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Biogenic mono-, di- and polyamine contents in Spanish wines and influence of a limited irrigation

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Abstract

The distribution of biogenic aromatic amines, and also those of aliphatic diamines and polyamines, in red and white Spanish wines, was examined. Moreover, a study was carried out to determine whether the degree of irrigation affects the endogenous amine contents in grape as well as its evolution during the winemaking process. Amine levels were variable, ranging from not detected to 100 mg/l. Putrescine, which constituted the major amine, tyramine and histamine contents were significantly higher in red than in white wine. The aromatic phenylethylamine, and the aliphatic cadaverine, agmatine, spermine and spermidine, were detected in very low amounts in a few samples. Both, diamines and polyamines, were the only amines observed in grape samples, showing similar levels, irrespective of the water-stress degree, and their levels decreased significantly during winemaking. Most probably, the low levels of di- and polyamines in wine arise from those endogenously found in grape berries and the water-stress does not seem to be a factor influencing the contents.

Keywords: Biogenic amines; Polyamines; Wine tyramine; Putrescine; Water-stress

1. Introduction

The occurrence of biogenic amines in wine is well documented and wide ranges of biogenic amine concentrations are reported among different types of wine. It is generally accepted that biogenic amines appear as a consequence of amino acid–decarboxylase activity of microorganisms, which can be those responsible for the fermentation process and/or those related to contamination or spoilage (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). The variability in biogenic amine contents of wine could be explained on the basis of differences in winemaking processes, time and storage conditions, raw material quality, and possible microbial contamination during winery operations (Vidal-Carou, Mariné-Font, & Codony-Salcedo, 1990). Some of these amines, especially the aromatic ones such as tyramine, histamine and phenylethylamine, have vasoactive and psychoactive properties and excessive intake is considered to be deleterious for human health.

Moreover, the aromatic amines, together with the aliphatic diamines, putrescine and cadaverine, can be produced by spoilage bacteria and thus they have been suggested as food spoilage indicators (Mariné-Font, Vidal-Carou, Izquierdo-Pulido, Veciana-Nogués, & Hernández-Jover, 1995). Mainly the polyamines spermidine and spermine, but also putrescine, are natural intracellular polycationic molecules which are ubiquitous in most plant and animal cells, playing an important role in cell growth and development (Bardócz, 1995). Also they may be a key protective factor for the stressed cell (Bouchereau, Aziz, Larher, & Martin-Tanguy, 1999). Particularly in higher plants, di- and polyamine metabolism is dependent on external conditions and a major

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shift in nitrogen and amine metabolism can occur when plants are starved of nutrients, or exposed to osmotic shock or atmospheric pollutants (Bouchereau et al., 1999). In the case of the grapevine, biotic stress, such as by *Botrytis cinerea*, can also alter the composition of grape berries, increasing amine contents (Hajós, Sass-Kiss, Szerdahelyi, & Bardócz, 2000). Indeed, despite the fact that biogenic amines of bacterial origin could be avoidable in wine, the occurrence of the physiologically endogenous amines is unavoidable.

So far, the study of biogenic amines in wine has mostly been focussed on aromatic amines, due to their toxicological implications. Much less research has been done on diamines, and especially polyamines, although the study of aliphatic di- and polyamines has become of increasing interest due to their numerous functions and properties, for instance their antioxidant effect, protecting DNA and cellular membranes from radical damage and oxidative stress (Matkovics, Kecskemeti, Varga, Novak, & Kertesz, 1993).

The aim of the present work was (1) to carry out a survey on the distribution of biogenic aromatic amine contents, and also those of aliphatic diamines and polyamines in red and white Spanish wines; (2) to study whether the degree of irrigation affects endogenous amine contents in grape as well as its evolution during the winemaking process.

2. Materials and methods

2.1. Samples

Wine samples for the retail market survey were purchased from local stores and they consisted of 30 red and 10 white Spanish wines.

To study the influence of irrigation on amine contents in grapes and their evolution during winemaking, Cabernet Sauvignon grapes were collected in a vineyard from Catalonia (Spain). In total, 16 batches were obtained through the combination of four different plots of land (I, II, III, and IV) randomly subjected to four different degrees of water-stress, measured as the percentage of potential evapotranspiration (pET) (no stress: 80% pET; weak stress: 65% pET; moderate stress: 35% pET; and maximum stress: 0%). Grape berries were processed by a local winery to elaborate red wine.

2.2. Biogenic amine determination

Aromatic amines (tyramine, histamine, phenylethylamine, tryptamine, serotonin, octopamine and dopamine), aliphatic diamines (putrescine and cadaverine) and polyamines (spermidine and spermine) were determined all together by ion-pair high performance liquid chromatography with spectrofluorometric detection after post-column derivatization with *ortho*-phthalaldehyde (Vidal-Carou, Lahoz-Portolés, Bover-Cid, & Mariné-Font, 2003).

2.3. Statistical analysis

Data were statistically treated using the procedures of the SPSS software for Windows (SPSS Inc.). Mean values were compared by applying analysis of the variance (ANOVA) and post-hoc contrasts (least significant difference, LSD). When data were not normally and symmetrically distributed, non-parametric tests were used (Kruskal–Wallis and Mann–Whitney, respectively). Linear regression analysis was performed to study the significance of the linear relationship between time and amine changes during winemaking, as well as the influence of water-stress and plot of land.

3. Results and discussion

3.1. Biogenic amine distribution in Spanish commercial wines

The distributions of biogenic mono-, di- and polyamine contents in samples of red and white wine are shown in Table 1. Amine levels were rather variable, ranging from not detected to 100 mg/l. The apparently random distribution of both the type and the amount of biogenic amines in red and white wines is remarkable. The variability of data can be attributed to numerous variables affecting biogenic amine formation by bacteria during winemaking and wine storage. This makes it difficult to set a definitive pattern to describe their occurrence and profile among different types of wines, or even the same type.

Table 1
Biogenic amine contents (mg/l) in Spanish red and white wines

	Red wine (*n* = 27)		White wine (*n* = 10)	
	Range[a]	Mean (SD)[b]	Range	Mean (SD)
Histamine	n.d.[c]–19.6	3.9 (5.6)	n.d.–1.1	0.2 (0.4)
Tyramine	n.d.–18.2	3.3 (4.4)	n.d.–2.3	0.2 (0.7)
Phenylethylamine	n.d.–1.4	0.5 (0.4)	n.d.–1.7	0.3 (0.5)
Octopamine	n.d.	–	n.d.	–
Dopamine	n.d.	–	n.d.–1.0	0.3 (0.3)
Tryptamine	n.d.–4.7	0.2 (0.9)	n.d.	–
Serotonin	n.d.–0.7	0.02 (0.1)	n.d.–0.6	0.1 (0.2)
Putrescine	3.7–99.9	27.9 (30.3)	2.1–9.7	4.0 (2.3)
Cadaverine	n.d.–1.0	0.2 (0.3)	n.d.–0.6	0.1 (0.2)
Agmatine	n.d.–3.2	0.2 (0.7)	n.d.	–
Spermidine	n.d.–2.64	0.3 (0.6)	n.d.–1.5	0.4 (0.5)
Spermine	n.d.	–	n.d.	–

[a] Range: minimum–maximum.
[b] Mean (standard deviation).
[c] n.d.: not detected.

Statistically significant differences were found among some biogenic amine contents, depending on the type of wine, though in all cases the diamine putrescine constitutes the major amine. Particularly, red wine contained relatively high amounts of the diamine putrescine as well as the biologically active amines, histamine and tyramine, their contents being significantly higher ($p < 0.002$) than those found in white wine. Moreover, it is noteworthy that the highest levels of biogenic amines were found in wines older than one year (data not shown), which most probably underwent the malolactic fermentation. By contrast, no white wine contained more than 10 mg/l of putrescine and only two white wine samples contained histamine (below 1.1 mg/l) and/or tyramine (below 2.3 mg/l). Levels of phenylethylamine were quite low in all samples, although red wine also contained significantly higher amounts than white wine ($p = 0.025$). Dopamine was only detected in a few white wines and not in red wines, whereas octopamine and tryptamine were not detected in any sample. From the safety point of view, levels of biogenic amines found in wines do not represent any health hazard for the consumers. Even taking into account that wine contains alcohol, aromatic monoamine levels were lower than those considered capable of producing physiological effects (20 mg/l for histamine, 25–40 mg for tyramine and 3 mg for phenylethylamine) (Soufleros, Barrios, & Bertrand, 1998). There are no legal maximum tolerable levels for any biogenic amine in wines, but there is a recommended maximum content for histamine, from 2 to 10 mg/ml, depending on the country (Lehtonen, 1996). Only five samples of red wine contained more than the maximum recommended histamine level of 10 mg/ml, although none of them surpassed the potentially toxic level of 20 mg/l. When compared with other fermented foods, such as cheese or meat sausages, wine and other fermented beverages show relatively low amounts of biogenic amines (Mariné-Font et al., 1995; Vidal-Carou, Mariné-Font, & Codony-Salcedo, 1989).

Concerning di- and polyamines, putrescine was the only amine detected in all samples and its concentration was much higher in red wines than in the white wines tested ($p < 0.001$), as was also observed in other Spanish wines from the "Rioja" region (Vázquez-Lasa, Íñiguez-Crespo, González-Larraina, & González-Guerrero, 1998). Sources of putrescine in wine could be multiple and sometimes difficult to distinguish. First, a certain amount of this polyamine might come from raw material, e.g., endogenous compounds in grapes. Red wine vinification is usually carried out in the presence of grape skin and pulp and thus putrescine from these parts can be released into the must, and this could explain, at least partially, the higher levels of this diamine in red wine. During winemaking, putrescine can also originate from the microbial decarboxylation of ornithine, which may also be formed by the microbial metabolism of amino acids such as citrulline or arginine. Also, putrescine can be formed from arginine via agmatine. All these pathways have been described in several wine lactic acid bacteria which can develop during malolactic fermentation (Arena & Manca de Nadra, 2001). This could explain the higher concentration of putrescine found in red wine than in white wine, since this secondary fermentation is less usual in white wines.

Cadaverine is the other diamine relatively common in food, although it is not so frequently found in alcoholic beverages. Levels of cadaverine in this study were always below 0.5 mg/l, either in red or white wine, without significant differences between them. These findings are in agreement with others published in the literature (Lehtonen, 1996; Soufleros et al., 1998; Vázquez-Lasa et al., 1998). Cadaverine is usually associated with decarboxylase activity of contaminant enterobacteria (Halász et al., 1994), and therefore high amounts of cadaverine and/or putrescine have been used as indicators of the degree of food spoilage, especially fish and meat (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996; Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997). However, these diamines do not seem to be good indicators of the hygienic degree of the vinification process, due to the multiple sources of putrescine as well as the association of cadaverine with enterobacteria, which are not usual contaminants in wine.

Agmatine was only found in some red wine samples, with a maximum value of 3.5 mg/l. Little is known about the significance of this polyamine in food and beverages. It is recognized as an intermediate of putrescine formation from arginine but it has also been related to food spoilage (Halász et al., 1994; Veciana-Nogués et al., 1997). However, the relationship between agmatine and spoilage cannot be applied to wine on the basis of the results obtained in the present study.

Finally, spermidine was only observed in some samples, the average content not being significantly different in red and white wine. By contrast, spermine was not detected in any sample. The fact that there were not differences between red and white wine is in agreement with the non-bacterial origin of these polyamines. Moreover, the contents observed agree with the fact that vegetal products usually contain more spermidine than spermine, while animals show more spermine than spermidine (Bardócz, 1995). Little is known about the occurrence of such physiological polyamines in wine. Although polyamines might come from grapes and/or from yeast lysis, different possibilities could explain the variability of polyamine contents among wines. For instance differences in grapevine culture conditions, temperature, rain, sun and soil nutrient availability, but also variables relating to changes during winemaking due to lysis of yeast and eventual consumption by microorganisms.

3.2. Red winemaking from grapes subjected to different degrees of water-stress

Both diamines and polyamines were the only amines observed in grape samples in all 16 batches studied. Putrescine was always the main aliphatic amine, with a mean value of 6.81 mg/kg (standard deviation, SD = 1.47), followed by 4.66 mg/kg (SD = 0.26) of spermidine, 2.50 mg/kg (SD = 0.23) of spermine and 1.16 mg/kg (SD = 0.37) of cadaverine. Spermidine and spermine disappeared during the alcoholic fermentation and were not detected in samples from the winemaking process, which could be explained by a potential consumption by the alcoholic fermentative yeast or by a spontaneous chemical degradation of polyamines. Diamines also decreased during the wine elaboration, following different patterns, depending on the amine. Putrescine decreased linearly throughout time, ending at 4.30 mg/l (SD = 0.96) with the linear correlation coefficient from the regression analysis being highly significant ($p < 0.0001$). By contrast, cadaverine significantly decreased at the maceration stage ($p < 0.0001$) and remarked at around 0.5 mg/l until the end of the malolactic fermentation. In any case, it is difficult to associate these amounts of di- and polyamines with a microbial production during alcoholic fermentation, since the contents of these di- and polyamines were low and also because it is generally accepted that yeasts are unable to liberate them in significant amounts. Therefore, the most probable sources of such low levels of di- and polyamines in wine are the endogenous amines found in grapevine.

The aromatic biogenic amines usually found in retail wines did not appear in must or during the alcoholic fermentation. Usually an increase of these amines, and also of the diamines, occurs during malolactic fermentation (Torrea-Goñi & Ancín-Azpilicueta, 2001; Vidal-Carou et al., 1990) because those amines appear as a result of bacterial activity. However, wines that have undergone this secondary bacterial fermentation, but without a significant biogenic amine accumulation, and even showing a polyamine decrease, have also been previously reported (Hajós et al., 2000).

The lack of accumulation of biogenic amines (either aromatic or aliphatic) during the winemaking process is in agreement with the proper hygienic and controlled conditions applied in all the 16 batches. Perhaps, these optimal conditions did not occur during the winemaking of all red wine from the retail samples. The results obtained here suggest that is feasible to produce wine with extremely low contents of amines, which would be aliphatic di- and polyamines originating from raw materials rather than being produced through amino acid–decarboxylation by microorganisms.

Despite the low biogenic amine levels found, data from all batches were statistically treated, taking into account the irrigation level of the grapevine. Contents of all di- and polyamines were very similar in all four groups of grape samples ($p > 0.05$), irrespective of the water-stress degree applied. Neither were there statistical differences between the evolutions of putrescine or cadaverine during the winemaking (Table 2). It has been reported that the direction and the intensity of di- and polyamine metabolism response to water-stress vary, depending on the species and the plant part (leaf, root, fruit) (Bouchereau et al., 1999). An increase in putrescine and cadaverine and a decrease in spermidine and spermine have been reported in cereal leaves subjected to osmotic stress (Flores & Galston, 1984). In the case of grape berries, the water-stress does not seem to be a factor able to modify aliphatic polyamine contents. According to our knowledge, this is the first study aiming to evaluate the possible influence of water-stress on polyamine metabolism in grapevine.

Therefore, further research is needed to investigate factors influencing the changes occurring in putrescine levels, as well as those of the other polyamines, during the winemaking process. Polyamines, but also putrescine, have a number of attributed physiological roles, including a protective effect against oxidative damage (Bardócz, 1995; Matkovics et al., 1993). In this sense, perhaps it would be interesting to investigate the influence of oxidative-stress on the amine contents.

In comparison with other foods, wine contains relatively low amounts of polyamines, but the consumption

Table 2
Mean (and standard deviation) values of putrescine and cadaverine contents (mg/l) during winemaking from grapes subjected to different water-stress degrees

	No stress (80% ETP)	Weak stress (65% ETP)	Moderate stress (35% ETP)	Maximum stress (0% ETP)
Putrescine				
Maceration	5.19 (1.13)	5.27 (0.74)	5.58 (0.88)	5.64 (1.19)
AF[a]	4.99 (1.46)	4.84 (0.98)	5.23 (1.42)	5.07 (1.29)
MLF[b]	4.25 (1.05)	4.28 (0.70)	4.39 (0.73)	4.31 (1.57)
Cadaverine				
Maceration	0.42 (0.07)	0.39 (0.02)	0.43 (0.08)	0.39 (0.03)
AF	0.68 (0.14)	0.61 (0.19)	0.38 (0.12)	0.63 (0.13)
MLF	0.50 (0.09)	0.51 (0.05)	0.46 (0.07)	0.43 (0.12)

[a] AF: end of alcoholic fermentation.
[b] MLF: end of malolactic fermentation.

of wine in southern Europe is relatively high. It would therefore be interesting to follow up the study of the compounds discussed here and their significance in the Mediterranean diet.

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