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Influence of malolactic fermentation, postfermentative treatments and ageing with lees on nitrogen compounds of red wines

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Abstract

A comparative study was conducted on nine batches of wine taken from the same initial wine, subjected to malolactic fermentation and ageing in barrels, under different technological conditions: malolactic fermentation in barrel or in tank, with or without wine clarification, ageing with or without lees, and stirring or no stirring of the lees. Samples were taken of the initial wine, of the wine at the end of malolactic fermentation, of the wines after clarifying treatments, and after 3, 6, 9, 12 and 14 months of ageing in the barrel, making a total of 51 wines. Only a very small decrease in amino acids was observed during malolactic fermentation, probably due to the wine releasing amino acids produced by the exocellular proteasic activity of the lactic acid bacteria. Ageing of the wine with lees modifies its nitrogen composition because amino acids are released by yeast and bacteria autolysis. The amount of amino acids released was greatest in the wines stirred weekly. All of the wines studied contained low concentrations of biogenic amines.

Keywords: Wine; Malolactic fermentation; Ageing on lees; Amino acids; Biogenic amines

1. Introduction

During the manufacture of red wines, after alcoholic fermentation has finished, malolactic fermentation (MLF) takes place, usually in the same tank. Its main purpose is to reduce wine acidity, transforming malic acid, a dicarboxylic acid, into lactic acid, a monocarboxylic acid. Moreover, during this process volatile compounds are also formed, which enrich the wine's aromatic quality (de Revel, Martin, Pripis-Nicolau, Lonvaud-Funel, & Bertrand, 1999; Henick-Kling, 1995). On the other hand, during MLF there is the risk of the lactic acid bacteria producing biogenic amines, mainly from the metabolism of amino acids, as occurs in other fermented food products. Biogenic amines produce undesirable physiological effects in the human organism, especially in sensitive individuals (Bauza et al., 1995), and their presence should be avoided.

After MLF has finished, the wine is submitted to different treatments, to clarify it and stabilise it, and is stored in oak barrels for ageing for a variable period of time, ranging from a few months to over a year. In recent years, in an attempt to obtain more complex wines from an organoleptic perspective, with their own distinguishing personality, new production technologies are being introduced in the wineries, including ageing of wine in barrels, to which the lees from the malolactic and alcoholic fermentations has been added. Another production method consists in carrying out the malolactic fermentation in the oak barrels in which the ageing takes place, after which the wine is aged further with its own lees. For ageing with lees, the wine is regularly stirred, with greater or lesser frequency, to facilitate the transfer of compounds from the lees to the wine.

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Traditional red wine production technology in which malolactic fermentation is carried out in tanks and ageing in barrels, has been widely studied (Henick-Kling, 1995). However, less research has been done on malolactic fermentation in barrels, in which the control of the process is reduced and, therefore, entails more risks. There are also few studies on the changes occurring during ageing in the barrel with the lees. Most research in this area has focused on the changes in phenolic composition (Hernández, Estrella, Carlavilla, Martín-Álvarez, & Moreno-Arribas, 2006) and in polysaccharides (Escot, Feuillat, Dulau, & Charpentier, 2001), and has been conducted in wines to which yeast walls or autolysed yeasts obtained in model solutions have been added (Guilloux-Benatier & Chassagne, 2003; Salmon, Vuchot, Doco, & Moutounet, 2003). To our knowledge there have been no studies conducted on the influence of this production method on changes in amino acid composition and on biogenic amine formation.

The formation of biogenic amines in wines depends on the presence of lactic acid bacteria, which can decarboxylate the corresponding amino acids. The pH of the wine and the amount of sulfur dioxide added at the start of vinification also affect the amount and the types of lactic acid bacteria present (Lonvaud-Funel, 2001; Vidal Carou, Cordony-Salcedo, & Mariné-Font, 1990). Therefore, for results to be comparable, studies on the influence of different MLF techniques and ageing in the barrel on the formation of biogenic amines should be conducted on the same base wine. To do this, nine batches of wine have been produced from a single base wine of the Tempranillo variety in a winery from Navarra, in northern Spain. MLF was carried out in tanks or in barrel, some of the wine was submitted to different clarification treatments and ageing was done with or without lees, stirred weekly or monthly or not stirred.

2. Material and methods

2.1. Manufacture of wines

A red wine was industrially prepared and submitted to different experiments in a winery. A description of the wines is shown in Table 1 and these were manufactured as follows. The initial wine used was a quality red wine from the AOC Navarra (Spain), from *Vitis vinifera* L., c.v. Tempranillo grapes, manufactured in 10 000 l stainless-steel tanks. After alcoholic fermentation, part of this wine (≈7000 l) underwent malolactic fermentation in stainless-steel tanks (MLF-T wines). After this second fermentation had finished, part of the wine was treated with the following postfermentation treatments: racking (T-R wine), racking and clarification with albumin and bentonite (T-C wine), and racking, clarification, cold stabilisation and filtration (T-S wine). These treated wines were transferred to oak barrels and were considered as control wines. Another part of the MLF-T wine was transferred to oak barrels, without removing the lees (T-L wines). The rest of the wine (≈3000 l) underwent malolactic fermentation in oak barrels with yeast lees (MLF-B wines). During ageing in barrels with lees, some of the wines were stirred weekly (T-L-Sw and B-L-Sw wines), others were stirred monthly (T-L-Sm and B-L-Sm wines), and stirring was not carried out in barrels T-L-S0 and B-L-S0. Malolactic fermentation was carried out by inoculation of a commercial lactic acid bacterium, *Oenococcus oeni* (ITV 04 A1) provided by Oenofrance (Rueil-Malmaison, France).

For storage of the wines during MLF and wine ageing, new 225 l barrels of French oak (*Quercus sessilis*) were used. During ageing, four barrels of each technological batch were considered and the samples from the barrels were mixed and homogenised before analysis. A total of 51 wine samples were analysed. Wine samples were taken before and after malolactic fermentation, after postfermentation treatments and at 3, 6, 9, 12 and 14 months of ageing. At each sampling time, wine samples were collected, centrifuged for 15 min at 5000*g* and immediately refrigerated until analysis. Each analytical assay was perform in duplicate.

2.2. Total nitrogen analysis

Total nitrogen was determined by the Kjeldahl method with a DK 20 Heating Digestor System and a UDK 142 Automatic Distillation Unit from VELP Scientifica (Milan,

Table 1
Technological procedures for the manufacture of wines and key labels

Wine that performed MLF in stainless-steel tank MLF-T	Wines aged without lees T (control wines)	T-R (with racking) T-C (with racking and clarification) T-S (with racking, clarification and cold stabilisation)
	Wines aged with lees T-L	T-L-S0 (without stirring) T-L-Sw (with weekly stirring) T-L-Sm (with monthly stirring)
Wine that performed MLF in barrel MLF-B	Wines aged with lees B-L	B-L-S0 (without stirring) B-L-Sw (with weekly stirring) B-L-Sm (with monthly stirring)

Italy) and a 702 SM Tritino Unit from Metrohm (Herisan, Switzerland).

2.3. Amino acid analysis

Amino acids were analysed in duplicate by reversed-phase HPLC using a liquid chromatograph, consisting of a Waters 600 Controller programmable solvent module (Waters, Milford, MA), a WISP 710B autosampler (Waters), and a HP 1046-A fluorescence detector (Hewlett Packard, Palo Alto, CA). Samples were submitted to automatic precolumn derivatization with *o*-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol. Solvents and gradient conditions were as described by Moreno-Arribas, Pueyo, Polo, and Martín-Alvarez (1998). Separations were performed on a Waters Nova-Pak C18 (150 × 3.9 mm i.d., 60 Å, 4 μm) column and the same type of precolumn. Detection was performed by fluorescence ($\lambda_{excitation} = 340$ nm, $\lambda_{emission} = 425$ nm) and chromatographic data were collected and analysed with a Millenium32 system (Waters). Variation coefficients of the amino acids determination were less than 6% (Martínez-Rodriguez & Polo, 2000).

2.4. Biogenic amines analysis

Biogenic amines were analysed by reversed-phase HPLC, according to the method described by Marcobal, Polo, Martín-Álvarez, and Moreno-Arribas (2005). The chromatographic system was the same as that used for the amino acid analysis. Samples were previously filtered through Millipore filters (0.45 μm) and then directly injected in duplicate onto the HPLC system. All reagents used were HPLC grade. The variation coefficients of the amine determination ranged from 0.20 for ethylamine to 9.95% for methylamine (Marcobal et al., 2005).

2.5. Statistical analysis

The statistical methods used for the data analysis were cluster analysis (Ward's method from standardised variables), to discover natural groupings of the wine samples, and principal component analysis (from standardised variables), to examine the relationship among the analysed variables. Two-way analysis of variance (ANOVA) was used to test the effects of the two factors studied (techno-

Table 2
Total nitrogen, free amino acids and biogenic amines content (mg/l) in initial wine and in wines after malolactic fermentation and after treatments

	Initial wine	Malolactic fermentation in tank				Malolactic fermentation in barrel
		MLF-T	T-R	T-C	T-S	MLF-B
Total nitrogen	226.8	197.7	204.9	232.4	215.6	192.1
Amino acids						
Aspartic acid	9.7	10.3	16.9	14.4	15.4	10.9
Glutamic acid	19.4	17.6	18.0	14.1	18.7	19.7
Asparagine	10.2	11.0	12.3	9.6	16.4	11.5
Serine	7.0	7.7	6.3	8.7	4.8	8.1
Glutamine	7.0	8.0	5.0	5.0	6.0	7.0
Histidine	7.5	7.9	5.3	n.d.[a]	n.d.	7.5
Glycine	10.9	10.0	5.9	9.8	7.7	8.6
Threonine	7.9	8.4	6.4	8.9	5.4	10.0
Arginine	14.4	n.d.	n.d.	n.d.	n.d.	n.d.
β-Alanine	n.d.	n.d.	1.0	4.0	1.0	n.d.
α-Alanine	14.5	14.6	14.6	18.2	18.7	17.1
γ-Aminobutyric acid	8.1	9.2	9.4	7.0	6.6	8.9
Tyrosine	9.3	n.d.	n.d.	n.d.	1.5	9.9
Methionine	5.6	5.4	4.6	3.6	6.6	5.6
Valine	6.9	7.4	8.7	2.7	6.2	8.2
Tryptophan	9.1	9.6	6.8	6.4	2.7	9.5
Phenylalanine	10.1	10.9	11.3	9.9	10.8	11.3
Isoleucine	6.2	6.1	5.2	5.9	2.2	7.0
Leucine	9.5	10.3	13.8	10.5	10.0	11.1
Ornithine	n.d.	8.0	7.0	7.0	9.0	3.0
Lysine	9.7	10.6	10.2	10.4	10.4	12.5
Total free amino acids	183.0	173.9	169.0	156.0	160.0	187.3
Amines						
Histamine	1.0	1.8	3.6	3.4	2.8	1.9
Methylamine	0.8	0.8	0.8	0.8	0.9	0.7
Ethylamine	0.8	0.8	0.8	0.8	1.2	0.8
Tyramine	1.7	2.0	3.7	3.6	5.4	2.1
Phenylethylamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Putrescine	2.3	4.1	5.9	5.9	5.9	4.1
Cadaverine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

For identity of samples, see Table 1.
[a] n.d. = not detected.

logical factor and ageing time) and the Scheffe test for the comparisons of means. STATISTICA for Windows (Version 7.1) was used for data processing (StatSoft, Inc., 2005, www.statsoft.com). This program was run on a personal computer.

3. Results and discussion

3.1. Changes in nitrogen compounds during malolactic fermentation, and clarification and stabilisation treatments

Table 2 shows the total nitrogen contents in the initial wine, the samples taken after malolactic fermentation in tanks (MLF-T) and in barrels (MLF-B), and the samples taken after racking (T-R wine), after clarification (T-C wine) and after tartaric stabilisation and filtration (T-S wine). Before malolactic fermentation, the wine had a relatively low total nitrogen content, 226.8 mg/l, lower than the mean value, 306 mg/l, reported by Cáceres, Polo, and Cabezudo (1987), in a study conducted on 45 Spanish red wines of different geographical origins. During malolactic fermentation, the total nitrogen content dropped to similar levels in the MLF-B wine (197.7 mg/l) and in the MLF-T wine (192.1 mg/l). In the control wine samples taken after the clarifying and stabilising treatments, the total nitrogen contents were higher, probably due to the release of nitrogen by the yeasts after fermentation has finished and before the different clarification and stabilising treatments had started.

Table 2 also shows the free amino acid contents of these wines. These are also lower than the mean values recorded in the literature (Cáceres, Barahona, & Polo, 1986). The major amino acids in the initial wine are glutamic acid, α-alanine, arginine, glycine, asparagine and phenylalanine, which are present at concentrations ranging from 10 to 20 mg/l. Aspartic acid, tyrosine, tryptophan, leucine and lysine are present at concentrations between 9 and 10 mg/l. The remaining amino acids determined are present at concentrations lower than 9 mg/l. It is interesting to note the absence of the amino acid ornithine, which is present in most red wines (Etiévant, Schlich, Bouvier, Symonds, & Bertrand, 1988; Marcobal, Martín-Álvarez, Polo, Muñoz, & Moreno-Arribas, 2006; Pozo-Bayón et al., 2005).

No important changes occurred in the free amino acid concentrations during malolactic fermentation, either in barrels or in tanks (see Table 2). The most relevant finding was the disappearance of arginine and the formation of ornithine. This suggests that the strain used can degrade arginine, producing ornithine via the arginine deiminase pathway, as described for other heterofermentative bacteria (Liu, Pritchard, Hardman, & Pilone, 1995; Remize, Augagneur, Guilloux-Benatier, & Guzzo, 2005). Arginine is an essential amino acid for *O. oeni*, which degrades it as a source of ATP (Fourcassie, Makaga-Kabinda-Massard, Belarbi, & Maujean, 1992). Tyrosine disappears completely during malolactic fermentation in tank. According to Garvic (1967) this amino acid is essential to *Oenococcus*, although Fourcassie et al. (1992) do not consider it essential for the strains they study. There is no consumption of phenylalanine, an amino acid precursor of phenylethyl-

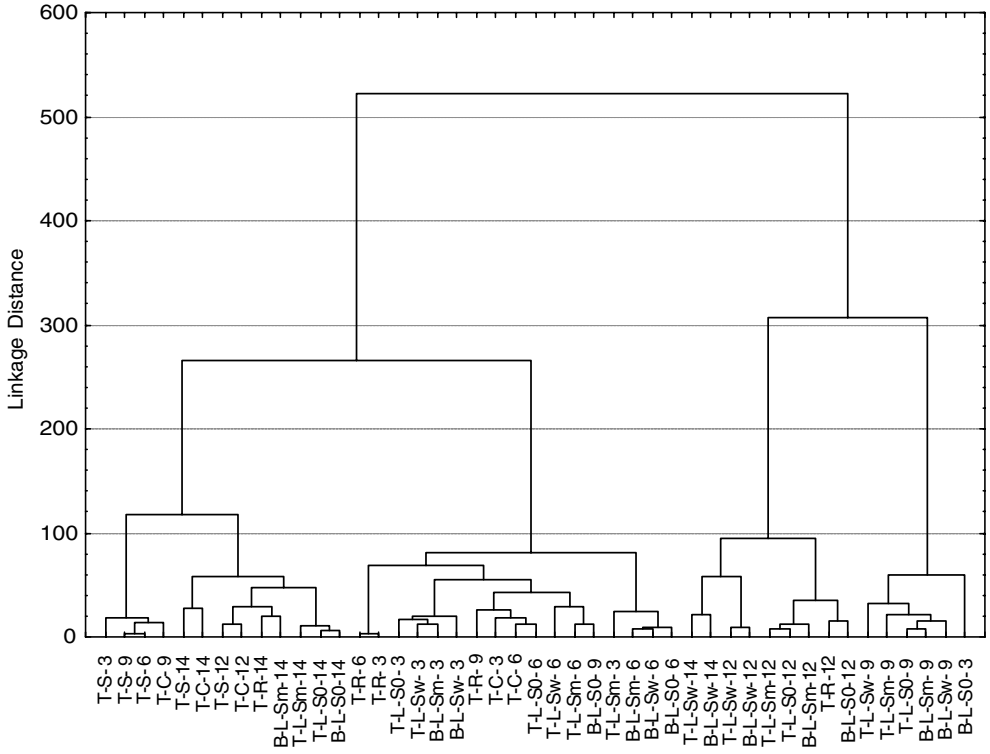

Fig. 1. Dendrogram of the wine samples according to the data of total nitrogen, free amino acids and biogenic amines. For identity of samples, see Table 1.

amine, 90% of which is consumed by *L. plantarum,* according to Liu, Davis, and Brooks (1995). The sum of the free amino acids in the wine after malolactic fermentation in the barrel is of the same order as that in the initial wine (187.3 mg/l) and the sum of the free amino acids in the wine undergoing malolactic fermentation in the tank is somewhat lower (173.9 mg/l). The difference in the sum of the free amino acids does not correspond with the decrease in total nitrogen content, which is lower. This suggests that amino acids have probably been released from the wine proteins or peptides by the proteases of the lactic acid bacteria. Exocellular proteasic activity of the lactic acid bacteria has been described in model solutions (Manca de Nadra, Farias, Moreno-Arribas, Pueyo, & Polo, 1999) and mainly occurs in nitrogen-poor media (Remize et al., 2005), such as the initial wine used here.

The initial wine does not contain the amines phenylethylamine or cadaverine and, compared to values recorded in the literature for red wines before malolactic fermentation (Landete, Ferrer, Polo, & Pardo, 2005; Marcobal et al., 2005; Soufleros, Barrios, & Bertrand, 1998), has a low content of histamine, methyl and ethylamine, tyramine and putrescine (Table 2). During malolactic fermentation, the concentration of histamine, tyramine and putrescine increased slightly to 0.9, 0.4 and 1.8 mg/l, respectively, in wines that had undergone malolactic fermentation in the barrel and to values of 0.8, 0.3 and 1.8 mg/l in the wines in which malolactic fermentation had taken place in a tank. Phenylethylamine was not detected in any of the wines, in agreement with no decrease occurring in phenylalanine during MLF. During the racking, clarification and tartaric stabilisation operations, there was a slight rise in histamine and tyramine content, probably because of the bacteria having conserved their enzymatic activity after completing MLF. There was also a decrease in some amino acids, especially histidine, which actually disappeared in the wines after clarification (wines T-C and T-S).

3.2. Changes in nitrogen compounds during wine ageing

Once the wines had finished malolactic fermentation, they were aged in barrels under different conditions (Table 1). Samples were taken from wines from each of the barrels at 3, 6, 9, 12 and 14 months of ageing. In order to establish whether any grouping of the wines occurred on the basis of the data of total nitrogen, free amino acids and biogenic amines, cluster analysis was applied. Fig. 1 shows the dendrogram obtained using these data. The square of the Euclidean distance was used to measure the similarity between samples on the basis of standardised variables and Ward's method for fusion of the groups. Two main groups were obtained: most of the 9 and 12 month-old wines appear in one group and the remaining wines appear in the other group. The second group is, in turn, divided into two subgroups, one mainly comprised of most 14-month old wines and the aged wines after racking, clarification and cold stabilisation (T-S) and the other mainly comprised of wines with shorter ageing times, of 3 and 6 months. The 9-month wines were included in all of the three groups. No clusters were observed due to the three ageing procedures: with or without lees, with or without stirring, and starting with clarified or non-clarified wines.

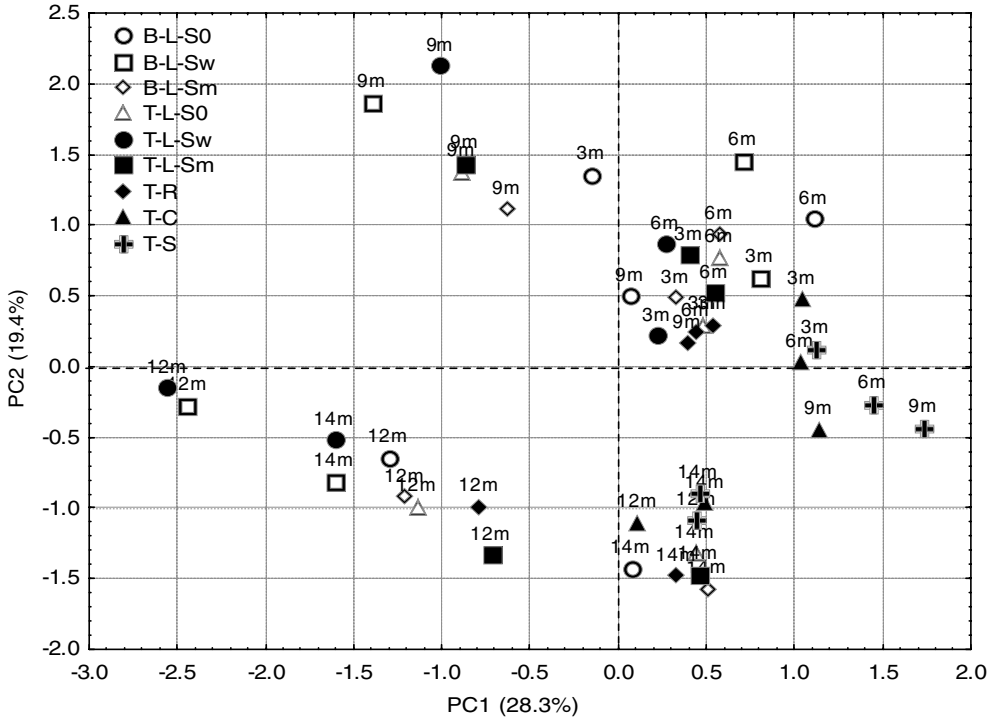

Fig. 2. Plot of the wine samples in the plane defined by the first two principal components.

Principal component analysis (PCA) was applied to establish which variables reveal the relationship among the nitrogen compounds analysed in the wines. The two first principal components explained 47.4% of the total variance of the data. The first principal component, which explains 28.3% of the total variance, was strongly correlated with threonine (−0.88), leucine (−0.88), glycine (−0.86), phenylalanine (−0.86) and valine (−0.76). The second principal component, which explains 19.4% of the total variance, was mainly correlated with ornithine (0.84), tryptophan (0.78), asparagine (0.75) and tyrosine (0.75). Fig. 2 shows the wines in the PCA plot defined by the first two principal components (PC). With negative values on PC1 and PC2 are the 12 months wines aged with lees, which have the highest levels of glycine, threonine, valine, phenylalanine and leucine. The 14 month and 12 month wines which have not been aged with lees (wines T-R, T-C and T-S) have positive values on PC1, in other words, are those with the lowest values of the amino acids glycine, threonine, valine, phenylalamine and leucine. The 12 and 14 month-old wines are resolved from the other lengths of time across PC2, possibly because they have lower ornithine values. In general, it can be observed that wines from experiments T-L-Sw and B-L-Sw, i.e., aged with lees and stirred weekly, have very similar values for all ageing times, i.e., they have similar values of the amino acids associated with the two principal components. A similarity can also be observed between the clarified wines T-C and T-S, and between wines T-L-S0, B-L-Sm and T-L-Sm.

To test the effects of the type of manufacture and the ageing time factors on the nitrogen composition of the wines, two-way ANOVA was carried out, to analyse the first order effects (the interaction and the within-error terms were pooled). This analysis was applied to the data of the total nitrogen, free amino acids and amines of the wines aged for 3–14 months. The results are shown in Table 3. There are significant differences in the values of the amino acids serine, glycine, threonine, α-alanine, phenylalanine, leucine, lysine and in the sum of the amino acids, related to the production technique used, and in all the amino acids due to ageing time. Scheffe's test was used to compare the means and the results are summarised in the

Table 3
Factors effect, mean ± standard deviation and range of concentration of nitrogen compounds (mg/l) of wines during ageing time (3–14 months)

	Factors effect		Mean ± SD	Range	Procedure		Ageing time	
	Procedure	Time			Higher values	Lower values	Higher values	Lower values
Total nitrogen	n.s.	**	215.8 ± 16.1	180.9–240.8	–	–	6 m; 9 m	3 m; 14 m
Amino acids								
Aspartic acid	n.s.	n.s.	13.4 ± 2.4	8.80–17.0	–	–	–	–
Glutamic acid	n.s.	**	24.2 ± 3.7	19.6–32.1	–	–	6 m	9 m; 14 m
Asparagine	n.s.	**	9.5 ± 3.9	4.0–15.4	–	–	3 m; 6 m; 9 m	12 m; 14 m
Serine	*	**	7.4 ± 1.7	4.2–10.8	B-L-Sw; T-L-Sw	T-C; T-S	3 m; 9 m	14 m
Glutamine	n.s.	**	1.0 ± 1.2	0.0–3.0	–	–	3 m; 6 m	12 m; 14 m
Histidine	n.s.	*	0.7 ± 1.1	0.0–3.5	–	–	6 m; 12 m	9 m; 14 m
Glycine	*	*	10.3 ± 1.8	8.0–16.9	B-L-Sm	T-S	12 m	3 m; 6 m; 14 m
Threonine	*	*	9.0 ± 2.4	6.4–15.9	B-L-Sw; T-L-Sw	T-C; T-S	12 m	3 m; 6 m
Arginine	–	–	n.d	n.d.	–	–	–	–
β-Alanine	n.s.	**	1.0 ± 1.1	0.0–4.0	–	–	3 m; 6 m; 9 m	12 m; 14 m
α-Alanine	*	**	22.2 ± 3.0	15.4–28.1	B-L-Sw; T-L-Sw	T-R; T-C; T-S	12 m	3 m
γ-Aminobutyric acid	n.s.	**	7.2 ± 0.9	5.3–9.0	–	–	12 m	6 m
Tyrosine	n.s.	**	1.9 ± 3.0	0.0-9.5	–	–	9 m	12 m; 14 m
Methionine	n.s.	**	3.0 ± 1.9	0.3–7.4	–	–	9 m	12 m
Valine	n.s.	**	7.1 ± 1.4	5.3–9.9	–	–	12 m	3 m; 6 m; 9 m; 14 m
Tryptophan	n.s.	**	5.3 ± 1.8	3.5–9.7	–	–	9 m	6 m; 12 m; 14 m
Phenylalanine	*	**	10.7 ± 1.9	7.0–14.6	T-L-Sw	T-S	12 m	6 m
Isoleucine	n.s.	**	5.1 ± 1.3	2.2–7.4	–	–	9 m; 12 m	3 m; 6 m
Leucine	*	**	11.2 ± 2.0	8.3–16.8	B-L-Sw; T-L-Sw	T-C; T-S	12 m	3 m; 6 m; 9 m
Ornithine	n.s.	**	3.2 ± 3.2	0.0–9.0	–	–	3 m; 6 m; 9 m	12 m; 14 m
Lysine	*	**	13.9 ± 3.1	9.2–21.5	B-L-Sw; T-L-Sw	T-R; T-C; T-S	9 m; 12 m	14 m
Total amino acids	*	**	168.4 ± 20.1	131.0–209.0	B-L-Sw; T-L-Sw	T-C; T-S	9 m;12 m	14 m
Amines								
Histamine	**	n.s.	3.0 ± 0.8	1.9–4.9	T-L-Sw; T-L-Sm	B-L-Sw; T-R; T-S	–	–
Methylamine	n.s.	n.s.	1.0 ± 0.3	0.0–0.3	–	–	–	–
Ethylamine	n.s.	n.s.	3.9 ± 2.2	0.8–7.6	–	–	9 m; 12 m	3 m; 6 m
Tyramine	**	n.s.	3.8 ± 0.8	2.3–5.3	T-R	B-L-S0; B-L-Sw	–	–
Phenylethylamine	–	–	n.d.	n.d.	–	–	–	–
Putrescine	**	**	6.5 ± 1.0	4.8–9.0	T-L-S0; T-L-Sw; T-L-Sm	T-S	12 m; 14 m	3 m
Cadaverine	–	–	n.d.	n.d.	–	–	–	–

The higher and lower values of these compounds in relation to the production technique and ageing time factors are also shown.

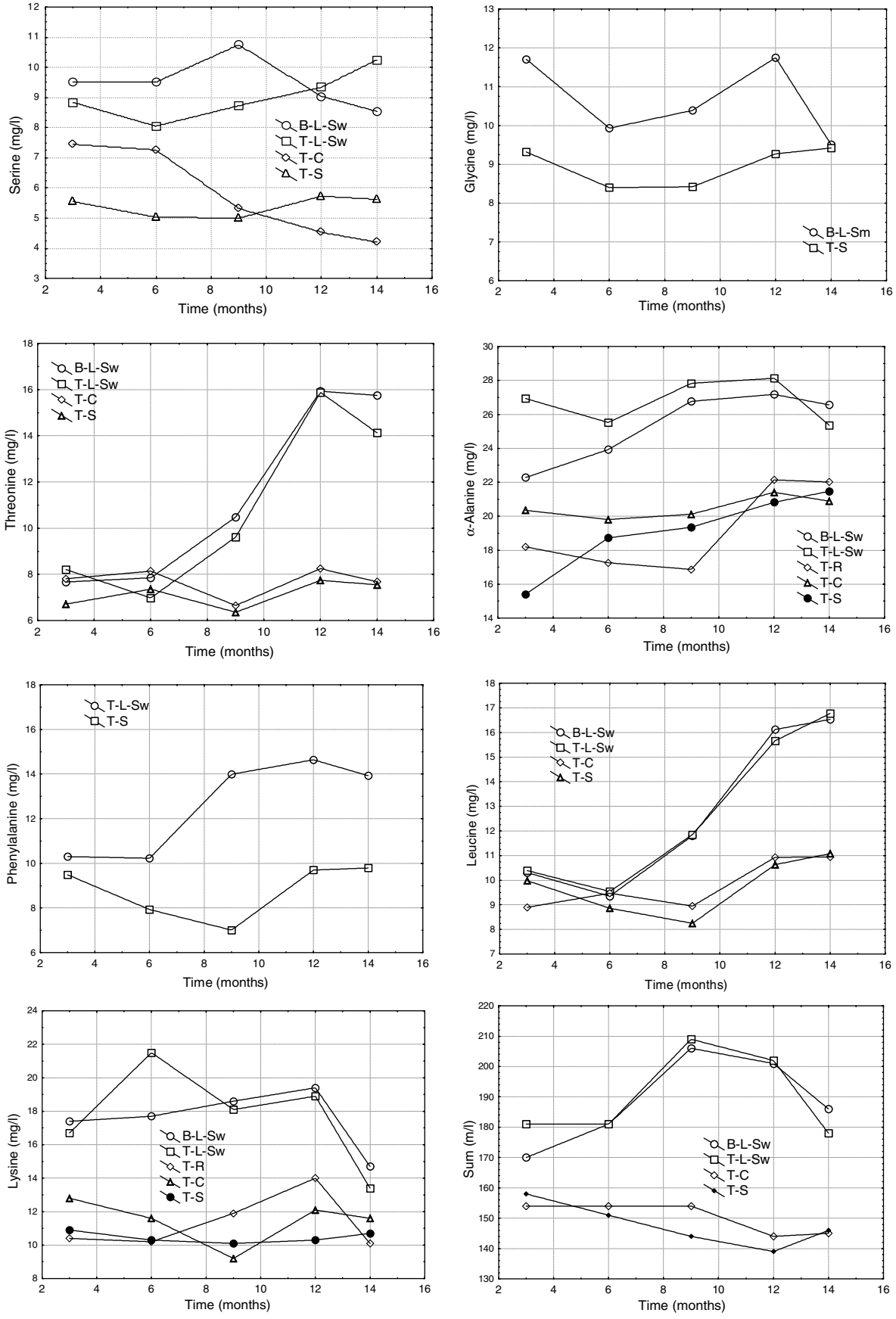

Fig. 3. Amino acids significantly affected by production techniques.

table. Generally, it can be said that the wines with the highest mean values of amino acids, in relation to the production technique, are those aged with lees and stirred weekly (T-L-Sw and B-L-Sw) and those with the lowest values of these amino acids correspond to the wines submitted to the different clarification and stabilising treatments in barrel (T-C and T-S and in some cases T-R). The lowest values of these amino acids, in relation to ageing time in the barrel, correspond to those wines aged for short times, such as the 3 and 6-month wines, and to some of those aged for 14 months.

Fig. 3 shows, the change in those amino acids influenced by the production technique. The main observation from the figure is that the concentration of these amino acids and the sum of all the free amino acids increase from 6 to 12 months ageing in wines aged with lees. However, this increase is not observed in wines aged without lees. This result shows that amino acids have been released by yeast autolysis, as demonstrated previously in autolysis experiments in model media (Martínez-Rodriguez, Carrascosa, & Polo, 2001; Perrot, Charpentier, Charpentier, Feuillat, & Chassagne, 2002), in sparkling wines aged with yeasts (Moreno-Arribas et al., 1998) and in wines aged with *flor* yeasts (Charpentier, Dos Santos, & Feuillat, 2004). The amino acids threonine and serine form part of the *O*-glycosidic bonds of mannoproteins with yeasts. The figure shows a very prominent rise in threonine during ageing. Between 12 and 14 months, there is a drop in most of the amino acids. This has also been observed during the ageing of sparkling wines and has been attributed to decarboxylation reactions of the amino acids or the formation of esters (Martínez-Rodríguez, Carrascosa, Martín-Álvarez, Moreno-Arribas, & Polo, 2002).

There are no large changes in amine content during ageing, compared to the concentration measured at the end of malolactic fermentation (Table 2). Therefore, these wines can be considered to have a very small amine content. There are significant differences in the amines histamine, tyramine and putrescine in relation to the production technique of these wines and significant differences in putrescine, in relation to ageing time (Table 3). These results agree with those obtained by Martín-Álvarez, Marcobal, Polo, and Moreno-Arribas (2006). Applying the Scheffe test to compare the mean values of the amine data, we find that the highest mean values for histamine are recorded in the wines T-L-Sw and T-L-Sm, i.e., those in which malolactic fermentation was done in tanks and have been aged with lees and stirred weekly or monthly. The wines with the lowest mean values of histamine are those racked and clarified before ageing.

Fig. 4 shows the evolution of amines significantly different in relation to the different production techniques. In general, the histamine content does not change during ageing. The mean value of tyramine (3.76 mg/l) is similar to those recorded in Spanish wines by Bover-Cid, Izquierdo Pulido, Mariné Font, and Vidal Carou (2006) and by Marcobal et al. (2005). The wine fermented in tanks and aged without lees (T-R) had a higher tyramine content than wines fermented in barrel. During ageing, the tyramine concentration diminished in the wine aged without lees, and at 14 months all the wines had similar tyramine concentrations. The reduction in tyramine contents could be due to the presence of bacterial oxidases. In some studies, the presence of tyramine oxidase has been found in fer-

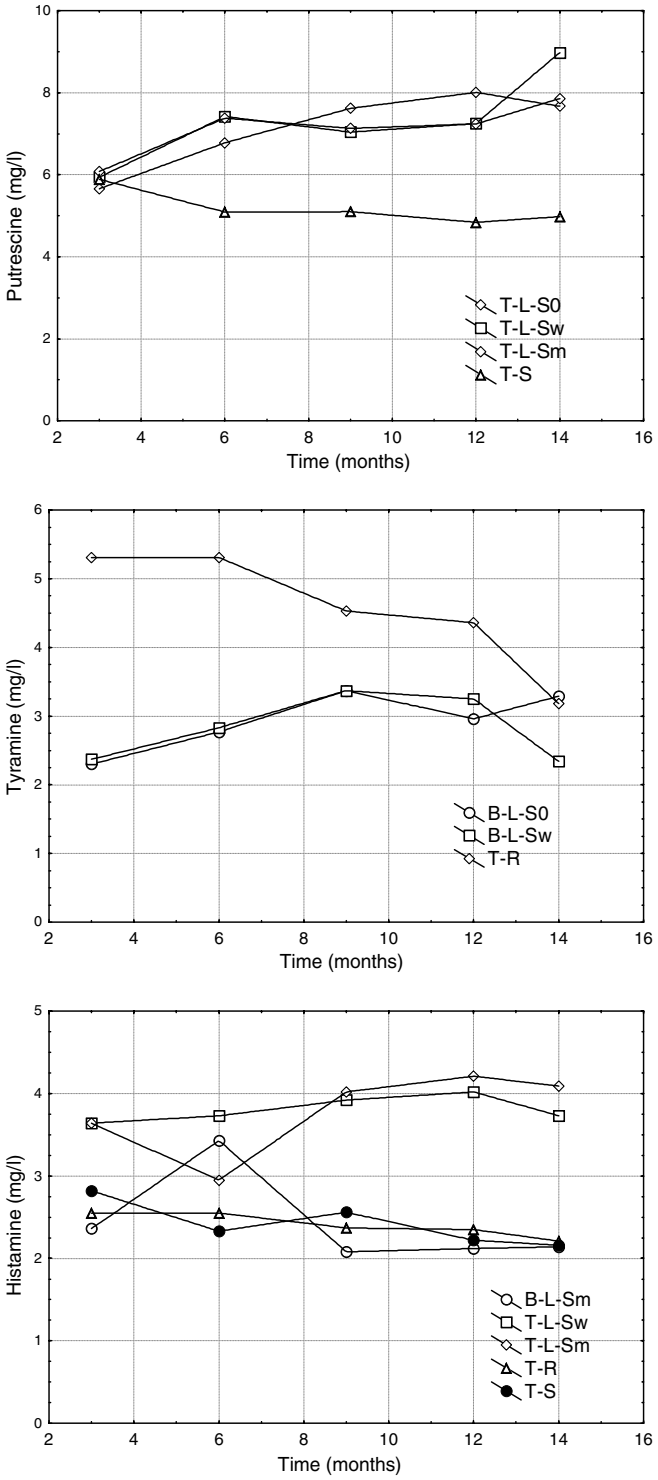

Fig. 4. Amines significantly affected by the production techniques.

mented drinks and also in wines, which prevents accumulation of this amine (Enes Dapkevicius, Nout, Rombouts, Houben, & Wymenga, 2000).

Putrescine is the major amine in all of the wines, with values ranging from 4.84 to 8.98 mg/l, with a mean value of 6.54 mg/l. The putrescine content of the wines aged with lees increases during ageing, although it remains stable in those aged without lees. Jimenez Moreno, Torrea Goñi, and Ancín Azpilicueta (2003) and González-Marco and Ancín-Azpilicueta (2006) did not observe a decrease in putrescine during ageing with wines either. It would, therefore, seem that there is no oxidation of this amine by the wine oxidases. All the values found are lower than the minimum values reported for French wines (Soufleros et al., 1998) and the mean values of 15 mg/l recorded by Herbert, Cabrita, Ratola, Laureano, and Alves (2005).

Methyl and ethylamine, with mean values of 0.98 and 3.90 mg/l, respectively, did not change significantly during ageing in the barrel and were not influenced either by the method of wine production. The amines phenylethylamine and cadaverine were not detected in any of the wines.

In summary, from the results obtained in this study, it can be concluded that there is only a slight reduction in amino acids during malolactic fermentation. This is probably due to the release of amino acids from the wine by the exocellular proteasic activity of lactic acid bacteria. During ageing of the wine with lees, amino acids are released by yeast and bacterial autolysis. This release of amino acids is more pronounced in wines aged with lees and stirred weekly. Therefore, precaution should be taken when wines are aged with lees, since this increases the risk of the formation of biogenic amines by residual decarboxylase activities of the lactic acid bacteria responsible for malolactic fermentation. The findings indicate that if this technology, which increases wine complexity, is to be used to make red wines, selected strains of lactic acid bacteria should be used, which do not produce biogenic amines.

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