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Study of polyamines and their precursor amino acids in Grenache noir and Syrah grapes and wine of the Rhone Valley

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Abstract

Polyamines and their amino acid precursors were determined in Grenache and Syrah grapes and in wines made from these grapes. The compounds analysed were the polyamines putrescine, spermidine, and spermine, in addition to their precursors, ornithine, agmatine and arginine. The analytes were determined by reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection using FMOC (fluorenylmethylchloroformate) as a pre-column derivatising agent Grape clusters were sampled from flowering to full maturity at different developmental stages. In addition, different berry parts were analysed separately from half veraison onwards. It appears that at berryset there is a decrease in the concentration of arginine, whereas polyamine concentrations remain constant at this stage. Concentrations of polyamines increased from must to alcoholic to malolactic fermentation; putrescine was the most abundant in wine (mean concentration after malolactic fermentation, MLF was 4.93 mg/l) followed by spermidine and then spermine (mean concentrations after MLF were 1.84 and 0.17 mg/l, respectively). In three of the four fermentation sites concentrations of all three compounds were greater in Syrah than in Grenache wines. In both varieties, it appears that polyamine biosynthesis occurs preferentially from arginine via agmatine. In all cases, concentrations of polyamines found in these grapes and wines were significantly below the levels typically found in other fermented foods.

Keywords: Polyamines; Putrescine; Spermidine; Spermine; Agmatine; Grapes; Wines

1. Introduction

Polyamines amines are found in a wide range of food products, particularly protein-rich foods (Taylor, Hui, & Lyons, 1984) of both animal and plant origin, as well as in fermented products generally (Joosten, 1987; Vidal-Carou, Izquierdo-Pulido, Martin, & Mariné Font, 1990). They occur at low concentrations in wines and beers (Aerny, 1990; Ingargiola & Bertrand, 1992; Vidal-Carou, Isla Gavin, Marine Font, & Codony Salcedo, 1989) where they are usually considered as markers of microbial spoilage during wine storage or during the technological stages in winemaking.

The demand by consumers for better and healthier foods has led to renewed interest in polyamines and biogenic amines, given their importance for human health and food safety. The aliphatic polyamines, putrescine, cadaverine, spermine and spermidine, are pharmacologically active and reportedly toxic (Tabor & Tabor, 1964; Til, Falke, Prinsen, & Willems, 1997). Putrescine and cadaverine play an important role in food poisoning as they can enhance the toxicity of histamine (Cinquina et al., 2004). They play an essential part in tissue growth, and because of this, it has been suggested that they may

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be involved in the development of tumors (Seiler, 1990; Tabor & Tabor, 1984). Furthermore, putrescine and cadaverine can react with nitrite to form heterocyclic nitrosamines which are carcinogenic (Hotchkiss, 1989).

The presence and role of polyamines in grape berries have been studied for some years. In the vine, potassium deficiency increases the putrescine content in leaves (Adams, Franke, & Christensen, 1990; Adams, Bates, Adams, & Franke, 1992; Ruiz & Moyno, 1998). In plants, and particularly in *Vitis vinifera*, it appears that in addition to their metabolic functions, polyamines may act as growth factors (Geny, Broquedis, Martin-Tanguy, & Bouard, 1997) or are produced as response factors to unfavorable conditions, such as excessive nitrogen fertilization, or mineral deficiency (Adams et al., 1990, 1992; Broquedis, Dumery, & Bouard, 1989); for example, elevated concentrations of putrescine were found in the leaves of potassium-deficient *V. vinifera* (Bertrand, Ingargiola, & Delas, 1991). Polyamines are found at all the stages of the vegetative cycle of the vine and particularly during berry development (Broquedis & Bouard, 1990; Lespy-Labaylette, Broquedis, Soyer, & Bouard, 1994), but they occur in low concentrations in wines (Bauza, Kanny et al., 1995): concentrations of spermidine, spermine and cadaverine rarely exceed mg/l levels, whereas the concentration of putrescine can increase considerably during fermentation.

Spermine and spermidine are derived from putrescine which is derived either from ornithine or from arginine, via the formation of agmatine. Which of these two biosynthetic pathways predominates depends on the species and on the stage of development within a given species (Bauza, Blaise, Daumas, & Cabanis, 1995).

There are several literature reports describing the presence of polyamines in grapes and wines (Aziz, 2003; Bover-Cid, Iquierdo-Pulido, Mariné-Font, & Vidal-Carou, 2006; Fernandes & Ferreira, 2000; Herbert, Cabrita, Ratola, Laureano, & Alves, 2005; Leitão, Marques, & San Romão, 2005; Lozanov, Petrov, & Mitev, 2004; Paschalidis, Aziz, Geny, Primikirios, & Roubelakis-Angelakis, 2001; Shiozaki, Ogata, & Horiuchi, 2000; Soleas, Carey, & Goldberg, 1999; Soufleros, Barrios, & Bertrand, 1998; Vidal-Carou, Lahoz-Portolés, Bover-Cid, & Mariné-Font, 2003) but little data are available on the concentrations of these compounds specifically in the grapes and wines of Syrah and Grenache noir, the principal cultivars in the Southern Rhone Valley. The climate in this area is characterized by dry, hot summers and drought conditions for at least three months. The soil is composed of sandy marl, alluvium and large, gravely clay and hard stony terraces.

The purpose of this study was to obtain a greater understanding of the evolution of polyamines during the growth stages of the grapeberry and during winemaking. Putrescine, spermidine, spermine, as well as their precursor amino acids agmatine, arginine and ornithine were determined in Grenache noir and Syrah at different points according to the Baggiolini (1952) stages of berry development and in wines produced from these grapes at four different fermentation sites.

The classical analytical method for the determination of biogenic amines in wines by HPLC uses precolumn derivatization with *o*-phthaldialdehyde (OPA). This derivatising agent gives good results for primary amines, but it was found that it gave a very poor response with the secondary amine functions of spermine and spermidine. The use of fluorenylmethylchloroformate (FMOC) for this type of application (Bauza, Kanny et al., 1995; Bellagamba et al., 1997; Ekegren & Gomes-Trolin, 2005; Herbert et al., 2005) allows the direct analysis of the polyamines putrescine, spermine and spermidine, simultaneously with other amines.

2. Materials and methods

2.1. Sampling and preparation of the plant material

For both cultivars, samples were taken from batches of 20 vinestocks selected at random in five plots; each plot was homogeneous in terms of vine development, soil composition, exposure to sunlight and cultural techniques. Samples were taken at the different phenological stages of berry development as defined by Baggiolini (1952): separate floral buds (III), clusters at flowering (IV), berryset (V), berries at half ripening (stage VI), full ripening (VII) and maturity (VIII).

From stage III to stage VIII, samples consisted of approximately 100 g whole of grape clusters accurately weighed. In addition, from stages VI to VIII, 200 berries were collected from each plot and divided into skin, pulp, stalks, and seeds for separate analysis. The weighed samples were homogenized in liquid nitrogen, freeze-dried and re-weighed in order to determine the dry weight. The dried samples were used for analysis and results expressed as mg/kg dry weight.

2.2. Wine sampling

Grapes harvested from the five plots were fermented at four different winemaking sites – G1–G4 for Grenache noir and S1–S4 for Syrah. Duplicate samples of the wines were analyzed first during alcoholic fermentation (AF) when the density reached the 1.04 then at the end of malolactic fermentation (MLF) which in this instance study occurred immediately after AF.

2.3. Extraction of amino compounds from plant material

The dried samples were extracted in duplicate by adding a 5% (v/v) perchloric acid solution according to the method of Flores and Galston (1982) for the extraction of free polyamines from plants (Geny et al., 1997). The volume of acid

solution was adapted to sample size and was equivalent to 10 ml of acid solution for 2 g of sample (fresh weight). After 12 h soaking at ambient temperature with intermittent agitation, the solid parts were separated by centrifugation and the supernatant containing the amines and their precursors was then derivatized for HPLC analysis.

2.4. Separation and quantification by HPLC

The amino acids and polyamines were supplied by Fluka (Busch, Switzerland). Two stock solutions were prepared in 0.1 M hydrochloric acid, one containing 1 g/l of the polyamines and the other containing 1 g/l of the amino acids. These solutions were stable for eight weeks when stored at 4 °C. Working solutions containing 10 mg/l of the amino acids and agmatine and 1 mg/l of the other polyamines were prepared daily by appropriate dilution of the stock solutions.

The compounds were analysed using a validated method which was previously reported for the determination of these nitrogen compounds by HPLC with pre-column derivatisation (Bauza, Blaise et al., 1995). This automated procedure may carried out in the autosampler of the HPLC chromatograph. A 20 µl aliquot of wine or acid extract of grape samples were placed in a 700 µl amber vial and mixed with 50 µl borate buffer pH 8.5 and 100 µl fluorenylmethyl-chloroformate FMOC (Fluka, Buchs, Switzerland) 8 mg/1 in acetonitrile. After 3 min, 50 µl of a 0.55 M ammonia solution was added followed by 300 µl of a quenching solution ($CH_3CN/CH_3COOH/H_2O$: 20/2/3).

The derivatised amines were separated on a Superspher 100 RP 18, 125 mm × 4 mm, 5 µm column (Hewlett-Packard), and detected by spectrofluorimetry ($\lambda_{exc.}$ 263 nm, $\lambda_{em.}$ 313 nm). A data analysis processor (type Vectra 286-S-20 Hewlett-Packard) controlled the quaternary pump (model 1050 Hewlett-Packard), the auto-sampler tray (model 1050 Hewlett-Packard) and the spectrofluorimetry detector (model 1046A Hewlett-Packard).

The two groups of compounds were separated with the same binary mobile phase operated under different gradient elution conditions. Solvent A consisted of acetonitrile–octanol 2 (100/1); solvent B consisted of [acetonitrile–phosphoric acid–*N*,*N*-dimethylcyclohexylamine–water (150/5/10/835)]. In both cases, the injection volume was 5 µl, and the flow rate 0.7 ml/min.

Gradient programme

Amino acids	Polyamines
85% B: 5 min	40–30% B: 0–20 min
85–70% B: 5–30 min	30–10% B: 20–30 min
70–30% B: 30–60 min	10–40% B: 30–40 min
30–85% B: 60–70 min	

Full details of the methods are available in a previously published report (Bauza, Blaise et al., 1995).

3. Results and discussion

3.1. Polyamine analysis in grapes

In order to test the repeatability of the method, the coefficients of variation (% CV) of the analyses were determined using seven extracts of whole clusters at half ripening. The % CV values obtained were similar to those reported in literature (Geny et al., 1997): arginine 1.8%, putrescine 7.3%, spermidine 8.8% and spermine 8.6%. The higher CV values may be explained by the low concentrations of putrescine, spermidine and spermine (approximately 1–10 mg/l) present in the samples.

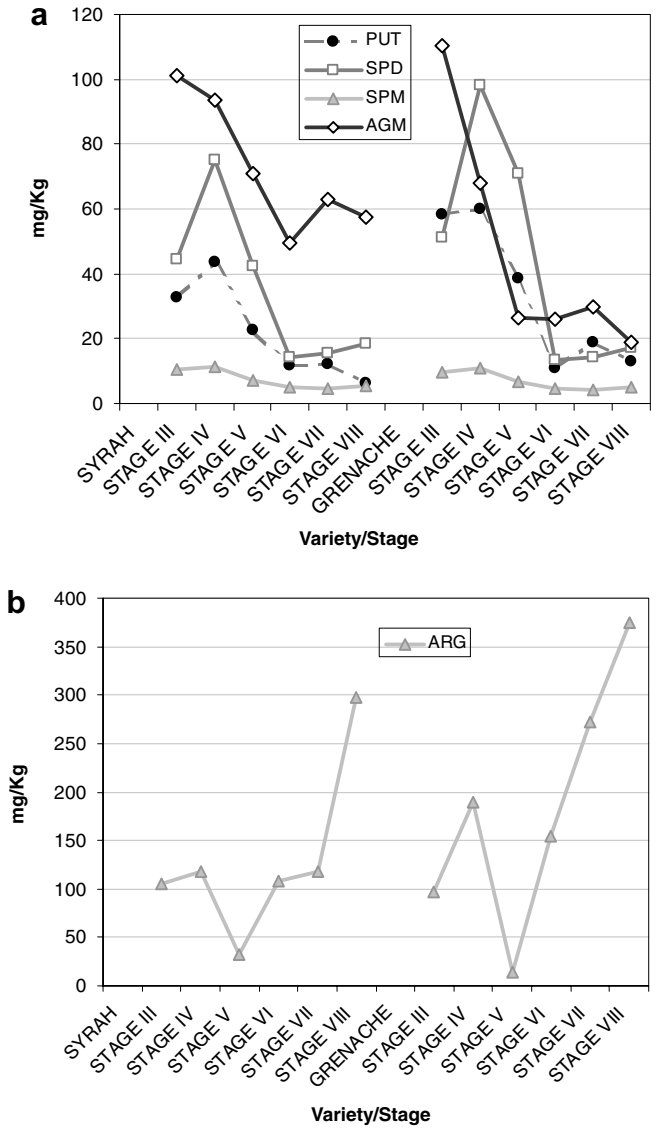

Fig. 1. (a) Evolution of putrescine (PUT), spermidine (SPD) spermine (SPM) and agmathine (AGM) in grape berries of Syrah and Grenache from floral buds to maturity, (b) evolution of arginine (ARG) in grape berries of Syrah and Grenache from floral buds to maturity. Separate floral buds (stage III), clusters at flowering (stage IV), berryset (stage V), berries at half ripening (stage VI), full ripening (stage VII) and maturity (stage VIII). Concentrations are in mg/kg of dry weight.

Fig. 1a shows the evolution of putrescine, spermidine, spermine and agmatine in plant parts of Syrah and Grenache from stage III (separate floral buds) to stage VIII (maturity). It may be observed that maximum concentrations of putrescine, spermidine and spermine occurs at stage IV (clusters at flowering) in both varieties, and also that the relative concentrations of the three compounds are spermidine > putrescine > spermine. These observations concur with data reported in previous studies on free polyamine levels in various organs of *V. vinifera* during anthesis (Geny et al., 1997). Spermidine increases significantly between stages III and IV and then decreases between stages IV and V after which its concentration does not change significantly. Spermine accounts for 7% of the total free polyamine (FPA) concentration at stage IV; its evolution is similar to that of spermidine, though at a concentration six to seven times lower. The reduction in the concentration of the polyamines from stage IV onwards may be explained by the fact that as the berry ripens these compounds become less essential to berry development. Interestingly, the highest concentrations of agmatine (a recognized precursor of putrescine) occur at stage III. Shiozaki et al. (2000) also found that putrescine, spermidine and spermine were the predominant free polyamines in the pericarp and seeds of grape berries and that putrescine, and particularly spermidine were found at higher levels in the early developmental stages. Elevated concentrations of free polyamines in the early phase of fruit development was also reported in fruits of other species (Biasi, Bagni, & Costa, 1988; Ponappa & Miller, 1996), in which the direct involvement of polyamines in cell division has been proposed. It has further been suggested that high levels of free putrescine and spermidine during early development maybe associated with cell proliferation in the pericarp. To establish the existence of possible correlations between these compounds during the growing stages of the vine, a non-parameter Spearman test was used as the variables were non-Gaussian. The correlations matrix obtained

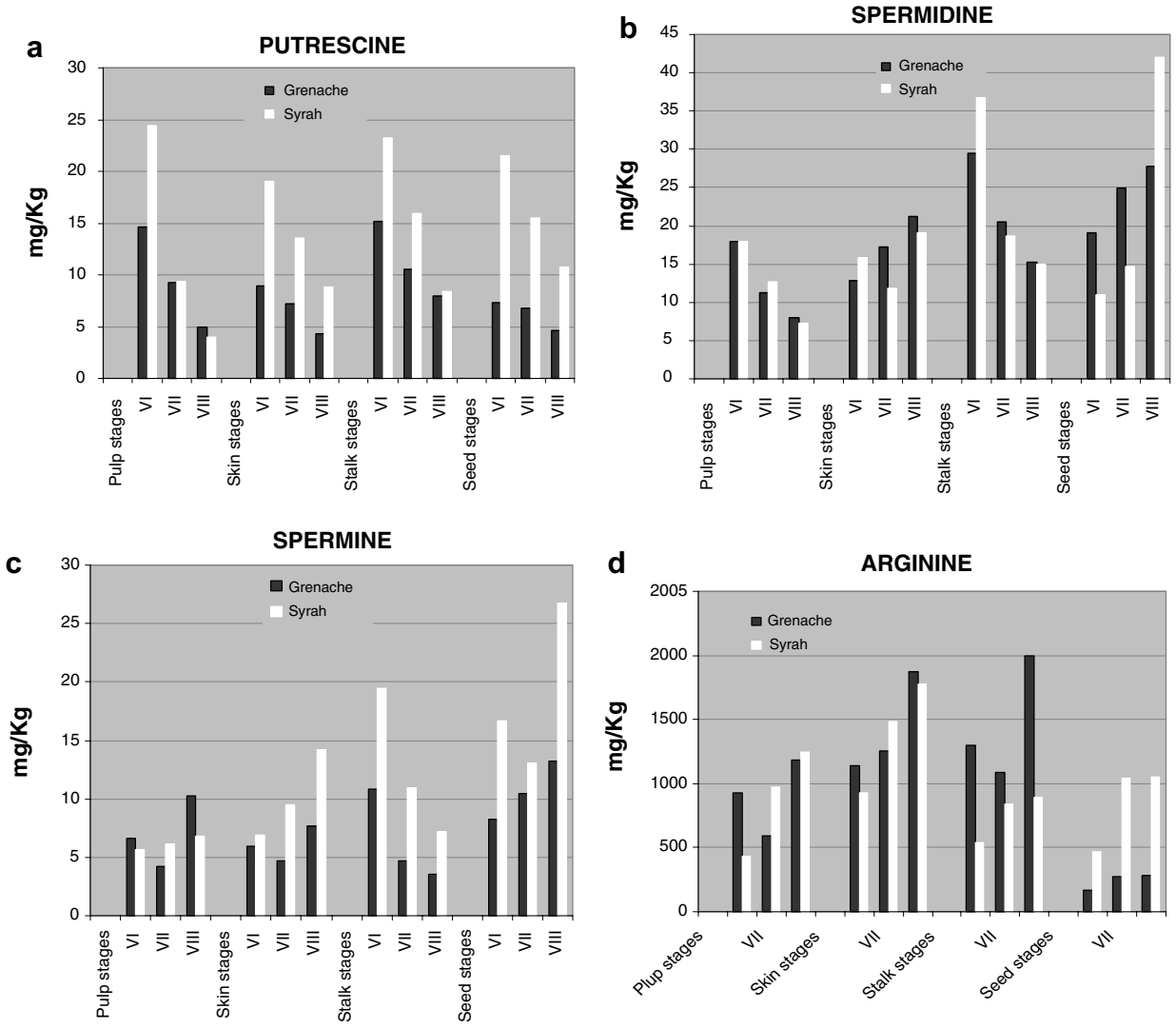

Fig. 2. Evolution of the concentrations (mg/kg dry weight) of individual biogenic amines in different berry parts of Syrah and Grenache grapes. (a) Putrescine; (b) spermidine; (c) spermine; (d) arginine. Stages as per Fig. 1.

showed that the results were similar for Grenache noir and Syrah grapes and that variations in the concentrations of putrescine are statistically correlated to those of spermidine, spermine and agmatine ($p < 0.01$).

The evolution of arginine concentrations are shown on a separate graph (Fig. 1b) in view of the large concentration difference between this and the other amines. It may be seen that there is a sharp increase in the concentration of arginine at the end of ripening reaching maxima of approximately 1400 and 1700 mg/kg in Syrah and Grenache grapes, respectively. Results also showed that there is probably an inverse correlation between the evolution of arginine and the various polyamines. Like the polyamines, there is a concentration peak for arginine at stage IV, though interestingly, concentrations decrease dramatically between stages IV and V before increasing sharply through

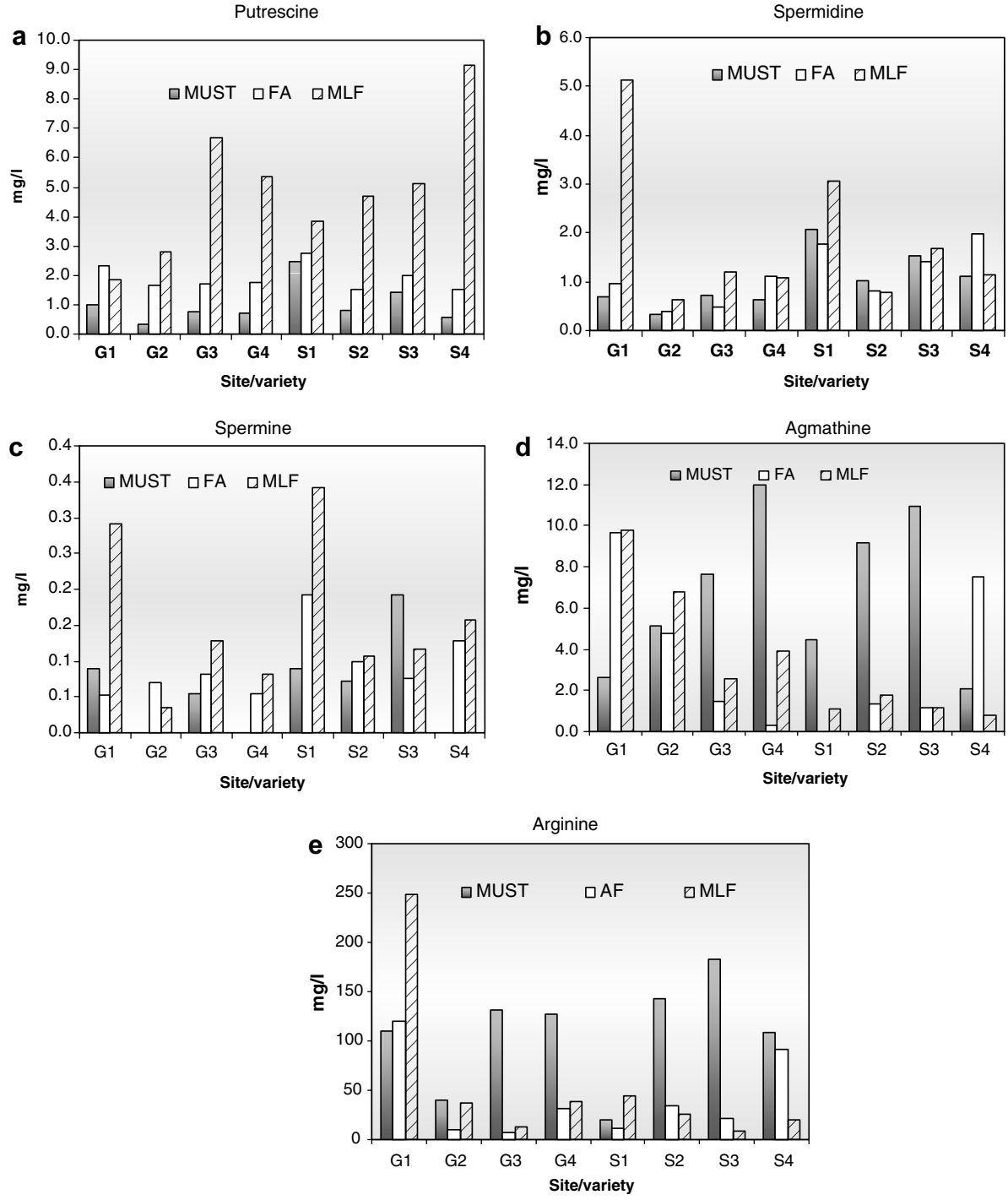

Fig. 3. Concentrations (mg/l) of polyamines from must to alcoholic fermentation (AF) to malolactic fermentation (MLF). (a) Putrescine; (b) spermidine; (c) spermine; (d) agmathine; (e) arginine.

stages VI–VIII. Stage V coincides with a key period in the ripening process: at this point large amounts of compounds start accumulating in the berry and it is possible that the amino acids present in the berry at this point are used for protein synthesis, which would explain the reduction in their concentration at this point.

The evolution of the concentrations of individual amines was studied in the different berry parts (pulp, skin, stalks seeds). The first observation that may be made from Fig. 2 is that the concentration of putrescine (Fig. 2a) decreases from stage VI to stage VIII in the pulp, skin, stalk and seeds of both grape varieties and concentrations are higher in Syrah than in Grenache grapes. Results are more variable for spermidine (Fig. 2b); concentrations of this compound decrease from stages VI to VII in the pulp and in the stalk, whereas concentrations increase in the seeds skin and with the exception of stage VII for Syrah grapes in the skins also. Grenache seeds are richer than Syrah seeds in spermidine whereas the pulp of Syrah contains slightly more of this compound. The distribution and evolution of spermine (Fig. 2c) follow similar patterns, in that concentrations increase in the pulp, skin and seeds from stages VI to VIII though the concentration of spermine decreases in the stalks. As in the case of putrescine, Syrah grapes are richer than Grenache in these compounds. The concentrations of arginine (Fig. 2d) are substantially higher (100–500 mg/l) than the other compounds, and as was seen with the whole berries, increase from stages VI to VIII although, highest concentrations are found in the skin and stalks, rather than in the seeds.

Agmatine was only detected in the seeds of Syrah grapes and concentrations halved between stages VI and VIII. This polyamine was detected at concentrations less than 5 mg/kg in pulp, skin and stalks Grenache noir grapes and then only at stage VI or VII.

The observed differences between the concentrations measured in the earlier and later stages of ripening may be explained by the complexity of the processes taking place and the fact that the pulp, stalks and seeds do not achieve optimum ripeness at the same time. Therefore it may be concluded that as the berry approaches maturity there is a general reduction in the concentration of putrescine and an increase in the concentration of arginine, and it is therefore likely, given the low concentrations of polyamines in the berry at maturity, that their presence in finished wine is due to their synthesis by microorganisms during the winemaking process.

3.2. Evolution of polyamines and amino acids during winemaking

Results are the mean values obtained for the duplicate sampling and analyses and are expressed as mg/l. Individual results are given for each of the four fermentation sites since it was found that there were significant variations in polyamine concentrations from one site to another.

Fig. 3 shows that putrescine levels increase during fermentation from must (mean concentration 1 mg/l), to alcoholic fermentation (AF) (mean concentration 1.97 mg/l) to malolactic fermentation (MLF) (mean concentration 5.37 mg/l). These findings concur with those of Soufleros et al. (1998), who found that concentrations are low in must and increase slightly after alcoholic fermentation, and more significantly during malolactic fermentation. The same general trends are observed for spermidine and spermine though at some sites, concentrations are greater after AF than after MLF. However the concentration of spermidine and spermine are significantly lower than those of putrescine, (mean concentrations after MLF of 1.37 and 0.17 mg/l for spermidine and spermine, respectively). These observations concur with the results of previous authors (Anli et al., 2004; Bauza, Blaise et al., 1995; Bauza, Kanny et al., 1995; Bover-Cid et al., 2006; Fernandes & Ferreira, 2000; Gloria, Watson, Simon-Sarkadi, & Daeschel, 1998) who found that the order of concentrations of these three amines in wines were putrescine > spermidine > spermine. It should, however be pointed out that in all these examples, as in the present study, significant variations in the amounts of polyamines found in the individual wine samples, as may be seen in Table 1 which presents the maximum, minimum, mean and standard deviation values of putrescine, spermidine and spermine found in this and other selected studies. It may be observed that frequently the values of the standard deviations exceed those of the

Table 1
Maximum, minimum, mean and standard deviations of putrescine, spermidine and spermine in selected studies

Reference	Wine type/region	Putrescine		Spermidine		Spermine	
		Max–min	Mean (±SD)	Max–min	Mean (±SD)	Max–min	Mean (±SD)
This study	Syrah and Grenache ($n = 8$)	1.85–6.67	5.37 (1.64)	0.64–3.07	1.37 (1.63)	0.13–0.29	0.17 (0.1)
Bover-Cid et al. (2006)	Spanish red wines ($n = 30$)	3.7–99.9	27.7 (30.3)	ND–2.64	0.3 (0.6)	ND	ND
Anli et al. (2004)	Turkish red wines ($n = 30$)	ND–5.92	0.88 (1.29)	ND 2.19	0.44 (0.53)	ND–1.75	0.46 (0.39)
Fernandes and Ferreira (2000)	Port wine, table wines	0.15–11.95		ND–0.70		ND–0.04	
Gloria et al. (1998)	Pinot noir ($n = 36$)	2.43–203.12	27.47 (39.95)	ND–2.35	0.6 (0.57)	ND–2.38	0.51 (0.84)
	Cabernet Sauvignon ($n = 23$)	3.15–23.6	10.63 (5.47)	ND–4.03	1.64 (1.26)	ND–1.17	0.08 (0.25)
Soufleros et al. (1998)	Burgundy ($n = 20$)	0.71–137.86	25.9				
	Alsace ($n = 20$)	0.48–29.92	4.43				
	Bordeaux ($n = 66$)	0.25–46.56	16.51				
	Champagne ($n = 6$)	1.2–59.2	14.17				

means, indicating the multiplicity of factors that determine the final concentration of these compounds in wine.

The increased concentrations of putrescine from must to wine may be explained by the fact that during winemaking, this compound can be formed either from the microbial decarboxylation of ornithine, or from amino acids such as arginine via agmatine. Although absolute concentrations appear to be strongly influenced by winemaking site, it was consistently observed that the concentration of putrescine, spermidine and spermine was greater in Syrah than in Grenache wines after MLF (Fig. 4).

It should be noted in relation to these compounds, that final quantities are low compared to other red cultivars (Gloria et al., 1998; Soufleros et al., 1998; also Table 1) and in all cases are less than 10 mg/l; the final concentrations of spermidine and spermine do not exceed 3 mg/l and 0.35 mg/l, respectively, which is favorable from the point of view the hygienic properties of the wine. It might be added that the extremely low concentrations of spermine are consistent with the fact that plant-derived products usually contain more spermidine than spermine, while animal-derived products contain more spermine than spermidine (Bardócz, 1995).

Agmatine is recognized as an intermediate of putrescine formation from arginine; initial values were the highest in musts, up to 12 mg/l though with a maximum value of 10 mg/l were found in one of the wines. Little is known about the significance of this polyamine in food and beverages, but it is a precursor of putrescine, spermine and spermidine that has also been related to food spoilage (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997). This pathway and the pathway via ornithine have been described in several wine lactic acid bacteria which can develop during malolactic fermentation (Coton, Rollan, Bertrand, & Lonvaud-Funel, 1998; Arena & Manca de Nadra, 2001). Which of the two pathways predominates in any given situation has not been determined and it is known that spermine (Lonvaud-Funel, 2001; Moreno-Arribas, Polo, Jorganes, & Muñoz, 2003) and spermidine synthesis is not the result of a simple amino acid decarboxylation, but it depends on a complex biosynthetic pathway (Bardócz, 1995). However, observation of Fig. 5 reveals that there appears to be a positive correlation between the concentration of agmatine in must and putrecine in the finished wine after MLF. It also seems that there is an inverse relationship between the concentrations of ornithine and agmatine. The fact that the concentrations of putrescine correlate with those of agmatine rather than those of ornithine supports the argument that the principal

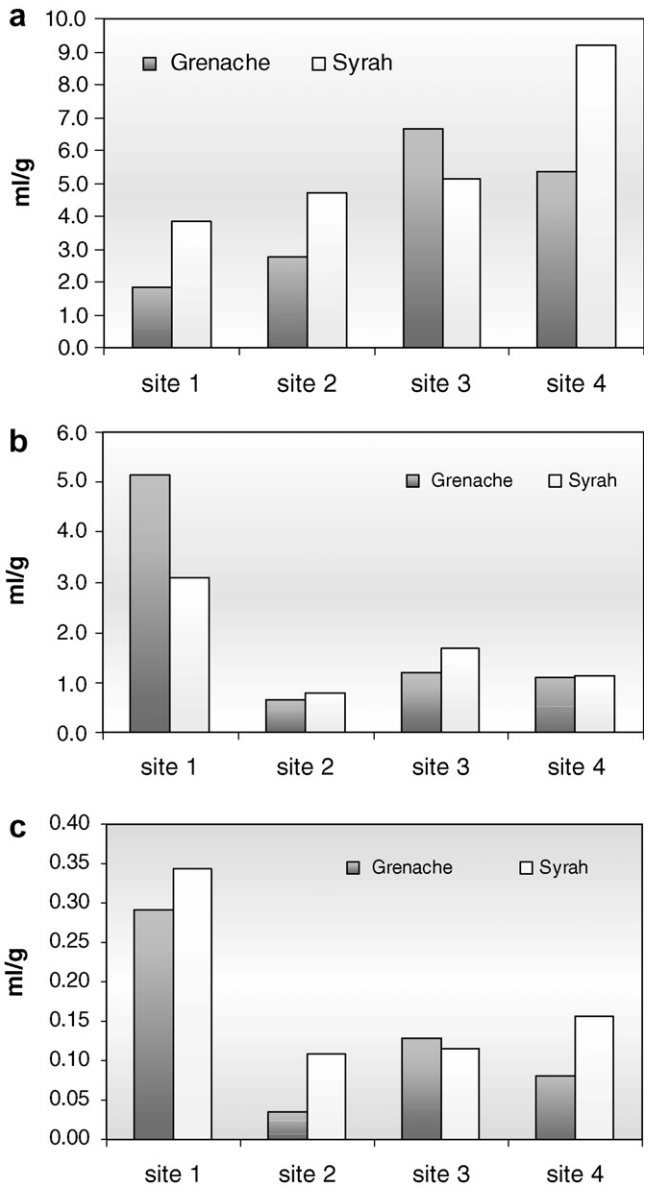

Fig. 4. Comparison of polyamine concentrations (mg/l) in Syrah and Grenache in wine after MLF. (a) Putrescine; (b) spermidine; (c) spermine.

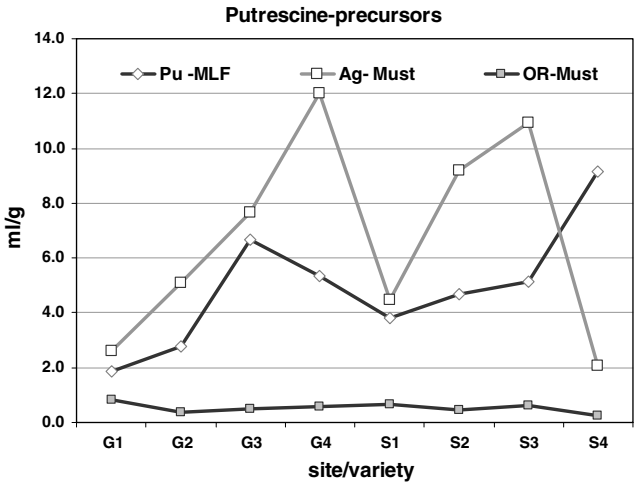

Fig. 5. Comparison between the concentration (mg/l) of agmathine (Ag-must) and ornithine (Or-must) in must and putrecine in wine after MLF (Pu-MLF).

biosynthetic pathway for putrescine is via arginine–agmatine rather than ornithine. This is also supported by the fact that with the exception of site 1, the concentration of arginine, (Fig. 3e) from which agmatine is derived is greater in must than in the finished wine.

Finally in the present study, it was also found that the lies contained substantial (i.e. greater than 5 mg/l) amounts of the target compounds, (data not shown as the lies were not analysed at all eight sites). This would possibly lead to higher levels of biogenic amines in wines which are produced with an extended lees contact.

4. Conclusion

The results suggest that the major pathway of FPA biosynthesis in grapes is via arginine and agmatine leading to putrescine, spermine and spermidine, which occur naturally in small quantities in grapes at maturity. Ornithine, does not appear to contribute to the biosynthesis of free polyamines in the grapes and musts chosen for this study. It is identified in wines after the MLF, contributing, via microbial action, to the general increase in the concentration of putrescine. The concentrations of spermidine and spermine remain low in wines and with the exception of one example, similar to concentrations found in grapes.

These data on polyamine concentrations in grapes and their evolution in wines are reassuring as regards the quality of wines and consumers' health. However, it should be emphasized that the experiments were conducted on healthy grapes presenting no visible signs of fungal attack other microbiological alteration. Since it is clear that certain microorganisms can sometimes significantly alter the concentrations in polyamines during winemaking, it would apposite to determine the concentration of these compounds in grapes that have been attacked by certain microorganisms before, during and after winemaking.

References

Adams, D. O., Franke, K. E., & Christensen, L. P. (1990). Elevated putrescine levels in grapevine leaves that display symptoms of potassium deficiency. *American Journal of Enology and Viticulture, 41*, 121–125.

Adams, D. O., Bates, D. J., Adams, D. F., & Franke, K. E. (1992). The effect of agmatine and other precursors on the accumulation of putrescine in grape leaves. *American Journal of Enology and Viticulture, 43*, 239–243.

Aerny, J (1990). Présence d'histamine et d'autres amines biogènes dans les vins. In *Feuillets Verts*; OIV Ed., Paris; France, 881.

Anli, R. E., Vural, N., Yilmaz, S., & Vural, H. (2004). The determination of biogenic amines in Turkish red wines. *Journal of Food Composition and Analysis, 17*(1), 53–62.

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

Aziz, A. (2003). Spermidine and related metabolic inhibitors modulate sugar and amino acid levels in *Vitis vinifera* L.: possible relationships with initial fruitlet abscission. *Journal of Experimental Botany, 54*(381), 355–363.

Baggiolini, M. (1952). Les stades repères dans le developpement annuel de la vigne et leur utilisation pratique. *Rev Romande Agric Vitic Arbor*(8), 4–6.

Bardócz, S. (1995). Polyamines in food and their consequences for food quality and human health. *Trends in Food Science and Technology, 6*(10), 341–346.

Bauza, T., Blaise, A., Daumas, F., & Cabanis, J. C. (1995). Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône, by HPLC with precolumn derivatization and fluorimetric detection. *Journal of Chromatography A*(707), 373–379.

Bauza, T., Kanny, G., Blaise, A., Daumas, F., Moneret Vautrin, D. A., & Cabanis, J. C. (1995). Les amines biogènes des vins; métabolisme et toxicité. *Bulletin OIV*(767–768), 42–67.

Bellagamba, F., Moretti, V. M., Mentasti, T., Albertini, A., Luzzana, U., & Valfrè, F. (1997). High-performance liquid chromatographic determination of polyamines in milk as their 9-fluorenylmethoxycarbonyl derivatives using a column-switching technique. *Journal of Chromatography A, 791*(1–2), 79–84.

Bertrand, A., Ingargiola, M. C., & Delas, J. (1991). Incidence de la fumure azotée de la vigne et du greffage sur la composition des vins de merlot, en particulier sur la présence du carbamate d'éthyle et des amines biogènes. *Revue Francaise d'Oenologie, 132*, 7–13.

Biasi, R., Bagni, N., & Costa, G. (1988). Endogenous polyamines in apple and their relationship to fruit set and fruit growth. *Physiology of Plants, 73*, 201–205.

Bover-Cid, S., Iquierdo-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*(1), 43–47.

Broquedis, M., Bouard, J. (1990). Les polyamines et leurs relations avec l'acide abscissique au cours du développement de la baie de raisin. In Dunod (Ed.). *Actualités Œnologiques* 89; Paris; France, pp. 117–121.

Broquedis, M., Dumery, B., & Bouard, J. (1989). Mise en évidence de polyamines putrescine, cadavérine, nor-spermidine, spermidine et spermine dans les feuilles et les grappes de *vitis vinifera* L. *Connaissances Vigne et Vin, 23*, 1–6.

Cinquina, A. L., Calì, A., Longo, F., De Santis, L., Severoni, A., & Abballe, F. (2004). Determination of biogenic amines in fish tissues by ion-exchange chromatography with conductivity detection. *Journal of Chromatography A, 1032*(1–2), 73–77.

Coton, E., Rollan, G., Bertrand, A., & Lonvaud-Funel, A. (1998). Histamine-producing lactic acid bacteria in wines: Early detection, frequency, and distribution. *American Journal of Enology and Viticulture, 49*(2), 199–204.

Ekegren, T., & Gomes-Trolin, C. (2005). Determination of polyamines in human tissues by precolumn derivatization with 9-fluorenylmethyl chloroformate and high-performance liquid chromatography. *Analytical Biochemistry, 338*(2), 179–185.

Fernandes, J. O., & Ferreira, M. A. (2000). Combined ion-pair extraction and gas chromatography–mass spectrometry for the simultaneous determination of diamines, polyamines and aromatic amines in Port wine and grape juice. *Journal of Chromatography A, 998*(1–2), 235–241.

Flores, H. E., & Galston, A. W. (1982). Analysis of polyamines in higher plants by high performance liquid chromatography. *Plant Physiology, 69*, 701–706.

Geny, L., Broquedis, M., Martin-Tanguy, J., & Bouard, J. (1997). Free, conjugated and wall-bound polyamines in various organs of fruiting cuttings of *Vitis vinifera* L. cv. cabernet sauvignon. *American Journal of Enology of Viticulture, 48*, 80–84.

Gloria, 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.

Halász, A., Baráth, A., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology, 5*, 42–49.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O., & Alves, A. (2005). Free amino acids and biogenic amines in wines and musts from the

Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. *Journal of Food Engineering, 66*(3), 315–322.

Hotchkiss, J. H. (1989). Preformed *N*-nitroso compounds in foods and beverages. *Cancer Surveys, 8*(2), 295–321.

Ingargiola, M. C., Bertrand, A. (1992). Origine des amines biogènes dans les vins. In *Feuillets Verts*; OIV Ed., Paris; France, p. 896.

Joosten, H. M. (1987). The biogenic amine content of Dutch cheese and their toxicological significance. *Netherlands Milk Dairy Journal, 42*, 25–42.

Lespy-Labaylette, P., Broquedis, M., Soyer, J. P., & Bouard, J. (1994). Influence d'une carence en potassium sur les polyamines libres des feuilles de vigne et des baies de raisin. *Journal International de la Science de la Vigne et du Vin, 28*, 383–388.

Leitão, M. C., Marques, A. P., & San Romão, M. V. (2005). A survey of biogenic amines in commercial Portuguese wines. *Food Control, 16*(3), 199–204.

Lonvaud-Funel, A. (2001). Biogenic amines in wines: role of lactic acid bacteria. *FEMS Microbiology Letters, 199*, 9–13.

Lozanov, V., Petrov, S., & Mitev, V. (2004). Simultaneous analysis of amino acid and biogenic polyamines by high-performance liquid chromatography after pre-column derivatization with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide. *Journal of Chromatography A, 1025*(2), 201–208.

Moreno-Arribas, M. V., Polo, M. C., Jorganes, F., & Muñoz, R. (2003). Screening of biogenic amine production by lactic acid bacteria isolated from grape must and wine. *International Journal of Food Microbiology, 84*(1), 117–123.

Paschalidis, K. A., Aziz, A., Geny, L., Primikirios, N. I., & Roubelakis-Angelakis, K. A. (2001). Polyamines in grapevine. In K. A. Roubelakis-Angelakis (Ed.), *Molecular biology and biotechnology of the grapevine* (pp. 109–151). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Ponappa, T., & Miller, A. R. (1996). Polyamines in normal and auxin-induced strawberry fruit development. *Physiologica Plantarum, 98*, 447–454.

Ruiz, S., & Moyno, S. (1998). Bunchstem necrosis in grapes and its relationship to elevated putrescine levels and low potassium content. *Australian and New Zealand Wine Industry Journal, 13*, 319–324.

Seiler, N. (1990). Polyamines metabolism. *Digestion, 46*, 319–330.

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*(1), 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., Barrios, M. L., & Bertrand, A. (1998). Correlation between the content of biogenic amines and other wine compounds. *American Journal of Enology and Viticulture, 49*, 266–278.

Tabor, H., & Tabor, C. W. (1964). Spermidine, spermine and related amines. *Pharmacology Reviews, 16*, 245–300.

Tabor, C. W., & Tabor, H. (1984). Polyamines. *Annual Review of Biochemistry, 53*, 749–790.

Taylor, C. W., Hui, J. Y., & Lyons, D. E. (1984). Toxicology of scombroïd poisoning. In E. P. Ragelis (Ed.), *Seafood toxins* (pp. 417–420). Washington, USA: American Chemical Society.

Til, H. P., Falke, H. E., Prinsen, M. K., & Willems, M. I. (1997). Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadavérine in rats. *Food and Chemical Toxicology, 35*, 337–348.

Veciana-Nogués, M. T., Mariné-Font, A., & Vidal-Carou, M. C. (1997). Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds, volatile amines, and organoleptic changes. *Journal of Agricultural and Food Chemistry, 45*, 2036–2041.

Vidal-Carou, M. C., Isla Gavin, M. J., Marine Font, A., & Codony Salcedo, R. (1989). Histamine and tyramine in natural sparkling wine, vermouth, cider and vinegar. *Journal of Food Composition and Analysis, 2*, 210–218.

Vidal-Carou, M. C., Izquierdo-Pulido, M. L., Martin, M. C., & Mariné Font, A. (1990). Histamina y tiramina en derivados carnicos. *Revista de Agroquimica Y Tecnologia de Alimentos, 30*, 102–108.

Vidal-Carou, M. C., Lahoz-Portolés, F., Bover-Cid, S., & Mariné-Font, A. (2003). Ion-pair high-performance liquid chromatographic determination of biogenic amines and polyamines in wine and other alcoholic beverages. *Journal of Chromatography A*(1–2), 235–241.