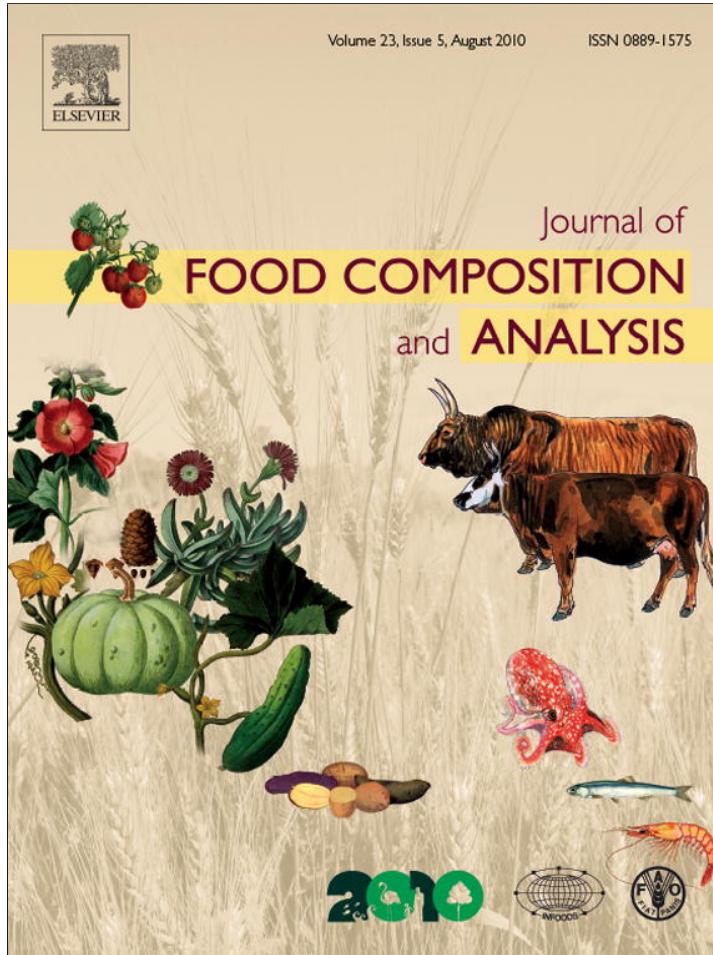


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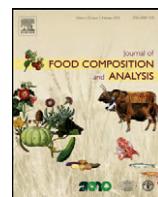
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Journal of Food Composition and Analysis

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

Original Article

Influence of oenological practices on the formation of biogenic amines in quality red wines

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ARTICLE INFO

Article history:

Received 13 January 2009

Received in revised form 29 January 2010

Accepted 18 February 2010

Keywords:

Red wine

Biogenic amines

Histamine

Organic wine

Yeast mannoproteins

Food composition

Food analysis

ABSTRACT

Changes in the contents of biogenic amines (histamine, putrescine, tyramine, cadaverine, agmatine, ethylamine, isobutylamine, phenylethylamine, isoamylamine, serotonin and tryptamine) were studied during the winemaking process of quality red wines, including an organic wine. The analytical method used was validated in terms of linearity, precision, coefficient of variation and recovery. The limits of detection and quantification of the amines were also calculated. The method involved pre-column automated derivatisation of the amines by treatment with o-phthalaldehyde, after which the derivatives formed were analysed by reverse-phase HPLC. Results showed that grape must already contains biogenic amines and this content tends to increase throughout winemaking and maturation. The organic wine showed higher levels of biogenic amines than the non-organic wine. The fact that malolactic fermentation occurs spontaneously in organic wines, together with low levels of SO₂ because of legal restrictions, could be responsible for the higher levels in biogenic amines found. For the non-organic wine, 2 oenological practices could increase the content in biogenic amines: the addition of press wine to the free run wine, and the treatment with yeast mannoproteins.

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1. Introduction

Biogenic amines (BAs) are organic bases of low molecular weight, which play an important role as endogenous regulators of diverse physiological processes in the human organism. Moreover, they can be present in foodstuffs in variable proportions as a result, generally, of the existence of fermentative processes or because of bacterial contamination. When foodstuffs containing biogenic amines are ingested, adverse effects, whose gravity depends on the quantity ingested, can be produced in the organism. The symptoms associated with the ingestion of BAs range from headaches, shortness of breath and tachycardia if small doses have been ingested, to vomiting, bronchial constriction, hyper and hypotension, kidney and vascular failure, etc. when the quantity ingested has been higher (Silla-Santos, 1996; Taylor, 1986; Rivas-Gonzalo et al., 1983).

Among the BAs, histamine is the most toxic and thus the most commonly sought after, although its effects can be potentiated by the presence of other amines, such as spermine, spermidine, putrescine or agmatine (Bauza et al., 1995; Chu and Bjeldanes, 1981). The sensitivity of individuals to the BAs varies in accordance with the individual capacity for detoxification (Bauza et al., 1995; Jung and Bjeldanes, 1979; Taylor and Lieber, 1979; Maynard and

Schenker, 1962). Therefore, when the toxic effects of the BAs are considered, as well as the total concentration of amines, the ingestion together with ethanol and/or determined medicines must be taken into account. In fact, whereas in products such as meat and fish derivatives the adverse effects of certain amines (phenylethylamine, for example) appear after the ingestion of 53 mg, in wines doses of 3 mg can be enough (Glória et al., 1998; Bauza et al., 1995; Sandler and Reynolds, 1976; Rivas-Gonzalo et al., 1983). The complexity of the interactions of the amines that take place among them and with other substances is the principal factor responsible for the difficulty in establishing maximum limits for these compounds in foodstuffs.

The BAs can be produced by the decarboxylation of determined amino acids (for example, histamine originates by the decarboxylation of histidine) by enzymes produced by some lactic bacteria (LB) responsible for fermentative processes undergone by foodstuffs such as cheeses, cold meats, pickles and wines (Bauza et al., 1995; Buteau et al., 1984; Vidal-Carou et al., 1990; Lonvaud-Funel, 2001).

The evolution and presence, both qualitative and quantitative, of BAs in wines is still not well defined and, at times, there is a lack of agreement between the published results (Gerbaux and Monamy, 2000; Coton et al., 1998; Soufleros et al., 1998; Pogorzelski, 1992; Ough et al., 1987). At present, the tendency is to accept the existence of malolactic fermentation (MLF) as one of the most important factors that determine their presence.

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The fact that the majority of white wines do not undergo MLF, together with the fact that, consequently, their pH is generally lower than that of red wines, results, frequently, in the content of BAs of white wines being smaller than that of red wines, which is between 0 and 130 mg/L (Gerbaux and Monamy, 2000; Soufleros et al., 1998; Cilliers and Van Wyk, 1985; Zee et al., 1983; Glória et al., 1998). Nonetheless, it does not appear to be the only factor to take into account. Among the LBs, not all of them have the enzymatic equipment necessary to decarboxylate the amino acids and, consequently, give rise to the formation of BAs (Moreno-Arribas et al., 2003; Coton et al., 1998). One of the factors that determine not only the biological activity of the bacteria in wine, but also its variety is the pH. With the objective of attending to the demands of the consumer, wines tend to be less acid than in the past. The ripening of the grape tends to be prolonged to the maximum possible with the aim of increasing the extractability of the phenolic compounds and the concentration of the aroma precursors. As a result the acidity of wines tends to be lower and the pH higher. Consequently, the number and type of bacterial microflora found present during winemaking increases and with it the possibilities of formation of BAs (Lonvaud-Funel and Joyeux, 1994).

Although the microbiology of the MLF in wines, at present, tends to be more controlled through the use of selected starters, in many cellars it continues to develop spontaneously from autochthonous bacteria, since they are better adapted to conduct the MLF of their wines than other commercial generics from strains that have not been isolated in the zone of production of the cellar.

Another factor to be borne in mind is the possible formation of BAs during the maturation and ageing of the wine. Indeed, in recent studies, a progressive increase in the content of certain amines has been demonstrated during ageing, particularly of histamine, tyramine, putrescine and diaminobutane (Herbert et al., 2004; Jiménez-Moreno et al., 2003; Gerbaux and Monamy, 2000). After the MLF the wine is sulphited with the objective of eliminating the yeasts and residual bacteria, but due to the rise in pH and also to the fact that it is found in part combined with the polyphenols, the activity of the SO₂ decreases and this can give rise to some lactic bacteria remaining viable months after the winemaking and conserving certain biological activity, fundamentally that which helps their survival.

Thus, the decarboxylation of the amino acids is a mechanism that allows the bacteria to obtain energy when other sources have already been metabolised (Lonvaud-Funel, 1999; Rollán et al., 1995), so the strains that present this activity can survive longer than those which do not.

Furthermore, such amines as putrescine, spermidine, methylamine, ethylamine, phenylethylamine, isoamylamine and cadaverine, among others, can already be found in the grapes and must (Lonvaud-Funel, 2001; Vidal-Carou et al., 1990; Buteau et al., 1984; Ough and Daudt, 1981) as well as being produced (and also degraded) during the winemaking. It has been demonstrated that many strains of yeasts present in wines (*Saccharomyces cerevisiae*, *Brettanomyces bruxellensis*, *Kloeckera apiculata* among others) can produce histamine, ethanolamine, agmatine, phenylethylamine and cadaverine (Caruso et al., 2002; Torrea and Ancín, 2002; Vidal-Carou et al., 1990; Pogorzelski, 1992; Buteau et al., 1984).

For the determination of BAs in foodstuffs diverse methods have been proposed in the bibliography, among them high performance liquid chromatography followed by fluorimetric detection or spectrophotometry is the most commonly used technique in recent years, especially for the analysis of wines (Busto et al., 1996; Soufleros et al., 1998; Glória et al., 1998; Soleas et al., 1999). Recently the application of more complex methods of detection, such as mass spectrometry, have also begun to be used (Loukou and Zotou, 2003). Due to the complexity of wine, generally the analysis of BAs is accompanied by the use of techniques of pre-

or post column derivatisation, which permit a significant advance in the sensitivity on eliminating interferences. Among the different reagents used are: dansyl chloride (Csomos and Simonne, 2002; Anli et al., 2004; Caruso et al., 2002), o-phthalaldehyde (Herbert et al., 2001, 2004; Glória et al., 1998; Iñiguez-Crespo and Vázquez-Lasa, 1994; Busto et al., 1997; Mafra et al., 1999; Vidal-Carou et al., 2003; Leitão et al., 2005; Moreno-Arribas et al., 2003; Martínez et al., 2000; Lavizzari et al., 2006; Del Prete et al., 2009), dabsyl chloride (Romero et al., 2000; Romero et al., 2002) and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Jiménez-Moreno et al., 2003; Torrea and Ancín, 2001; Torrea and Ancín, 2002; Martínez et al., 2000).

Of all of them, o-phthalaldehyde (OPA) is most habitual. Its principal advantages being it offers high selectivity and sensitivity for the primary amines (Izquierdo-Pulido et al., 1993), as well as the fact that the reaction of derivatisation is performed in a short period of time, thus preventing errors associated with long manipulations.

In this work the determination of BAs in quality wines of Castilla y León (Spain) has been carried out by HPLC using derivatisation with o-phthalaldehyde. For this, the principal variables involved in the derivatisation reaction and chromatographic separation were initially studied. The method developed was validated in terms of precision, repeatability and linearity. With the aim of contributing to the knowledge of the origin and evolution of BAs in wines, the analytical method has been applied to the determination of BAs during the winemaking process of quality red wines from the Spanish "Denominación de Origen" (DO) Toro, including an organic wine, as well as other DO wines from the "Comunidad Autónoma de Castilla y León" (Spain).

2. Materials and methods

2.1. Standard and reagents

The biogenic amines cadaverine (Cad), agmatine (Ag), ethylamine (Et), isobutylamine (Iso), phenylethylamine (Phe), putrescine (Put), and isoamylamine (Isoa) were supplied by Sigma-Aldrich (Steinheim, Germany); serotonin (Ser), histamine (His), tyramine (Tyr) and tryptamine (Tryp) by Merck (Darmstadt, Germany). The internal standard (IS), L-norvaline, was supplied by Sigma-Aldrich (Steinheim, Germany). o-Phthalaldehyde (OPA), from Merck, Darmstadt, Germany, was used as the derivatisation reagent.

The preparation of the OPA was performed weekly from 500 mg of o-phthalaldehyde and 22.5 mL of methanol in 25 mL of borate buffer 0.4 M pH 10, with the final addition of 400 µL of mercaptoethanol.

All the reagents used were of HPLC quality. The ultra-pure water of HPLC grade was obtained using a MilliQ-alpha Millipore-Waters system (Bedford, MA).

2.2. Samples

For the study of the evolution in the content of BAs during the elaboration of quality wines, *Vitis vinifera* L. cv Toro fresh grapes processed by wineries from the Spanish DO Toro were used. Wine made from organically certified vineyards, organic wine (OW), was obtained by regulated winemaking methods. The wine corresponding to the first fraction obtained through a direct pressing pomace, quality press wine (QPW), and the free run wine, quality wine (QW), were inoculated with selected yeast and malolactic bacteria. Samples were taken during the winemaking and maturation process and are summarised in Table 1.

Three sub-samples were taken from each step, and their analyses were performed in triplicate. All samples were immediately stabilised with sodium fluoride and stored in refrigeration

until their analysis in the laboratory. Moreover, 28 Spanish red wines were analysed (Table 5) belonging to different DO's of Castilla y León (Ribera de Duero, Vinos de la Tierra de Castilla y León, Bierzo, Cigales and Vino de Calidad de Arribes). These wines were acquired in the market. The analyses were performed in triplicate.

2.3. Preparation of standard and wine samples

Both the samples of wine and the standard solutions were filtered through 0.45 µm filters. 1000 µL of filtrate were mixed with 800 µL of borate buffer 0.4 M (pH 10) and 200 µL of the solution of the I.S. (l-norvaline, 100 mg/L). 300 µL of the solution obtained were later mixed automatically in the injector with 100 µL of derivatisation reagent (OPA). After the reaction time (one minute) 10 µL of the mixture were automatically injected into the chromatographic system for their analysis. All the analyses were performed in triplicate.

2.4. Analysis by HPLC

Analyses were performed with a Varian HPLC equipped with a 9012Q quaternary pump, a 9100 automatic injector, a 9075 fluorescence detector and using a Synergi-Hydro® RP-80 Å column with a particle size of 4 µm (150 mm × 4.6 mm) (Phenomenex, Torrance, CA). The system was controlled by a Varian Star Workstation version 5.52. Chromatographic conditions were: solvent (A): sodium acetate 0.05 M adjusted to pH 6.6/tetrahydrofuran (99:1); solvent (B): methanol/acetonitrile (50:50).

The elution gradient established was: isocratic 40% B for 5 min, 40–50% B for 9 min, 50–60% B for 12 min, 60–75% B for 16 min, 75–85% B for 3 min and 85–100% B for 1 min, at a flow rate of 1 mL/min. Detection was carried out at 340 nm and 426 nm as excitation and emission wavelengths, respectively. For the quantitative analysis, linear calibration curves of each biogenic amine were obtained in the concentration range 1–30 mg/L.

2.5. Statistical analysis

For comparison of wines, data were presented as mean ± standard deviation (SD) of analyses performed in triplicate. Statistical bilateral correlation studies, with the correlation coefficient of Pearson (significance $P < 0.05$), were carried out using SPSS 13.0 statistical software.

3. Results and discussion

For method development, different internal standards (l-norvaline and 1,7-diaminoheptane) and solvent systems to dissolve the amines (ultrapure water acidified 0.1N (pH 3.6), borate buffer and acetate buffer at pH 10), as well as different eluents for the chromatographic runs (for phase A: sodium phosphate 10 mM (pH 7.3)–methanol–tetrahydrofuran (80:19:1) and Na₂HPO₄·12H₂O

10 mM; and for phase B: sodium phosphate 10 mM (pH 7.3)–methanol (20:80) and 1% 2-octanol in acetonitrile–Na₂HPO₄·12H₂O 10 mM (70:30)), were assayed.

With the conditions selected (see Section 2) a good chromatographic separation of the peaks corresponding to the internal standard (l-norvaline) and to the 11 BAs studied (Ag, His, Ser, Et, Tyr, Isob, Tryp, Phe, Put, Isoa and Cad) was obtained in an acceptable elution time. Fig. 1 shows chromatograms corresponding to a biogenic amine standard solution of 10 mg/L and to a red wine sample.

3.1. Figures of merit

The method proposed was validated in terms of linearity, precision, coefficient of variation and recovery. The limits of detection and quantification of the amines were calculated.

The equations of regression, coefficients of determination and the ranges of linearity for the 11 compounds analysed are presented in Table 2. The goodness of fit of the regression model was also evaluated by the F-test. For this, regression variance and residual variance were obtained by means of an ANOVA analyse. The p-values in the F-test were lower than 0.05 indicating significance. Also, the lack of fit F-test was performed. For this, the lack of fit and the pure error were obtained by means of an ANOVA analysis of the residual variance. The p-values obtained were greater than 0.1, indicating that the fitted equation is statistically valid. The linear range of the response obtained is sufficient in all the amines considered, no amine being found in quantity superior to the upper limit in the samples. Furthermore, the limits of detection (LODs) and quantification (LOQs) were studied.

The limits of detection were calculated under the Glaser criteria (Glaser et al., 1981). LOD = $t(N - 1, 1 - \alpha = 0.99) \times SD$, where $t(N - 1, 1 - \alpha = 0.99)$ is the Student t-value for a one-tailed test at the 99% confidence level with $N - 1$ degrees of freedom. SD is the standard deviation of seven replicate analyses with concentrations from twice to five fold the estimated LOD according to the relationship between the instrumental signal/noise. The LOQs were three times the LODs. The limits of detection of the amines studied lies within an interval between 0.015 and 0.110 mg/L and the limits of quantification between 0.046 and 0.331 mg/L (Table 2).

Repeatability and recovery of the method were also determined (Table 3). Both parameters were calculated by carrying out seven consecutive injections of the standards of BAs at three different concentrations (1, 10, 25 mg/L) in the selected conditions.

The repeatability was expressed as the coefficient of variation (%) and the calculation was based on $(SD/x) \times 100$, where SD is the relative standard deviation and x is the mean of the different injections. The percentage of repeatability for low concentrations (1 mg/L) varied between 3.09 and 8.66%, for medium concentrations (10 mg/L) between 2.09 and 4.89 and for high concentrations (25 mg/L) between 2.96 and 6.28%.

Table 1

Samples taken in QW, QPW and OW wines.

	Samples	QW	QPW	OW
Step 1	Grape must	20/09/2005	–	–
Step 2	End alcoholic fermentation	10/10/2005	–	–
Step 3	Before malolactic fermentation	24/10/2005	24/10/2005	08/11/2005
Step 4	End malolactic fermentation	16/11/2005	16/11/2005	04/01/2006
Step 5	First racking	24/01/2006	–	15/02/2006
Step 6	One month in oak barrel	24/02/2006	–	03/04/2006
Step 7	QW/OW	24/03/2006	–	15/05/2006
Step 8	QW/OW	24/04/2006	–	15/06/2006

7 QW: two months in oak barrel; 7 OW: before bottling.

8 QW: three months in oak barrel; 8 OW: one month in bottle.

Table 2

Regression analysis equations, coefficients of determination (*R*), limits of detection (LODs) and quantification (LOQs), precision and linear ranges of the method for determination of biogenic amines in wine.

Compounds	Regression equation $y = (a \pm SD_a) + (b \pm SD_b)x$	<i>R</i> ²	LODs (mg/L) ^a	LOQs (mg/L) ^b	Precision ($\pm SD$)	Linear range (mg/L)
Agmatine	$y = (-0.01 \pm 0.04) + (0.36 \pm 0.02)x$	0.998	0.066	0.197	0.021	0.066–30
Isoamylamine	$y = (0.04 \pm 0.04) + (0.61 \pm 0.03)x$	0.994	0.029	0.086	0.009	0.029–25
Putrescine	$y = (-0.03 \pm 0.04) + (0.39 \pm 0.02)x$	0.995	0.038	0.113	0.012	0.038–30
Isobutylamine	$y = (0.08 \pm 0.07) + (0.57 \pm 0.04)x$	0.991	0.085	0.254	0.027	0.085–25
Cadaverine	$y = (0.01 \pm 0.09) + (0.57 \pm 0.05)x$	0.995	0.023	0.069	0.007	0.023–30
Histamine	$y = (-0.01 \pm 0.04) + (0.42 \pm 0.02)x$	0.998	0.068	0.203	0.022	0.068–30
Serotonin	$y = (-0.01 \pm 0.01) + (0.11 \pm 0.01)x$	0.995	0.094	0.282	0.030	0.094–30
Tryptamine	$y = (-0.04 \pm 0.03) + (0.39 \pm 0.02)x$	0.998	0.015	0.046	0.005	0.015–30
Ethylamine	$y = (0.07 \pm 0.05) + (0.47 \pm 0.03)x$	0.996	0.110	0.331	0.035	0.110–30
Tyramine	$y = (-0.02 \pm 0.05) + (0.47 \pm 0.03)x$	0.997	0.025	0.075	0.008	0.025–30
Phenylethylamine	$y = (0.04 \pm 0.06) + (0.56 \pm 0.04)x$	0.991	0.040	0.121	0.013	0.040–25

y, peak area ratio of each biogenic amine to internal standard (mean of three determinations); x, concentration in mg/L; a, intercept; b, slope; SD_a and SD_b , standard deviations of intercept and slope, respectively.

^a Limit of detection (mg/L).

^b Limit of quantification (mg/L).

The recovery was calculated by comparison of the initial concentration of the sample with that obtained after injection. The percentages of recovery obtained at low concentration (1 mg/L) varied from 56 to 108%, at medium concentration (10 mg/L) from 83 to 117% and at high concentration (25 mg/L) was situated between 104 and 109%. The lowest values of recovery were those obtained at low concentration (1 mg/L) for Tryp and Cad, in which it was situated below 60%. The His, a biogenic amine of great interest, presents very good proportions of recovery (from 93 to 104%) at the three concentrations studied.

3.2. Application to wine samples

The method was used for the determination of BAs in samples of quality wines collected during the winemaking process from the Spanish DO Toro. The average value of BAs in the analysed samples is reported in Table 4. His, Et, Put and Cad were found both in organic and non-organic wine. Tyr, Phe and Tryp were only present in the samples of OW wine. The amines Ag, Ser, Isob and Isoa were not found in quantifiable concentrations in any of the wines.

In the QW wine, the sample corresponding to grape must already contains BAs (8.35 mg/L) and this content tends to increase

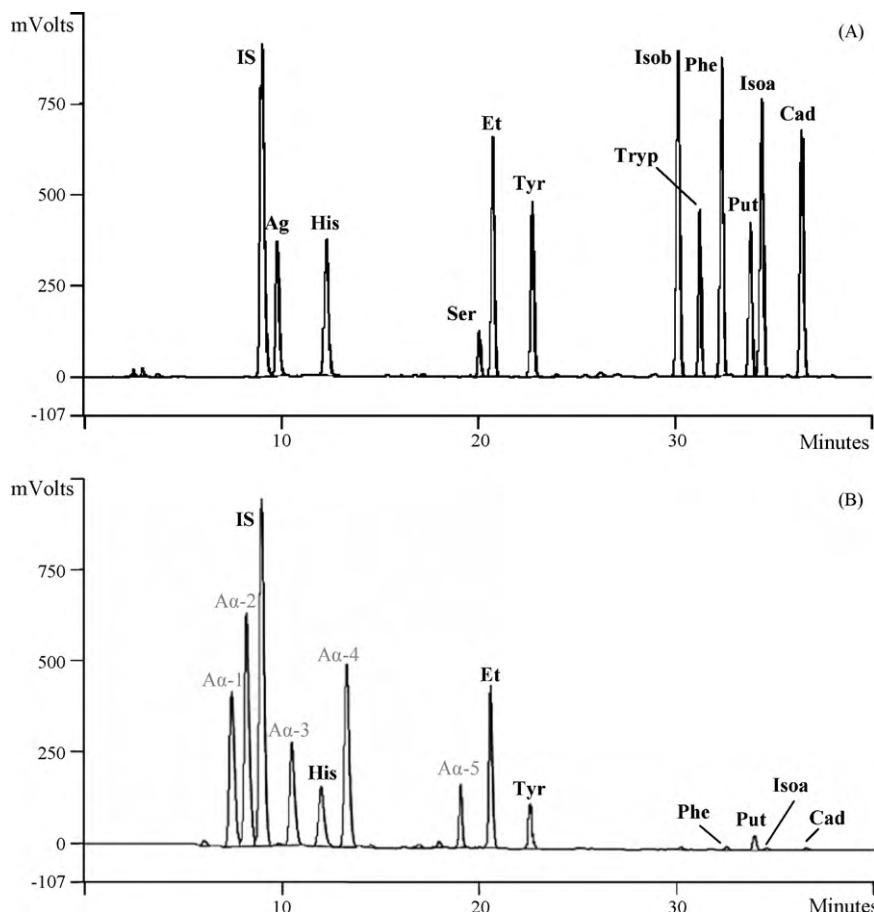


Fig. 1. Chromatograms corresponding to a biogenic amine standard solution of 10 mg/L (A) and to a red wine sample (B). Aα-1 to Aα-5 are wine amino acids.

Table 3

Coefficient of variation (CV%) and recovery of the standards of biogenic amines at low (1 mg/L), medium (10 mg/L) and high (25 mg/L) concentrations.

Compounds	CV(%, n=7)			Recovery (%) \pm SD ^a (n=7)		
	Low ^b	Medium ^c	High ^d	Low ^b	Medium ^c	High ^d
Agmatine	4.756	2.904	3.734	89 \pm 4	98 \pm 3	104 \pm 4
Isoamylamine	6.755	3.940	3.096	108 \pm 7	116 \pm 5	103 \pm 3
Putrescine	8.340	4.895	6.280	72 \pm 4	95 \pm 5	105 \pm 7
Isobutyramine	8.666	3.338	3.548	91 \pm 17	104 \pm 5	109 \pm 4
Cadaverine	7.721	3.150	3.812	56 \pm 2	115 \pm 4	105 \pm 4
Histamine	3.672	2.371	3.863	93 \pm 3	99 \pm 2	104 \pm 4
Serotonin	8.207	4.149	5.778	64 \pm 5	83 \pm 3	114 \pm 7
Tryptamine	4.679	3.330	5.576	59 \pm 3	87 \pm 3	105 \pm 6
Ethylamine	3.520	3.307	2.960	63 \pm 9	117 \pm 4	109 \pm 3
Tyramine	3.089	2.094	4.386	88 \pm 3	95 \pm 2	104 \pm 5
Phenylethylamine	4.341	2.400	3.372	99 \pm 4	127 \pm 3	106 \pm 4

^a Standard deviation.^b Low concentration: 1 mg/L.^c Medium concentration: 10 mg/L.^d High concentration: 25 mg/L.

throughout winemaking and maturation (Fig. 2). Some authors have reported the presence of ethanolamine, spermidine, Et, Put and His in grapes and musts (Del Prete et al., 2009; Broquedis et al., 1989; Vidal-Carou et al., 1990; Ough and Daudt, 1981; Broquedis et al., 1989) and it has been postulated that the variety of the grape,

vine nutrition and the climatic conditions play a role in the accumulation of these compounds (Soufleros et al., 1998; Lonvau-Funel, 2001; Del Prete et al., 2009).

The greatest increases in BAs in QW correspond to the AF (step 2) and to the last step of the maturation process sampled (step 8). The notable increase observed after the AF can be explained by the fact that several *S. cerevisiae* strains can produce significant levels of BAs (Caruso et al., 2002; Torrea and Ancín, 2002) and in our study is due to the important increase produced in Et. This result is contrary to that obtained by Del Prete et al. (2009) who found that this amine diminishes during the AF, possibly due to its incorporation by the fermentation yeasts to their metabolism.

In our case, the increase in the content of this amine in the wine also seems linked to the contact of the wine with the solids of winemaking, since the pressed wine (QPW) has much higher contents than the QW wine considering the same stages of winemaking (steps 3 and 4).

After MLF no appearance of new amines was observed and there was no increase in their total content during MLF, probably because the selected lactic bacteria were inoculated. Only a significant, though slight, increase in His was observed.

During maturation (steps 5–8), a decrease in the BAs content can be observed in the samples except in the last step (step 8). This step coincides with the addition of yeast mannoproteins, which is why we think that this practice could be responsible for the increase observed, since it would permit an increase in the content of nitrogen compounds which, probably, in the presence of residual enzyme activity, could act as precursors for the BAs synthesis.

QPW is the press wine obtained from QW pomace. We can observe in Table 4 and in Fig. 2 that (for the same step), press wine has higher levels of BAs than the free run wine (27% more). This wine is added to QW after MLF and that may be why the levels of BAs in QW increase from step 4 to step 5. Nevertheless, the contents in Et in step 5 of the QW wine are lower than expected, considering the concentration of this amine in the QPW wine, although this could be due to the fact that Et is an aromatic amine and, therefore, volatile.

In the OW wine, the MLF exerts a remarkable effect in the BAs' content. In contrast to QW, OW was not inoculated with selected lactic bacteria, because it is subject to organic wine restrictions and in this wine MLF occurs spontaneously.

His, Et and Put are the most abundant BAs in all samples. His content is relatively elevated in OW, being higher than in QW (Fig. 2). A great increase of this amine in OW is produced after MLF (steps 4 and 5), which is accompanied by the emerging of other amines: Tyr, Tryp, Phe, absent in QW. The increase in the BAs' levels

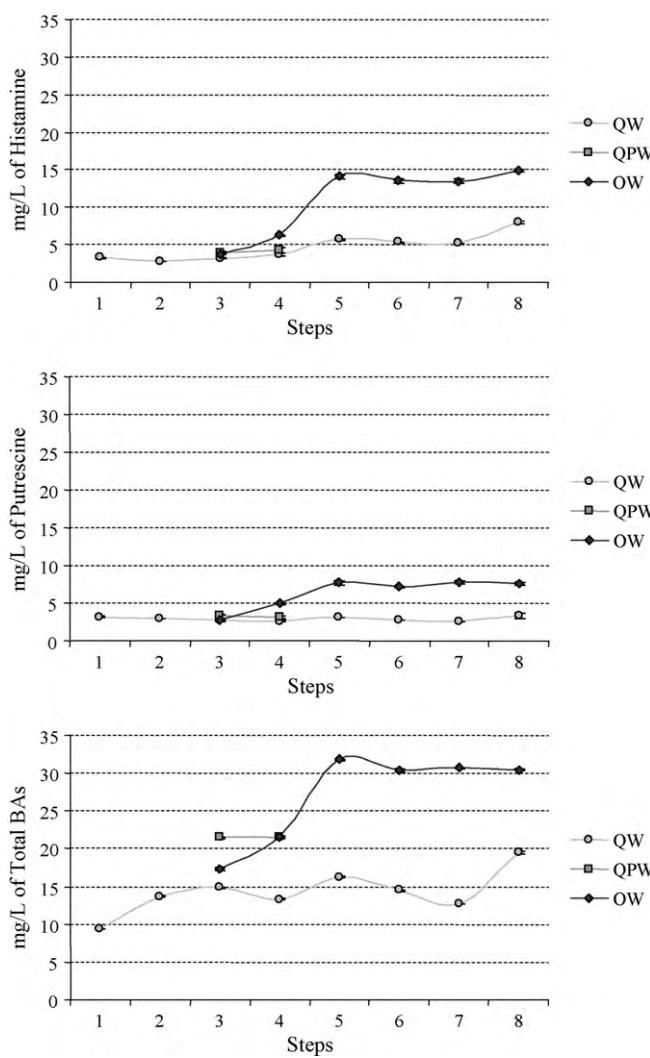


Fig. 2. Evolution of the contents of histamine, putrescine and total BAs during winemaking.

Table 4

Quantification of biogenic amines in wine samples (mg/L) during winemaking.

Ag	His	Ser	Et	Tyr	Isob	Trp	Phe	Put	Isoa	Cad	Total
Quality wine (QW)											
Step 1	nc	3.26 ± 0.16 ^{b/A}	nd	1.03 ± 0.06 ^{a/A}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	3.21 ± 0.09 ^{d/e/A}	nc	0.85 ± 0.07 ^{a/A}
Step 2	nc	2.79 ± 0.05 ^{a/A}	nd	5.44 ± 0.16 ^{a/A}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	2.97 ± 0.06 ^{b/cd/A}	nc	1.96 ± 0.01 ^{c/A}
Step 3	nc	3.25 ± 0.01 ^{b/A}	nd	6.29 ± 0.09 ^{d/A}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	2.80 ± 0.03 ^{abc/AB}	nc	1.93 ± 0.01 ^{c/B}
Step 4	nc	3.78 ± 0.20 ^{c/A}	nd	6.31 ± 0.12 ^{a/A}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	2.68 ± 0.01 ^{ab/A}	nc	0.00 ± 0.00 ^{-/-}
Step 5	nc	5.68 ± 0.06 ^{e/A}	nd	6.18 ± 0.08 ^{d/B}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	3.12 ± 0.04 ^{de/A}	nc	1.30 ± 0.05 ^{b/A}
Step 6	nc	5.31 ± 0.16 ^{de/A}	nd	5.22 ± 0.11 ^{bc/B}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	2.72 ± 0.03 ^{ab/A}	nc	1.30 ± 0.06 ^{b/B}
Step 7	nc	5.21 ± 0.05 ^{d/A}	nd	4.84 ± 0.14 ^{b/A}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	2.64 ± 0.03 ^{a/A}	nc	0.00 ± 0.00 ^{-/-}
Step 8	nc	8.02 ± 0.20 ^{f/A}	nd	6.18 ± 0.27 ^{d/A}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	3.35 ± 0.30 ^{e/A}	nc	1.98 ± 0.04 ^{c/A}
Quality press wine (QPW)											
Step 3	nc	4.00 ± 0.13 ^{a/B}	nd	11.04 ± 0.10 ^{a/C}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00	3.25 ± 0.24 ^{d/B}	nc	2.05 ± 0.10 ^{a/B}
Step 4	nc	4.27 ± 0.31 ^{a/A}	nd	10.90 ± 0.14 ^{a/C}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00	3.14 ± 0.13 ^{a/B}	nc	2.02 ± 0.09 ^{a/B}
Organic wine (OW)											
Step 3	nc	3.78 ± 0.13 ^{a/B}	nd	9.23 ± 0.30 ^{d/B}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00 ^{-/-}	2.78 ± 0.21 ^{a/A}	nc	0.65 ± 0.13 ^{a/A}
Step 4	nc	6.24 ± 0.02 ^{b/B}	nd	9.00 ± 0.10 ^{c/B}	0.00 ± 0.00 ^{-/-}	nc	0.00 ± 0.00 ^{-/-}	0.00 ± 0.00	4.93 ± 0.11 ^{b/C}	nc	0.65 ± 0.12 ^{a/A}
Step 5	nc	14.10 ± 0.31 ^{d/B}	nd	4.54 ± 0.03 ^{a/A}	2.71 ± 0.07 ^{b/A}	nc	0.60 ± 0.03 ^{a/A}	0.06 ± 0.01 ^{a/A}	7.71 ± 0.30 ^{a/B}	nc	1.30 ± 0.12 ^{b/B}
Step 6	nc	13.54 ± 0.25 ^{c/B}	nd	4.75 ± 0.20 ^{a/A}	2.85 ± 0.20 ^{b/A}	nc	0.60 ± 0.04 ^{a/A}	0.06 ± 0.01 ^{a/A}	7.13 ± 0.10 ^{c/B}	nc	0.66 ± 0.13 ^{a/A}
Step 7	nc	13.48 ± 0.30 ^{c/B}	nd	4.78 ± 0.08 ^{a/A}	1.87 ± 0.21 ^{a/A}	nc	0.60 ± 0.04 ^{a/A}	0.06 ± 0.01 ^{a/A}	7.78 ± 0.21 ^{d/B}	nc	1.30 ± 0.12 ^{b/A}
Step 8	nc	14.94 ± 0.19 ^{c/B}	nd	5.27 ± 0.20 ^{b/A}	2.57 ± 0.08 ^{b/A}	nc	0.00 ± 0.00 ^{-/-}	0.13 ± 0.02 ^{b/A}	7.56 ± 0.20 ^{d/B}	nc	0.00 ± 0.00 ^{-/-}

For each step, data are the mean ± S.D. of 3 sub-samples analysed in triplicate. Different lower case letters indicate significant differences ($P < 0.05$) between steps for each wine. Different upper case letters indicate significant differences ($P < 0.05$) between wines for each step. nd: non-detectable; nc: non-quantifiable.

in OW samples can, therefore, be attributed to the fact that MLF happens spontaneously. In addition, it should be noted that at this step, the pH was higher in OW than in QW (3.6 and 3.3, respectively) and in these conditions both the number and type of bacterial microflora increase and the protective activity of SO₂ decreases. Another amine with an important quantitative presence in the samples is Et. The level of this amine grows in QW after AF and the highest level in OW and QPW is before MLF, which seems to indicate that in the samples analysed its presence is linked to the development of the AF more than to MLF.

Regarding Put, this amine was present in the samples of QW and OW obtained before MLF (Fig. 2). The noticeable increase that was detected in OW samples shows that spontaneous MLF is a factor that could enhance its content. The content of Cad evolves

irregularly during the study and no tendency which allowed us to elucidate its possible origin was found. Similar observations have been made by other authors (Lonvaud-Funel, 2001) who indicate that Cad is already present in grape must and is produced and degraded during winemaking.

Tyr, Trp and Phe were only detected in samples from OW after the MLF, which is why its presence could be linked to the spontaneous MLF. The low levels of SO₂ in organic wines, due to legal impositions, could be responsible for not only the presence of these last amines but also the highest levels in total BAs found in OW.

The method was also used for the study of BAs in samples of commercial wines of different denominations of origin of the Comunidad Autónoma de Castilla y León (Table 5). His, Et Tyr and Put were found in all the wines analysed.

Table 5

Concentration (mg/L) of individual biogenic amines in samples of commercial wines.

Wines	Ag	His	Ser	Et	Tyr	Isob	Trp	Phe	Put	Isoa	Cad	Total	±SD
1	nc	6.66	nd	1.90	2.69	nc	nc	nc	7.97	nc	1.93	21.16	2.30
2	nc	8.86	nd	3.30	2.45	nc	nc	nc	7.95	nc	nc	22.57	1.66
3	nc	6.50	nd	2.36	4.11	nc	nc	nc	8.63	nc	2.03	23.63	3.37
4	nc	5.46	nd	2.37	1.79	nc	nc	nc	5.55	nc	1.90	17.08	1.57
5	nc	8.52	nd	2.58	3.60	nc	nc	nc	11.09	nc	1.96	27.76	1.09
6	nc	7.88	nd	2.35	2.01	nc	nc	0.23	8.88	0.67	1.96	25.89	0.60
7	nc	8.73	nd	3.33	4.40	nc	nc	0.36	18.50	0.85	1.97	40.07	0.00
8	nc	4.27	nd	1.80	2.84	nc	nc	0.30	5.55	0.54	1.87	19.10	2.17
9	nc	4.99	nd	1.91	1.35	nc	nc	0.13	3.39	0.54	1.87	16.11	0.67
10	nc	4.04	nd	2.14	1.98	nc	nc	0.13	5.13	0.54	1.90	17.79	1.52
11	nc	4.73	nd	2.17	1.90	nc	nc	0.13	3.31	0.54	1.87	16.57	0.50
12	nc	8.81	nd	2.44	4.28	nc	nc	0.20	6.02	0.54	1.87	26.09	1.62
13	nc	5.47	nd	2.95	3.35	nc	nc	0.13	5.81	0.54	1.89	22.07	1.80
14	nc	4.81	nd	3.93	2.53	nc	nc	0.22	6.82	0.80	1.87	22.90	4.15
15	nc	6.71	nd	3.31	3.19	nc	nc	0.16	8.32	0.54	1.91	26.06	3.88
16	nc	4.26	nd	1.50	1.98	nc	nc	0.21	3.63	1.11	1.88	16.50	1.97
17	nc	4.95	nd	3.30	1.63	nc	nc	0.16	4.25	0.60	1.88	18.68	2.22
18	nc	8.76	nd	3.45	4.88	nc	nc	0.13	25.03	0.54	1.87	46.59	8.11
19	nc	3.10	nd	4.38	1.65	nc	nc	3.34	nc	nc	12.44	5.50	
20	nc	1.72	nd	3.00	1.32	nc	nc	0.18	1.16	0.21	nc	7.51	2.00
21	nc	2.91	nd	3.59	3.11	nc	0.25	0.10	1.95	0.31	nc	12.07	4.17
22	nc	2.07	nd	3.16	1.17	nc	nc	0.24	0.98	0.52	nc	8.07	2.14
23	nc	5.24	nd	5.83	4.37	nc	nc	4.71	nc	nc	20.08	4.75	
24	nc	2.80	nd	4.44	4.03	nc	nc	1.35	1.65	2.21	nc	16.42	3.65
25	nc	3.30	nd	4.43	0.04	nc	nc	0.23	2.77	0.49	nc	11.20	3.55
26	nc	5.24	nd	6.56	1.55	nc	nc	nc	2.69	0.20	nc	16.18	5.11
27	nc	3.21	nd	6.66	4.44	nc	nc	0.69	0.51	0.72	nc	16.16	2.57
28	nc	8.20	nd	5.95	0.48	nc	nc	nc	3.54	nc	nc	17.97	4.06

Data are the mean ± SD of the samples analysed in triplicate. nd: non-detectable; nc: non-quantifiable.

The amines Ag, Ser and Isob were not found in quantifiable concentrations in any of the wines. His, Put and Et were those which were found in highest concentrations, followed by Tyr, although its content was variable, depending on the wine. The concentration of His in the samples analysed varied between 1.72 and 8.86 mg/L, Put between 0.51 and 25.03 mg/L and Et between 1.50 and 6.66 mg/L. These concentrations lie within the range found by other authors who have analysed the content of these compounds in Spanish red wines (Bover-Cid et al., 2006; García-Villar et al., 2007).

4. Conclusions

The main reasons why consumers choose organic food are concern for their health and for the environment. However, organic wines could have higher levels of BA than non-organic wines. This is probably due to the fact that MLF occurs spontaneously, and also due to the low levels of SO₂ as a result of legal restrictions in organic wines. In non-organic wines, 2 oenological practices could increase the content in BA: the addition of press wine to the free run wine, and the treatment with yeast mannoproteins. On the one hand, the intense contact with the pomace, or solid parts of the grape, which the press wine undergoes, enhances the presence of BA. On the other hand, the addition of mannoproteins would permit an increase in the content of nitrogen compounds which, probably in the presence of residual enzyme activity, could act as precursors for the synthesis of BAs.

Acknowledgements

Financial support received from Junta de Castilla y Leon (Grant ref. SA050A05) and from Fundación Vinos de Zamora. Thanks are due to Mr. G.H. Jenkins for his help with the English version of the manuscript.

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