, volatile and total acidity and levels of total SO_2 in Merlot wines made with different alcoholic and malolactic cultures.

Alcoholic fermentation	Values/malolatic fermentation			
	Spontaneous	*O. oeni* DSM 7008	*O. oeni* DSM 12923	*L. plantarum* DSM 4361
Alcohol (%v/v)				
Spontaneous	10.1 ± 0.10^{x,a}	10.1 ± 0.06^{x,a}	10.0 ± 0.10^{xy,a}	9.87 ± 0.25^{x,a}
S. bayanus	10.1 ± 0.10^{x,a}	9.93 ± 0.12^{x,a}	10.3 ± 0.10^{x,a}	10.2 ± 0.10^{x,a}
S. cerevisiae	9.77 ± 0.15^{x,a}	9.93 ± 0.06^{x,a}	9.80 ± 0.10^{y,a}	9.83 ± 0.12^{x,a}
Volatile acidity (meq/l)				
Spontaneous	9.80 ± 0.35^{x,ab}	9.33 ± 0.83^{x,ab}	10.3 ± 0.12^{x,a}	8.27 ± 0.76^{x,b}
S. bayanus	8.53 ± 0.81^{xy,a}	8.27 ± 0.23^{x,a}	8.27 ± 0.12^{y,a}	7.27 ± 0.23^{x,a}
S. cerevisiae	7.80 ± 0.72^{y,a}	7.93 ± 0.64^{x,a}	7.30 ± 0.26^{y,a}	7.07 ± 0.50 ^{x,a}
Total acidity (meq/l)				
Spontaneous	72.7 ± 2.31^{y,b}	72.7 ± 4.62^{y,b}	72.0 ± 2.00^{y,b}	84.7 ± 2.31^{x,a}
S. bayanus	72.0 ± 2.00^{y,a}	72.7 ± 1.15^{y,a}	69.3 ± 1.15^{y,a}	76.0 ± 0.00^{y,a}
S. cerevisiae	84.7 ± 1.15^{x,a}	84.0 ± 0.00^{x,a}	86.0 ± 0.00^{x,a}	87.3 ± 1.51^{x,a}
SO_2				
Spontaneous	16.5 ± 1.69^{xy,a}	15.4 ± 0.76^{y,a}	14.7 ± 0.45^{x,a}	15.7 ± 2.26^{x,a}
S. bayanus	13.2 ± 1.75^{y,c}	14.3 ± 1.46^{y,bc}	18.6 ± 1.66^{x,ab}	20.3 ± 0.78^{x,a}
S. cerevisiae	19.1 ± 0.58^{x,ab}	20.9 ± 0.78^{x,a}	15.8 ± 2.27^{x,ab}	15.4 ± 1.27^{x,b}

Mean levels for each amine with different letters (xyz in the columns and abcd in the lines) are significantly different (Tukey test, $p < 0.05$).

Table 4
Range and mean values for the sensory evaluation, using seven trained panelists, of the Merlot wines produced.

Aspects	Values[c]	
	Range	Mean
Visual attributes[a]		
Clarity (turbid–brilliant)	2.48–4.02	3.64 ± 0.45
Intensity (weak – intense)	2.36–3.11	2.87 ± 0.21
Colour (violet-red–brick-red)	2.48–3.70	3.08 ± 0.33
Olfactory[a]		
Intensity (weak–strong)	2.14–3.40	2.89 ± 0.35
Balance (low–high)	2.11–3.00	2.46 ± 0.29
Quality (none–high)	2.00–3.07	2.42 ± 0.34
Undesirable odour (none–accentuated)	0.96–2.04	1.56 ± 0.37
Taste[a]		
Intensity (weak–intense)	2.45–3.26	2.88 ± 0.24
Body (thin–dense)	2.40–2.79	2.59 ± 0.15
Astringency (none–strong)	2.32–2.83	2.57 ± 0.17
Acidity (flat–acid)	3.23–4.06	3.53 ± 0.34
Balance (low–high)	1.93–2.76	2.43 ± 0.25
Quality (none–high)*	1.93–3.01	2.59 ± 0.33
Undesirable taste (none–accentuated)	0.92–2.26	1.43 ± 0.41
Olfactory-taste[a]		
Balance (low–high)	2.04–2.80	2.53 ± 0.22
Persistence (short–long)	2.23–2.91	2.56 ± 0.23
Varietals characteristic[a] (none–high)	2.13–2.86	2.49 ± 0.25
General quality[b] (defective–high)	8.24–11.43	10.11 ± 0.95

[a] Hedonic scale from 1 to 7.
[b] Scale from 1 to 20: defective = 1–2; below average = 3–7; average = 8–13; above average = 14–18; high = 19–20.
[c] Range and mean values for the different treatments ($n = 12$).
* Significant difference was observed among treatments at 5% probability, Tukey test.

was no production of these biogenic amines, irrespective of the use of starter culture.

The biogenic amines can be formed and build up by contaminating microorganisms, especially enteric bacteria (Kiss et al., 2006). However, the use of sulphur dioxide could prevent the production of histamine and tyramine (Vidal-Carou et al., 1990; Yildirim et al., 2007). The lack of accumulation of biogenic amines during the winemaking process is in agreement with the proper hygienic and controlled conditions used during winemaking. Therefore, the profile and levels of biogenic amines in the wines suggest that they were produced under adequate hygienic sanitary conditions.

The use of *S. bayanus* and *S. cerevisiae* allowed putrescine and cadaverine accumulation at low levels in the wine, except when associated with *L. plantarum*. These amines were not detected in wines made with indigenous yeasts. Since the production and accumulation of these amines is undesirable in wines, a screening of starter cultures, based on their potential to produce amines, should be performed.

Significant interaction between yeast and malolactic bacteria was not observed for serotonin and total amine levels; however, no significant difference was observed among treatments.

3.3. Physicochemical characteristics of the wines

Physicochemical characteristics of the wines produced are indicated on Table 3. There were no significant differences ($p > 0.05$) among treatments with regard to reducing sugars (1.94–2.25 g/l), dry extract (17.0–20.2 g/l) and pH (3.34–3.42) (data not shown).

Even though there were significant differences among treatments, no relevant tendency was observed with respect to the alcoholic content of the wines, except that when *O. oeni* DSM 12923 was used, lower alcohol contents were detected when *S. cerevisiae* was used than when *S. bayanus* was used. Volatile acidity was higher when indigenous yeasts were used; however, total acidity was higher when *S. cerevisiae* was used. Even though malolactic fermentation has a function of deacidification of the wine (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004), the different malolactic bacteria did not significantly affect the acidity of the wine, except when spontaneous alcoholic fermentation was used. The total levels of SO_2 varied among wines with higher levels occurring when *S. bayanus* and *L. plantarum* were used. Wines made with *S. bayanus* and *L. plantarum* contained more SO_2 than did reference wine made with *S. cerevisiae*.

3.4. Sensory characteristics of the wines and correlation with amines levels

Overall, the wines were poorly rated with regard to the sensory evaluation (Table 4). This is probably due to the low age of the wines (50 days after bottling). Among the 17 parameters evaluated, no significant difference was observed among treatments, except for the attribute taste quality.

When spontaneous alcoholic fermentation was performed, better taste was observed if *O. oeni* DSM 12923 or *L. plantarum* were used as malolactic cultures (Table 5). When spontaneous malolactic fermentation was used, better taste was observed if *S. bayanus* was used as yeast.

3.5. Correlation between amines levels, physicochemical and sensory characteristics of the wine

According to the literature, several factors can affect the levels of amines in wines, among them, alcoholic content, pH, sulphur dioxide and wine making procedures (Garde-Cerdán & Ancín-Azpilicueta, 2007; Marcobal et al., 2006; Soufleros et al., 2007; Yildirim et al., 2007). In the present study, with the exception of the microorganisms used for alcoholic and malolactic fermentations, all of the conditions were standardised; therefore, the changes observed were solely affected by the microorganisms.

Even though there were significant differences among some of the variables investigated in the wines, no significant correlation was observed between the physicochemical and sensory characteristics and the levels of bioactive amines. This result suggests that the differences caused by the type of microorganisms on these parameters were not enough to cause a significant change on the levels of amines in the wines.

Table 5
Scores from the evaluation of the taste quality of Merlot wines made with different alcoholic and malolactic cultures.

Alcoholic fermentation	Scores/malolatic fermentation			
	Spontaneous	*O. oeni* DSM 7008	*O. oeni* DSM 12923	*L. plantarum* DSM 4361
Taste quality				
Spontaneous	$1.93^{y,b}$	$2.68^{x,ab}$	$3.01^{x,a}$	$2.91^{x,a}$
S. bayanus	$2.96^{x,a}$	$2.69^{x,a}$	$2.65^{x,a}$	$2.74^{x,a}$
S. cerevisiae	$2.48^{xy,a}$	$2.45^{x,a}$	$2.13^{x,a}$	$2.40^{x,a}$

Scores were obtained from a hedonic scale of 1 to 7.
Mean levels for each amine with different letters (xyz in the columns and abcd in the lines) are significantly different (Tukey test, $p < 0.05$).

4. Conclusions

The influence of different alcoholic and malolactic microorganisms on the levels of bioactive amines, physicochemical and sensory characteristics of Merlot wine was investigated for the first time. Among the ten amines investigated, only four were detected: spermidine, putrescine, cadaverine and serotonin. None of the amines which are hazardous to human health were detected, among them, histamine, tyramine and tryptamine.

The type of microorganism used during alcoholic and malolactic fermentations significantly affected the profile and levels of amines in Merlot wines. It proved to be possible to produce Merlot wines with no bioactive amines. Furthermore, the profile of amines in the wine could be predicted or optimised by the types of alcoholic and malolactic microorganisms used.

There were significant differences among physicochemical parameters and sensory characteristics of the wines. But the differences were not enough to affect the levels of bioactive amines in the wines.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq and Fundação de Amparo a Pesquisa do Estado de Minas Gerais – Fapemig for financial support.

References

Alcaide-Hidalgo, J. M., Moreno-Arribas, M. V., Martín-Álvarez, P. J., & Pólo, M. C. (2007). Influence of malolactic fermentation, post fermentative treatments and ageing with lees on nitrogen compounds of red wines. *Food Chemistry, 103*, 572–581.

Alexandre, H., Costello, P. J., Remize, F., Guzzo, J., & Guilloux-Benatier, M. (2004). *Saccharomyces cerevisiae–Oenococcus oeni* interactions in wine: Current knowledge and perspectives. *International Journal of Food Microbiology, 93*, 141–154.

Amerine, M. A., & Roessler, E. B. (1983). *Wines and their sensory evaluation*. San Francisco: Freeman.

Arena, M. E., & Manca de Nadra, M. C. (2001). Biogenic amine production by *Lactobacillus*. *Journal of Applied Microbiology, 90*, 158–162.

Bouchereau, A., Aziz, A., Larher, F., & Martin-Tanguy, J. (1999). Polyamines and environmental challenges: recent development. *Plant Science, 140*, 103–125.

Bover-Cid, S., Izquierdo-Pulido, M., Mariné-Font, A., & Vidal-Carou, M. C. (2006). Biogenic mono-, di- and polyamine contents in Spanish wines and influence of a limited irrigation. *Food Chemistry, 96*, 43–47.

Caruso, M., Fiori, C., Contursi, M., Salzano, G., Paparella, A., & Romano, P. (2002). Formation of biogenic amines as criteria for the selection of wine yeasts. *Journal of Microbiology and Biotechnology, 18*, 159–163.

Darrieumerlou, A., Geny, L., Broquedis, M., & Doneche, B. (2001). Evolution of polyamine composition in grape berries during infection with *Botrytis cinerea*. *Vitis, 40*, 11–15.

García-Villar, N., Hernández-Cassou, S., & Saurina, J. (2007). Characterization of wines through the biogenic amine contents using chromatographic techniques and chemometric data analysis. *Journal of Agricultural and Food Chemistry, 55*, 7453–7461.

Garde-Cerdán, T., & Ancín-Azpilicueta, C. (2007). Effect of SO_2 on the formation and evolution of volatile compounds in wines. *Food Control, 18*, 1501–1506.

Gloria, M. B. A. (2005). Bioactive amines. In Y. H. Hui (Ed.), *Handbook of food science, technology and engineering*. Melbourne: CRC Press. p. 38.

Gloria, M. B. A., Tavares-Neto, J., Labanca, R. A., & Carvalho, M. S. (2006). Influence of cultivar and germination on bioactive amines in soybeans (*Glycine max* L. Merril). *Journal of Agricultural and Food Chemistry, 53*, 7480–7485.

Gloria, M. B. A., & Vieira, S. M. (2007). Technological and toxicological significance of bioactive amines in grapes and wines. *Food, 1*(2), 258–270.

Gloria, M. B. A., Watson, B. T., Simon-Sarkadi, L., & Daeschel, M. A. (1998). A survey of biogenic amines in Oregon Pinot noir and Cabernet Sauvignon wines. *American Journal of Enology and Viticulture, 49*, 279–282.

González-Marco, A., & Ancín-Azpilicueta, C. (2006). Amine concentration in wine stored in bottles at different temperatures. *Food Chemistry, 99*, 680–685.

Guerrini, S., Mangani, S., Granchi, L., & Vincenzini, M. (2002). Biogenic amine production by *Oenococcus oeni*. *Current Microbiology, 44*, 374–378.

Hajós, G., Sass-Kiss, A., Szerdahelyi, E., & Bardócz, S. (2000). Changes in biogenic amine content of Tokaj grapes, wines, and Aszú-wines. *Journal of Food Science, 65*, 1142–1144.

Kiss, J., Korbáz, M., & Sass-Kiss, A. (2006). Study of amine composition of botrytized grape berries. *Journal of Agricultural and Food Chemistry, 54*, 8909–8918.

Landete, J. M., Ferrer, S., & Pardo, I. (2007). Biogenic amine production by lactic acid bacteria, acetic bacteria and yeast isolated from wine. *Food Control, 18*, 169–174.

Marcobal, A., Polo, M. C., Martín-Álvarez, P. J., Polo, M. C., Muñoz, R., & Moreno-Arribas, M. V. (2006). Formation of biogenic amines throughout the industrial manufacture of red wine. *Journal of Food Protection, 69*, 397–404.

Marques, A. P., Leitão, M. C., & San Romão, M. V. (2008). Biogenic amines in wines: influence of oenological factors. *Food Chemistry, 107*, 853–860.

Meyer, C. R., & Leygue-Alba, N. M. R. (1991). *Manual de métodos analíticos enológicos* (p. 51). Caxias do Sul: UCS.

mo Dugo, G., Vilase, F., la Torre, G. L., & Pellicanò, T. M. (2006). Reverse phase HPLC/DAD determination of biogenic amines as dansyl derivatives in experimental red wines. *Food Chemistry, 95*, 672–676.

Moinard, C., Cynober, L., & de Bandt, J.-P. (2005). Polyamines: Metabolism and implications in humans diseases. *Clinical Nutrition, 24*, 184–197.

OIV (1990). *Office International de la Vigne et du Vin*. Paris: Recuell des méthodes internationales d'analyse des vins et des mo uts. 368p.

Pillate, E. (1998). Maîtrise de la fermentation malolatique: Mieux gérer et anteciper. *Revue des Enologues, 88*, 21–22.

Pramateftaki, P. V., Metafa, M., Kallithraka, S., & Lanaridis, P. (2006). Evolution of malolactic bacteria and biogenic amines during spontaneous malolactic fermentations in a Greek winery. *Letters in Applied Microbiology, 43*, 155–160.

Shiozaki, S., Ogata, T., & Horiuchi, S. (2000). Endogenous polyamines in the pericarp and seed of the grape berry during development and ripening. *Scientia Horticulturae, 83*, 33–41.

Soleas, G. J., Carey, M., & Goldberg, D. M. (1999). Method development and cultivar-related differences of nine biogenic amines in Ontario wines. *Food Chemistry, 64*, 49–58.

Soufleros, E., Bouloumpasi, E., Zotou, A., & Loukou, Z. (2007). Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration. *Food Chemistry, 101*, 704–716.

Souza, S. C., Theodoro, K. H., Souza, É. R., Motta, S., & Glória, M. B. A. (2005). Bioactive amines in Brazilian wines: Types, levels and correlation with physico-chemical parameters. *Brazilian Archives of Biology and Technology, 48*, 53–62.

Vidal-Carou, M. C., Codony-Salcedo, R., & Mariné-Font, A. (1990). Histamine and tyramine in Spanish wines: Relationship with total sulfur dioxide level, volatile acidity and malolactic fermentation intensity. *Food Chemistry, 35*, 217–227.

Yildirim, H. K., Üren, A., & Yücel, U. (2007). Evaluation of biogenic amines in organic and non-organic wines by HPLC OPA derivatization. *Food Technology and Biotechnology, 45*, 62–68.

Zhijun, L., Yongning, W., Gong, Z., Yunfeng, Z., & Changhu, X. (2007). A survey of biogenic amines in Chinese red wines. *Food Chemistry, 105*, 1530–1535.