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Technological and Toxicological Significance of Bioactive Amines in Grapes and Wines

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ABSTRACT

Bioactive amines in grapes and wines are important from both technological and toxicological points of view. Polyamines are essential for optimum grape productivity and quality. Biogenic amines can, at high amounts, cause undesirable physiological effects in sensitive individuals. They can also have a negative effect on wine flavor and aroma. Furthermore, they can be used as an index of hygienic-sanitary conditions during production. One aspiration of the wine industry is to reduce the risk of biogenic amines. Updated information is provided on the biochemical and physiological aspects of bioactive amines, their formation and roles in grape production and winemaking, as well as ways to prevent their formation and accumulation in wines.

Keywords: alcoholic fermentation, biogenic amines, grape production, hygienic conditions, malolactic fermentation, polyamines, winemaking

Abbreviations: AGM, agmatine; **CAD**, cadaverine; **DAO**, diaminoxidases; **HIM**, histamine; **LAB**, lactic acid bacteria; **MAO**, monoaminoxidases; **MAOI**, monoaminoxidase inhibitor; **nd**, not detected; **PAO**, polyaminoxidases; **PHM**, phenylethylamine; **PUT**, putrescine; **SPD**, spermidine; **SPN**, spermine; **TRM**, tryptamine; **TYM**, tyramine

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INTRODUCTION

Amines are among the major factors determining the quality of wine and other fermented beverages. Several types of amines have been detected in wines, among them the volatile aliphatic and the bioactive amines. The volatile amines are widespread in plants, and play important roles in flavor and aroma. The most widely reported in wines are butyl-

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Invited Review

Table 1 Types and levels of free bioactive amine in wines.

Wine	Range or mean amine levels (mg/L) [1]									
Reference	**SPD**	**SPN**	**AGM**	**PUT**	**CAD**	**HIM**	**TYM**	**TRM**	**PHM**	**SRT**
Bordeaux										
Zee *et al.* 1983	---	---	---	4.03	0.88	4.91	7.31	---	---	---
Cabernet Franc										
Souza *et al.* 2005	0.07-0.30	nd	nd	0.77-1.43	nd	nd-1.37	0.30-0.83	nd	0.17-0.50	0.20-0.50
Cabernet Monreale										
mo Dugo *et al.* 2006	0.2	0.2	---	0.4	0.5	0.1	0.1	0.2	0.2	---
Cabernet Sauvignon										
Glória *et al.* 1998	nd-4.03	nd-1.17	nd-1.61	3.15-23.6	nd-1.51	nd-10.10	nd-7.53	nd	nd-0.14	nd-0.49
Souza *et al.* 2005	0.10-1.63	nd	nd	1.27-4.33	nd	0.23-1.73	0.40-1.07	nd	0.20-1.37	0.23-0.60
mo Dugo *et al.* 2006	0.1	0.1	---	0.2	0.4	nd	0.4	0.3	0.1	---
Soufleros *et al.* 2007	0.02	0.00	---	0.14	0.15	0.12	0.03	---	---	---
Yildirim *et al.* 2007	---	---	nd	4.38	0.49	1.25	nd	nd	nd	---
Merlot										
Souza *et al.* 2005	0.03-0.23	nd	nd	0.97-1.10	nd	0.07-1.67	0.33 - 0.50	nd	0.20-0.50	0.20-1.13
Yildirim *et al.* 2007	---	---	nd	4.54	nd	1.78	1.42	nd	nd	---
mo Dugo *et al.* 2006	0.1	0.2	---	0.3	0.2	nd	0.2	0.1	0.2	---
Soufleros *et al.* 2007	0.06	0.10	---	0.75	0.12	0.51	0.07	---	---	---
Petit Verdot										
mo Dugo *et al.* 2006	nd	nd	---	0.7	0.4	0.1	0.1	0.2	0.1	---
Pinot noir										
Glória *et al.* 1998	nd-2.35	nd-2.38	nd-8.37	2.43-20.3	nd-2.07	nd-23.98	nd-8.31	nd-5.51	nd-0.89	nd-2.23
mo Dugo *et al.* 2006	0.1-0.2	0.1-0.2	---	0.3-0.5	0.4-0.5	0.4-0.8	0.2-0.3	0.1-0.3	0.5-0.6	---
Porto wines										
Zee *et al.* 1983	---	---	---	3.33	0.23	3.48	2.17	---	---	---
Rioja										
Millán *et al.* 2007	0.08-1.10	0.09-0.19	---	0.06-13.0	0.07-0.68	0.40-8.22	0.03-3.20	0.04-0.98	0.09-4.02	---
Syrah										
mo Dugo *et al.* 2006	nd	nd	---	0.4	0.3	0.2	0.1	0.1	0.1	---
Soufleros *et al.* 2007	0.62	0.36	---	2.06	0.78	0.61	0.76	---	---	---
Tannat										
mo Dugo *et al.* 2006	0.1	0.1	---	0.2	0.2	0.1	0.3	0.1	0.2	---
Tempranillo										
Hernández-Orte *et al.* 2006	---	---	---	nd-55.0	nd-14.0	nd-25.0	nd-19.0	---	16.3	---
mo Dugo *et al.* 2006	nd	nd	---	0.1	0.2	nd	nd	nd	0.1	---

[1] nd - not detected; --- - not determined. SPD - spermidine, SPN - spermine, AGM - agmatine, PUT - putrescine, CAD - cadaverine, HIM - histamine, TYM - tyramine, TRM - tryptamine, PHM - phenylethylamine, SRT - serotonin.

amine, 1,3-diaminopropane, diethylamine, ethanolamine, ethylamine, hexylamine, methylamine, pentylamine, and propylamine (Radler and Fath 1991; Sass-Kiss *et al*. 2000; Caruso *et al*. 2002; González-Marco and Ancín-Azpilicueta 2006a; Hernández-Borges *et al*. 2007; Soufleros *et al*. 2007).

This review will be focused on the bioactive amines. Several of them have been found in wines: spermidine, spermine, agmatine, putrescine, cadaverine, histamine, tyramine, phenylethylamine, tryptamine, serotonin, dopamine and octopamine. In **Table 1** there is a compilation of data on the types and levels of bioactive amines in wines.

Histamine was first detected in wines in 1954, and the first report on the levels in wines was available in 1965. In the 1980's the main interest was on the presence and levels of histamine, tyramine, putrescine and cadaverine, due to the toxicological and technological aspects associated with these compounds (Zee *et al*. 1983; Broquedis *et al*. 1989; Vidal-Carou *et al*. 1989a, 1989b, 1990a, 1990b). From a technological point of view, high levels of these amines were related to a low quality product or to a defective processing. The toxicological interest was based on the development of direct toxicological problems such as 'histamine poisoning' and headache, caused by histamine and tyramine, respectively, and the potentiating effect of putrescine and cadaverine.

Recently, other amines have begun to attract renewed attention, among them, spermidine, spermine, tryptamine, phenylethylamine (mo Dugo *et al*. 2006; Millán *et al*. 2007; Soufleros *et al*. 2007), agmatine and serotonin (Glória *et al*. 1998; Souza *et al*. 2005; Yildirim *et al*. 2007; Manfroi *et al*. 2008). The polyamines spermidine and spermine have been associated with plant morphogenesis. They play important roles as growth regulators and are also implicated in plant response to environmental challenges (Geny *et al*. 1997; Bouchereau *et al*. 1999; Darrieumerlou *et al*. 2001; Kuznetsov and Shevyakova 2007; Pang *et al*. 2007). Agmatine can be a precursor of polyamines. Serotonin might be associated with plant defense mechanisms. Tryptamine and phenylethylamine can cause vasoconstriction and headache, as reported for tyramine (Coutts *et al*. 1986; Glória 2005).

According to **Table 1**, histamine, tyramine, putrescine and cadaverine were investigated in 100% of the studies; spermidine, spermine, tryptamine and phenylethylamine in 73%; agmatine in 32%, and serotonin in 23% of the studies. The levels and types of amines present in wines varied widely among wine types and also among samples of the same type.

Some amines, namely spermidine and putrescine, are normal constituents of grapes with amounts varying with grape variety, degree of maturation as well as soil type and composition, cultivation practices and climatic conditions. During wine making, several amines can be formed and accumulate. Several factors can affect amine levels in wines including precursor free amino acids, must treatment, contact time of must and skin, initial microbial population on the fruit, alcohol content, sulfur dioxide, added nutrients, pH, temperature, quantity and type of finings, and microbial contamination in wineries (Glória *et al*. 1998; Garcia-Villar *et al*. 2007).

Some wines are reported to contain higher levels of amines, among them red and botrytized wines. Overall, red wines have shown higher amine content, especially histamine, tyramine and putrescine, than rosé and white wines, in which malolactic fermentation does not take place or occurs to a lesser extent, as determined by several studies (Ough 1971; Cilliers and van Wyk 1985; Vidal-Carou *et al*.

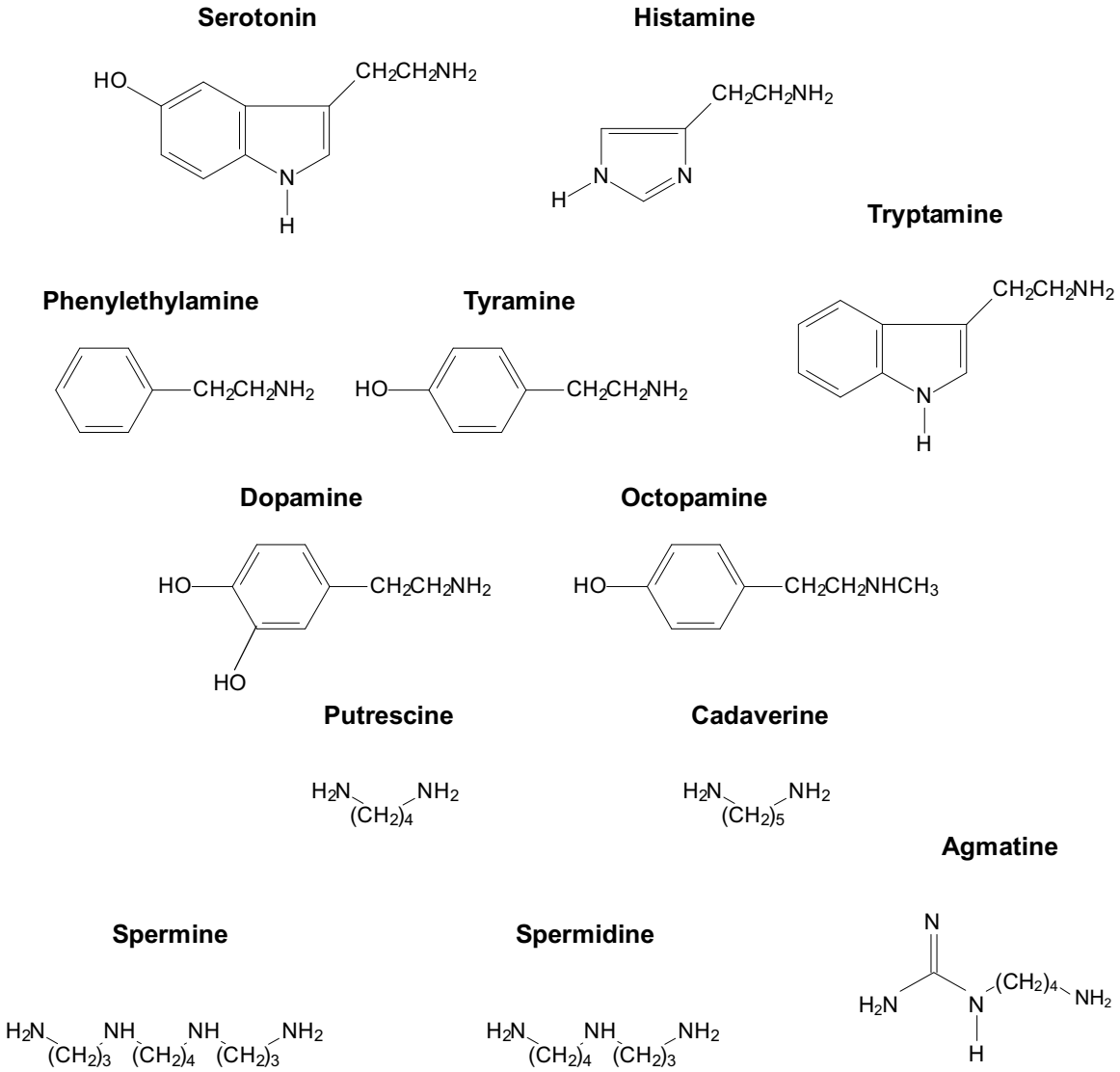

Fig. 1 Chemical structure of some bioactive amines.

1990a; Bauza *et al*. 1995; Lehtonen 1996; Fernandes and Ferreira 2000; Romero *et al*. 2002; Leitão *et al*. 2005; Bover-Cid *et al*. 2006; Hernández-Orte *et al*. 2006; Garcia-Villar *et al*. 2007; Hernández-Borges *et al*. 2007; Soufleros *et al*. 2007; Yildirim *et al*. 2007). Botrytized wines, such as Tokaj Aszú wines are produced from grapes grown in the Tokaj region of Hungary, which are infected with noble rot *Botrytis cinerea*. The amine composition and content of Aszú grapes were significantly different from those of intact grapes as well as from gray and green rots. The composition and concentration of bioactive amines in wines can support the authenticity of Tokaj Aszú-wines (Hajós *et al*. 2000; Geny *et al*. 2003; Kiss *et al*. 2006).

The presence of large amounts of some bioactive amines is an important food safety problem because of the implication of these compounds in food intolerance and intoxication (Ferreira and Pinho 2006). High levels of amines can indicate poor hygienic conditions during processing. Furthermore, amines can be significant in terms of aroma and flavor (González-Marco and Ancín-Azpilicueta 2006a; Garcia-Villar *et al*. 2007). In general, a weakening of the flavor impression is attributed to amines, whereby an unpleasant, bitter aftertaste has been noted and described as off-taste in wine with high amine levels (Glória 2005).

An overview of bioactive amines biochemistry will be provided, as well as the factors which may affect their formation and build up in wines and their metabolism and toxicological significance to human health.

BIOACTIVE AMINES

Definition, classification and physiological importance

Bioactive amines are aliphatic, aromatic or heterocyclic organic bases of low molecular weight (**Fig. 1**). The bioactive amines are produced through the normal metabolism of animals, plants and microorganisms, and participate in important metabolic and physiological functions in living organisms. Most of the amines have been named after their precursor amino acids, e.g. histamine originates from histidine, tyramine from tyrosine, tryptamine from tryptophan, and so on. However, the names cadaverine and putrescine are associated with decomposition and putrefaction, whereas spermine and spermidine with seminal fluids where they were found for the first time.

Bioactive amines can be classified on the basis of the number of amine groups, chemical structure, biosynthesis or physiological functions. According to the later, amines are classified as polyamines and biogenic amines. Polyamines play an important role in growth while biogenic amines are neuro- or vaso-active (Bardócz 1995). This is the most widely used classification.

A summary of the physiological importance of bioactive amines is described in **Table 2**. The polyamines spermine and spermidine are indispensable components of all living cells. Polyamines have various electrostatic interactions with macromolecules, especially DNA, RNA and proteins,

Table 2 Metabolic and physiological functions of bioactive amines.

Bioactive amines	Functions
Spermidine Spermine	- Regulation and stimulation of DNA, RNA and protein synthesis - Stimulation of cell differentiation - Permeability and stability of cell membranes - Free radical scavenger - Maintenance of the high metabolic activity of the normally functioning and healthy gut - Reduce mucosal permeability to macromolecules and prevent food allergy - Physiological processes in higher plants root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development; response to abiotic stress, synthesis of secondary metabolites, senescence, plant responses to pathogens
Putrescine Cadaverine	- Free radical scavenger
Histamine	- Strong capillary dilator - Hypotensive effects - Psychoactive - Protective effect in deterring predators
Serotonin	- Vaso and bronchoconstrictor - Neurotransmitter - Protective effect in deterring predators
Tyramine	- Pressor amines
Tryptamine Phenylethylamine	- Precursor of compounds with biological significance
Conjugated amines (cinnamic acids)	- Antifungal and antiviral agents - Plant growth and developmental processes - Plant responses to pathogens

Source: Drolet *et al.* 1986 ; Bardócz 1995 ; Shiozaki *et al.* 2000; Glória 2005.

and are involved in the regulation and stimulation of their synthesis. Polyamines stimulate cell differentiation, interacting and modulating various intracellular messenger systems. They are important in the permeability and stability of cellular membranes and reduce mucosal permeability to macromolecules and allergenic proteins, preventing food allergies (Drolet *et al*. 1986; Bardócz 1995; Löser 2000). According to Drolet *et al*. (1986) and to Bardócz (1995), spermine and spermidine, as well as the diamines putrescine and cadaverine are efficient free radical scavengers in a number of chemical and *in vitro* enzyme systems. They could inhibit lipid peroxidation and prevent senescence.

In higher plants, the polyamines are involved in several physiological processes including morphogenesis, rooting, flowering and senescence (Shiozaki *et al*. 2000). Polyamines can be used as organic nitrogen sources and can play a critical role in several processes, among them, root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development and response to abiotic stress, such as potassium deficiency, osmotic shock, drought and pathogen infection (Kuznetsov and Shevyakova 2007; Pang *et al*. 2007). They are also important in the synthesis of secondary metabolites of biological interest, for example, nicotine and alkaloids (Flores *et al*. 1989; Walters 2003). Polyamines are associated with cell walls and membranes. They modulate pectin esterase and bind to pectin, delaying fruit softening and senescence (Leiting and Wicker 1997). The firming effect of the polyamines is similar to that of calcium chloride, and may be due to its ability to bind to cell walls and membranes, stabilizing them, or by making cell walls less accessible to wall-softening enzymes (Bouchereau *et al*. 1999).

Polyamines can occur in three different forms: free, bound electrostatically to negatively charged molecules, or conjugated to small molecules, such as cinnamic acids, e.g. *p*-coumaric, ferulic and caffeic acids. These compounds have been implicated in a variety of plant growth and developmental processes and in plant responses to pathogens and stress (Bouchereau *et al*. 1999; Walters 2003).

Biogenic amines are either neuro- or vasoactive. Neuroactive amines, such as histamine and serotonin, affect the nervous system by acting on neural transmitters in the central nervous system. Vasoactive amines act directly or indirectly on the vascular system. Pressor amines – tyramine, tryptamine and phenylethylamine – cause a rise in blood pressure by constricting the vascular system and increasing the heart rate and force of contraction of the heart (Shalaby 1996). Histamine is a strong capillary dilator and can produce hypotensive effects. It also mediates primary and immediate symptoms in allergic responses (Taylor 1986; Shalaby 1996). Serotonin is vaso- and bronchoconstrictor. It is involved in the regulation of a number of important functions, including sleep, thirst, hunger, mood and sexual activity (Coutts *et al*. 1986; Glória 2005).

Some biogenic amines may have a protective role in deterring predators. Serotonin and histamine are some of the active principles that occur in the stinging hair of plants. Some amine conjugates are important as antifungal and antiviral agents. Some amines are quite important as precursors of compounds of biological significance. For instance, the plant hormones indol-3-yl-acetic acid and phenyl acetic acid are derived from tryptamine and phenylethylamine, respectively. The tryptamines are also precursors of beta-carboline alkaloids (Coutts *et al*. 1986).

Synthesis, metabolism and toxicological significance

The synthesis of the biogenic amines histamine, tyramine, tryptamine, phenylethylamine and cadaverine occur through decarboxylation of the precursor amino acids histidine, tyrosine, tryptophan, phenylalanine and lysine, respectively (**Fig. 2**). In the synthesis of serotonin, tryptophan is transformed by tryptophan hydrolase in 5-hydroxytryptophan, which is decarboxylated by aromatic amino acid decarboxylase in 5-hydroxytryptamine or serotonin. Tyrosine is the precursor of phenolic amines such as octopamine and dopamine (Glória 2005).

The decarboxylation of amino acids can be a result of high temperatures or microbial enzymes. Decarboxylase-positive microorganisms may constitute part of the associated population or may be introduced by contamination before, during and after processing. Prerequisites for the formation of amines are the availability of free amino acids, high processing temperatures, or the presence of decarboxylase-positive microorganisms and favorable conditions for microbial growth and decarboxylase activity. Free amino acids occur as such in foods, but may also be released from proteins as a result of proteolytic activity or thermal degradation (Halász *et al*. 1994; Glória 2005).

The production of amines by bacteria is affected by pH, temperature, oxygen tension, presence of vitamins and cofactors, availability of free amino acids and of fermentable sugars. In pH values of 2.5 to 6.5, the production of amines by the bacteria is stimulated as a protection against the acidic environment (Lucas *et al*. 2003; González-Marco and Ancín-Azpilicueta 2006b). The activity of decarboxylases depends on the microorganism's growth phase, being higher at the stationary phase. With regard to the temperature, decarboxylases are more active at temperatures lower than 30°C and without action above 40°C. However, at temperatures between 0 and 10°C, the activity will depend on the microorganisms present (Halász *et al*. 1994).

Polyamine synthesis is a more complex process, although the first few steps also include decarboxylation reactions (**Fig. 2**). In plants and some microorganisms, the first step is decarboxylation of ornithine to putrescine by ornithine decarboxylase. Alternative pathways exist to produce putrescine from arginine via agmatine by arginine decarboxylase and from citrulline. Putrescine is an obligate intermediate in polyamine synthesis (Flores *et al*. 1989; Bardócz 1995; Walters 2003; Glória 2005).

Spermidine and spermine are formed by the addition of an aminopropyl moiety to putrescine and spermidine, respectively. The aminopropyl group is derived from methio-

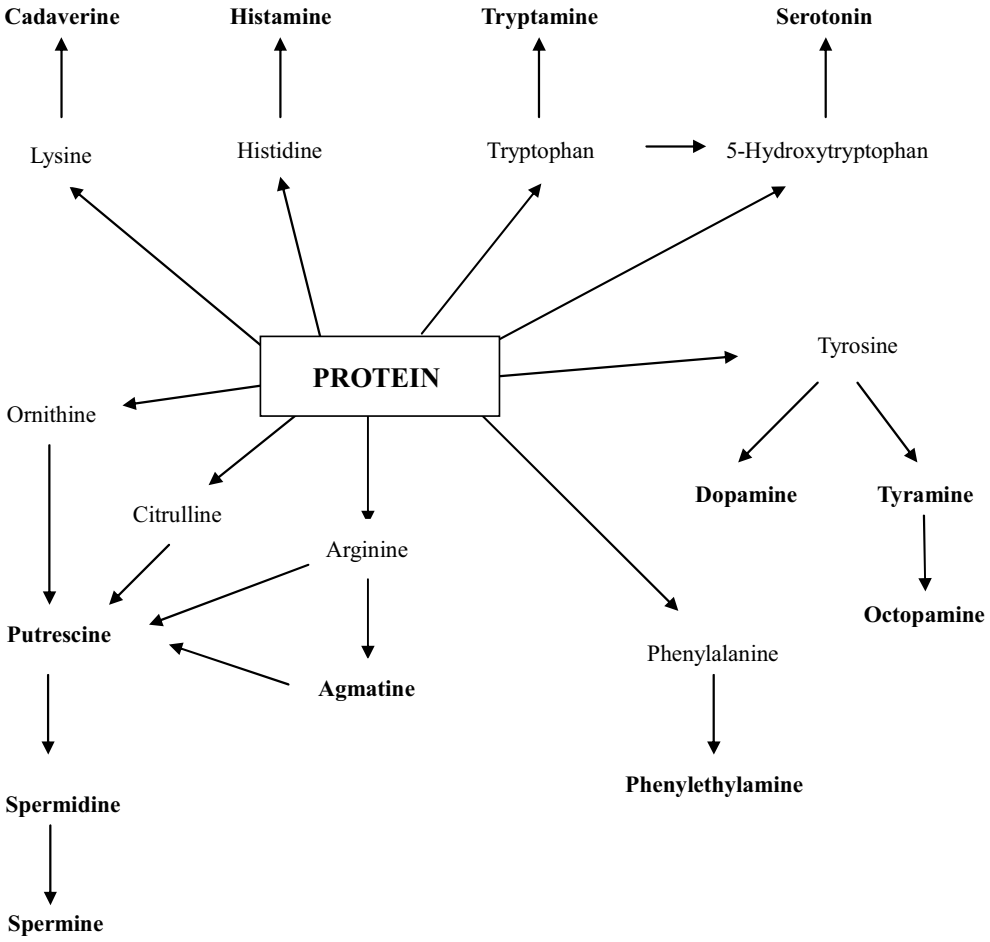

Fig. 2 Metabolic pathways for the formation of bioactive amines.

nine via *S*-adenosyl-L-methionine. The resulting 5′-methylthioadenosine is converted in methylthioribose and in methionine, recycling the $–SCH_3$ group which warrants the synthesis of polyamines (Flores *et al*. 1989: Walters 2003).

Health individuals can metabolize amines present in foods by acetylation and oxidation. Biogenic amines are oxidized by monoaminoxidases (MAO, EC 1.4.3.4) and diaminoxidases (DAO, EC 1.4.3.6). Polyamines are usually acetylated first, and then oxidized by polyaminoxidases (PAO, EC 1.5.3.11) (Glória 2005).

Low levels of biogenic amines in wines and foods do not usually represent any health hazard to individuals unless excessive amounts are ingested or the natural mechanism for their catabolism is genetically deficient or impaired by diseases or pharmacological agents. Individuals with respiratory and coronary problems, hypertension, vitamin B_{12} deficiency, and gastrointestinal problems (gastritis, irritable bowel syndrome, Crohn's disease, stomach and colonic ulcers) are at risk since the activity of aminoxidases in their intestines is usually lower than in healthy individuals. Patients taking medications that are inhibitors of MAO, DAO and PAO can also be affected, as such drugs prevent amines catabolism. These MAO and DAO inhibitors are used for the treatment of stress, depression, Alzheimer's and Parkinson's diseases, pulmonary tuberculosis, malaria, panic syndrome and social phobia (Fuzikawa *et al*. 1999; Glória 2005).

At high levels, biogenic amines have been implicated in a number of food poisoning episodes (**Table 3**), particularly histamine and tyramine toxicity. Histamine intoxication manifests several minutes to a few hours after ingestion of the histamine containing food. At first, a flushing of the face and neck is observed, accompanied by a feeling of heat and general discomfort. Often it is followed by intense throbbing headache. Other symptoms may be cardiac palpitations, dizziness and faintness, thirst, swelling of the lips, urticaria, rapid and weak pulse and gastrointestinal complaints. However, the most common symptoms are rash, diarrhea, sweating and headache. In severe cases, bronchospasms, suffocation and severe respiratory distress are reported. Recovery is usually complete within 8 hours (Taylor 1986; Shalaby 1996; Glória 2005; Landete *et al*. 2007a).

Tyramine is the second type of amine involved in intoxication. When foods containing high tyramine levels are ingested, large amounts of non-metabolized tyramine can reach the blood stream. This causes release of noradrenalin from the sympathetic nervous system, leading to a variety of physiological reactions (**Table 3**). There is an increase in blood pressure by peripheral vasoconstriction and by increasing the cardiac output (Shalaby 1996). Tyramine can also dilate the pupils and the palpebral tissue, cause lacrimation, salivation, fever, vomit, headache and increase respiration and blood sugar (Fuzikawa *et al*. 1999). When consuming tyramine rich foods, about 30% of the individuals with classical migraine, can have headaches (Coutts *et al*. 1986).

Ingestion of foods rich in tyramine by individuals under MAO inhibitor (MAOI) drugs results in hypertensive crisis. Several cases have been reported with cheese and alcoholic beverages (Fuzikawa *et al*. 1999). The attacks last from 10 minutes to 6 hours, during which hypertension and headache fluctuate. There can be visual alterations, nausea, vomit, muscle contraction, mental confusion or excitation. Chest pain simulating angina pectoris, acute heart failure, pulmonary edema, neuronal sequel, and cerebral hemorrhage have also been described. Fatal incidents have been reported in the literature (Shalaby 1996).

Tryptamine has pharmacological action similar to tyramine. High levels can exert direct effect on smooth muscles, cause headache and increase blood pressure by constriction of the vascular system. Phenylethylamine, like tyramine, causes an increase in blood pressure by liberating noradrenalin from tissue stores. It may be the precipitant of migraine headache. Putrescine and cadaverine have less pharmacological activity than the aromatic amines; however, they can potentiate the toxic effect of histamine, tyramine

Table 3 Toxic effects of bioactive amines.

Toxic effects	Amines involved	Symptoms
Histamine intoxication	Histamine (toxic effect is potentiated by putrescine, cadaverine, spermine, tryptamine, tyramine, phenylethylamine and by ethanol)	**Gastrointestinal:** nausea, vomiting, diarrhea, abdominal cramps **Neurological:** throbbing headache, palpitation, face and neck flushing, burning throat, itching, rapid and weak pulse, dizziness, faintness, tingling **Hemodynamic:** hypotension, capillary dillatation **Cutaneous:** rash, urticaria, edema, localized inflamation **Severe cases:** bronchospasms, suffocation, severe respiratory distress
Tyramine intoxication	Tyramine	Headache, fever, increased blood pressure, vomiting, perspiration, pupils and palpebral tissue dilatation, salivation, lacrimation, increased respiration, palpitation, dyspnea
Cheese reaction or hypertensive crisis (associated with patients under MAOI[1] drugs)	Tyramine Phenylethylamine	Hypertensive crisis, severe headache, cerebral hemorrhage, neuronal sequel, cardiac failure, pulmonary edema, visual alterations, palpitation, nausea, sweat, vomit, muscle contractions, excitation, mental confusion, high blood pressure, fever, perspiration
Migraine	Tyramine Phenylethylamine Tryptamine Serotonin	Throbbing headache, migraine attack

[1] MAOI - monoaminoxidase inhibitor.
Source: Glória 2005.

and phenylethylamine as they deplete a part of detoxification capacity of MAO and DAO (Glória 2005).

The determination of the exact toxicity threshold of amines is a hard task as it is dependent on the efficiency of the detoxification mechanism of different individuals. Upper limits of 10 mg of histamine, 10 mg of tyramine and 3 mg of phenylethylamine in 100 g of foods have been suggested (Halász *et al*. 1994). However, ingestion of foods containing 6 mg of tyramine can cause migraine and 10 to 25 mg can cause hypertensive crisis in individuals taking MAOI drugs (Fuzikawa *et al*. 1999).

Health problems with wines have been related mainly to high levels of histamine, tyramine and phenylethylamine (Lehtonen 1996). A selective sensitivity to red wine has been shown in patients with migraine and tension type headache (Goldberg and Confino-Cohen 2005; Holzhammer and Wöber 2006).

Because the toxic effect of biogenic amines can also be potentiated by the presence of ethanol, acetaldehyde and other amines (Taylor 1986; Lehtonen 1996; Soufleros *et al*. 1998), histamine levels above 2 to 8 mg/L and tyramine above 8 mg/L may cause headache when large amounts of wine are consumed (Battaglia and Frolich 1978; Radler and Fath 1991; Lehtonen 1996). Therefore, the content of bioactive amines in wines may be regulated in the future following the implemented regulations by FDA for fish. Moreover, some countries have established limits for histamine in wines. Switzerland recommends 10 mg/L as maximum level, Germany 2 mg/L, Belgium 5 mg/L and France 8 mg/L (Lehtonen 1996).

SOURCES OF BIOACTIVE AMINES IN WINES

Different studies have been carried out to examine the levels of bioactive amines in wines throughout the world (Vidal-Carou *et al*. 1990a, 1990b; Bauza *et al*. 1995; Lehtonen 1996; Glória *et al*. 1998; Soufleros *et al*. 1998; Vazquez-Lasa *et al*. 1998; Mafra *et al*. 1999; Csomós *et al*. 2002; Romero *et al*. 2002; Anli *et al*. 2004; Souza *et al*. 2005; Marcobal *et al*. 2005; Bover-Cid *et al*. 2006; Herbert *et al*. 2006; Martín-Álvarez *et al*. 2006; Pramateftaki *et al*. 2006; Rupasinghe and Clegg 2007; Soufleros *et al*. 2007). In these different studies, there were varying profiles and levels of amines in the wines. The variability on amines levels could be explained on the basis of differences in raw material origin and quality, winemaking practices, possible microbial contamination during winery operations and time and storage conditions.

Bioactive amines in grapes

Bioactive amines are inherent to living organisms and are, therefore, present in grapes. They are synthesized in several parts of *Vitis vinifera*, including berries and leaves (Broquedis *et al*. 1989; Adams 1991; Miklós and Sarjala 2002; mo Dugo *et al*. 2006). Furthermore, they can be present at the free, bound and conjugated forms (Shiozaki *et al*. 2000).

Some amines are normal constituents of grapes. As indicated on **Table 4**, the polyamine spermidine is usually abundant in the pericarp followed by its obligate precursor putrescine. Other amines, e.g., spermine, agmatine, cadaverine, histamine, tyramine and phenylethylamine have also been found in minute contents (Ough 1971; Broquedis *et al*. 1989; Vidal-Carou *et al*. 1990a; Radler and Fath 1991; Hajós *et al*.

Table 4 Profile and levels of free bioactive amines in the pericarp of grapes.

Reference	Mean (± standard deviation) or range of amines levels (mg/kg)							
Grape variety/vintage	**SPD**	**SPN**	**AGM**	**PUT**	**CAD**	**HIM**	**TYM**	**PHM**
Bover-Cid *et al.* 2006								
Cabernet Sauvignon	4.66 ± 0.26	2.50 ± 0.23	---	6.81 ± 1.47	1.16 ± 0.37	---	---	---
Broquedis *et al.* 1989*								
Ugni blanc	0.35-0.43	0.02-0.04	---	0.16-0.21	0.04	---	---	---
Kiss *et al.* 2006**								
Furmint/2003	3.69-5.33	---	nd	2.47-4.94	0.37-0.55	0.10-0.22	nd	nd-0.15
Hárslevelü/2003	6.33-6.37	---	nd	3.16-5.45	0.22-1.02	0.19-0.34	nd	0.08-0.10
Furmint/2004	10.1-12.3	---	nd-0.10	4.36-7.24	0.32-1.33	0.46-0.49	nd	0.16-0.23
Hárslevelü/2004	8.46-9.19	---	nd	0.70-1.67	0.01-0.04	0.63-0.86	nd	0.11-0.14
Yellow Muscat/2004	8.22	---	0.08	2.44	0.10	0.55	nd	0.17
Zéta/2004	7.48	---	nd	2.00	0.45	0.54	nd	0.45
White grape/2004	9.29	---	nd	4.26	0.01	0.31	nd	0.11
Sass-Kiss *et al.* 2000								
Furmint/1998	35.4	---	nd	3.56	1.04	0.13	0.50	0.31
Hárslevelü/1998	26.3	---	nd	3.82	1.34	0.24	0.37	0.19
Muscat Ottonel/1998	30.3	---	nd	3.79	1.34	0.40	0.60	0.23

* nmol/kg ** Dry weight basis.
nd - not detected; --- - not determined. SPD - spermidine, SPN - spermine, AGM - agmatine, PUT - putrescine, CAD - cadaverine, HIM - histamine, TYM - tyramine, PHM - phenylethylamine. Tryptamine and serotonin were not determined.

2000; Sass-Kiss *et al*. 2000; Landete *et al*. 2005; Kiss *et al*. 2006). Grape seeds also contain spermidine, putrescine and cadaverine at high concentrations (Shiozaki *et al*. 2000; Kiss *et al*. 2006).

The types and amounts of bioactive amines in grapes can vary with the phase of development and ripening, variety, vintage, microbiota of the grape, soil type and composition, fertilization, and climatic conditions during growth.

Factors affecting amine formation and build up in grapes

Grape development and ripening stage

The influence of grape development and ripening stage on the levels of free, bound and conjugated amines in the pericarp and seeds of Muscat Bailey A grapes was investigated by Shiozaki *et al*. (2000). It was observed that free spermidine and putrescine levels were higher during the early development of the grape, decreasing afterwards, which may be associated with cell proliferation after anthesis in the pericarp. Putrescine showed an increase at 30 days after full bloom, followed by a decrease. The increase in the pericarp occurred at the same time as polyamine levels in the seed increased, suggesting that this amine can be excreted from the seed into the pericarp. Bound and conjugated polyamines were also found in the pericarp of grapes. The conjugated polyamines increased at 30 days after full bloom and quickly decreased. Bound polyamines exhibited changes similar to conjugated polyamines. Conjugated and bound putrescine slightly increased 50 days after full bloom. The levels of free, bound or conjugated spermine were low and the changes were negligible throughout the grape development.

Guo *et al*. (2007) observed that the polyamines in ovules of seedless grapes (*Vitis vinifera* L.) during embryo development and abortion were lower than those of seeded grapes in 40-60 days after anthesis. The ratios of '(spermidine+spermine)/putrescine' and 'spermine/polyamines' were closely related with grape embryo development, and low ratios were disadvantageous to embryo differentiation.

Grape variety

The grape variety has been observed to affect the levels and profile of amines in grapes and wines (Kiss *et al*. 2006; mo Dugo *et al*. 2006; Yildirim *et al*. 2007; Marques *et al.* 2008). Sass-Kiss *et al*. (2000) observed significant differences on the levels of spermidine, tyramine, histamine and phenylethylamine in three varieties of Hungarian grapes (Furmint, Hárslevelü and Muscat Ottonel) from the 1998 vintage (**Table 4**). Kiss *et al*. (2006) reported different levels of spermidine and histamine in two varieties of Hungarian grapes – Furmint and Hárslevelü – from the same vineyards. However, the levels varied widely for different vintages of the same variety. Yildirim *et al*. (2007) observed significant differences on histamine, tyramine and cadaverine levels among *Vitis vinifera* varieties grown in Turkey (**Table 1**). Mo Dugo *et al*. (2006) investigated the presence of amines in wines produced by the same vinification procedure using Sicilian and French viticultures. Therefore, the differences on amines types and levels in grapes and wines are not solely dependent on the grape variety but on cultivation area as well.

Table 5 Levels of free bioactive amines in intact, sun-dried, Aszú, gray rotten and green rotten grapes.

Grape	Amines levels (mg/kg) on a dry weight basis						
	SPD	**AGM**	**PUT**	**CAD**	**HIM**	**TYM**	**PHM**
2003 Vintage							
Intact							
Furmint	3.69-5.33	nd	2.47-4.94	0.37-0.55	0.10-0.22	nd	nd-0.15
Hárslevelü	6.33-6.37	nd	3.16-5.45	0.22-1.02	0.19-0.34	nd	0.08-0.10
Dried							
Furmint	5.37-6.46	0.23-1.10	6.69-9.42	0.52-0.55	0.30-0.57	nd	0.07-0.40
Hárslevelü	8.96	1.06	7.56	0.06	0.85	0.07	0.93
Aszú							
Furmint	8.49-11.0	0.32-1.12	2.42-7.67	0.84-1.16	0.14-0.30	0.16-0.98	7.33-9.28
Hárslevelü	10.5-11.5	1.34-1.46	5.72-7.94	0.35-1.35	0.33-0.42	0.11-0.53	8.40-8.47
2004 Vintage							
Intact							
Furmint	10.1-12.3	nd-0.10	4.36-7.24	0.32-1.33	0.46-0.49	nd	0.16-0.23
Hárslevelü	8.46-9.19	nd	0.70-1.67	0.01-0.04	0.63-0.86	nd	0.11-0.14
Yellow Muscat	8.22	0.08	2.44	0.10	0.55	nd	0.17
Zéta	7.48	nd	2.00	0.45	0.54	nd	0.45
White grape	9.29	nd	4.26	0.01	0.31	nd	0.11
Dried							
Furmint	7.96	nd	6.15	0.74	0.42	nd	0.11
Hárslevelü	7.15	0.07	4.50	nd	0.88	0.34	0.20
Yellow Muscat	7.60	0.08	5.48	0.06	0.65	0.42	0.15
Aszú							
Furmint	22.1-32.1	2.71-4.93	2.94-14.3	0.39-0.46	0.30-0.40	0.42-0.87	2.87-12.4
Hárslevelü	25.0-29.8	2.61-3.07	1.67-7.53	0.07	0.34-0.43	0.47-0.49	2.46-8.84
Yellow Muscat	26.3	3.33	15.3	0.61	0.31	0.64	9.70
Zéta	20.3	1.08	1.52	0.36	0.28	0.38	1.76
White grape	10.6	3.03	4.25	0.09	0.37	0.46	4.46
Gray rotten							
Furmint	8.33	0.72	7.74	1.57	0.34	0.22	8.73
Hárslevelü	8.39	0.44	6.45	0.05	0.60	0.32	5.14
Yellow Muscat	7.25	0.33	6.76	0.79	0.44	nd	4.62
Green rotten							
Furmint	15.8	4.77	9.64	1.56	0.57	0.71	8.54
Hárslevelü	17.5	4.00	9.21	0.13	0.62	0.71	5.16
Zéta	11.2	2.19	7.22	0.51	0.31	0.38	3.20

[1] nd - not detected. SPD - spermidine, AGM - agmatine, PUT - putrescine, CAD - cadaverine, HIM - histamine, TYM - tyramine, PHM - phenylethylamine.
Source: Kiss *et al.* 2006.

Microbiota of the grape

The microbiota of the grape is another factor which may affect amine profile in grapes. Kiss *et al*. (2006) investigated the microbial population and distribution of molds of different grape varieties. Various morphologically different colonies were detected and microscopic investigations revealed that they were *Penicilium* spp. and *Botrytis* spp. Similar mold species were found by Bene and Magyar (2004), who also identified *Aspergillus* spp. in botrytized grapes in the Tokaj wine region.

Intact grapes contained less amines compared to those infected with molds. Intact grapes contained mainly spermidine and putrescine, with very low levels (if any) of other amines (**Table 5**). Raisin-like grapes infected mainly with *Botrytis cinerea* - Aszú grapes - showed a different profile of amines. Further amines appeared, such as tyramine and agmatine and the concentrations of spermidine and phenylethylamine increased as compared to intact grapes (Kiss *et al*. 2006). Sass-Kiss *et al*. (2000) found similar results when comparing intact with Aszú grapes. On grapes infected mainly with *Penicillium* spp., the so called green rotten grapes, the most prevalent amines were spermidine, putrescine, agmatine and phenylethylamine. In gray rotten grapes, spermidine, putrescine and phenylethylamine were the prevalent amines, followed by agmatine. The total amine content of intact and dried grapes did not reach 21 mg/kg, whereas those of Aszú, rotten grapes and grapes with green molds usually ranged from 26 to 69 mg/kg. These results indicate that the microbiota living in/on the berries has a great effect on the composition and concentration of amines. Furthermore, based on PCA studies, the different groups of grapes could be distinguished. Therefore, the study of amines could be used to determine the authenticity of Aszú wines.

Vintage

Some studies indicated that the vintage could affect significantly the levels of amines in grapes and wines (Héberger *et al*. 2003; Herbert *et al*. 2006; Kiss *et al*. 2006; Martín-Álvarez *et al*. 2006). According to Kiss *et al*. (2006), the levels of amines varied widely in intact Furmint grapes with higher levels of every amine in 2004, compared to 2003 (**Table 5**). However, for Hárslevelü grapes, higher spermidine and histamine and lower putrescine and cadaverine were detected in grapes from 2004 compared to 2003 vintage. The differences observed in the grapes were not observed in the botrytized grapes, confirming that the microbiota living in/on the grape played a major role on the final amine profile of the grapes.

Martín-Álvarez *et al*. (2006) compared wines from different vintages and observed significantly higher levels of histamine, tyramine, putrescine and phenylethylamine in wines from 2001 compared to 2002. The results were explained by the higher concentration of the precursor amino acids observed in 2001. Furthermore, the 2001 wines had higher pH, and greater complexity of the bacterial microflora. Based on these results, the authors concluded that the diversity of the wine microorganisms selected each year could also play a role.

Degree of irrigation

The influence of water stress on amine profile in plants has been described in the literature (Coelho *et al*. 2005). However, Bover-Cid *et al*. (2006) did not find significant influence of the degree of irrigation on the levels of amines in Cabernet Sauvignon grape and its evolution during wine making. The grapes were subjected to four different degrees of water stress, measured as the percentage of potential evapotranspiration (pET): no stress, weak stress, moderate stress and maximum stress (80, 65, 35 and 0% pET, respectively). The levels of putrescine, spermidine, spermine and cadaverine were similar in all four groups of grape samples, irrespective of the water stress applied. Furthermore, no significant difference was observed on the evolution of putrescine and cadaverine during winemaking.

Soil type and fertilization, cultivation practices and climatic conditions

Low potassium (K) concentrations in soil have been reported to be responsible for high putrescine levels in plants, mainly leaves (Adams 1991; Bouchereau *et al*. 1999; Vaz de Arruda *et al*. 2001; Miklós and Sarjala 2002; Walters 2003). According to Geny *et al*. (1997), polyamines (especially conjugated and wall-bound forms) were strongly affected by K nutrition in several organs of Cabernet Sauvignon grapes before visual nutrient deficiencies appeared in the leaves. They suggested that polyamines could be used as a sensitive biochemical marker to distinguish the optimum K levels for grapevines before appearance of nutrient deficiency symptoms.

According to Ingargiola and Bertrand (1991), Bauza *et al*. (1995) and Bell and Henschke (2005), nitrogen supply increases the levels of amino acids and, consequently, of amines and yeast-assimilable nitrogen. Amounts of 100 kg N/ha/year doubled the concentration of amines in comparison to grapevines where no nitrogen fertilizer had been applied (Bertrand *et al*. 1991).

Other factors that may affect amine levels in grape and wines are cultivation practices and climatic conditions. Lower amines levels were observed in grapes grown in cooler and rainier seasons (Sass-Kiss *et al*. 2000). When comparing organic and non-organic grape varieties, Yildirim *et al*. (2007) found significantly higher putrescine levels in organic wines, however, since the wines were produced using different processes, the difference observed could not be solely attributed to the organically grown grapes.

Influence of fermentation on amine formation and build up

The levels of amines in grapes can increase during wine making, affected by microorganisms intentionally added (starter culture) or contaminants (Ough 1971; Radler and Fath 1991; Glória *et al*. 1998; Hajós *et al*. 2000; Sass-Kiss *et al*. 2000).

Investigators have questioned which microorganisms are responsible for biogenic amine production. It is important to know the ability to produce amines by microorganisms involved in fermented foods in order to establish the potential risk of toxicological disorders to consumers (Landete *et al*. 2007a).

During fermentation or spoilage, spermine and spermidine levels can decrease. Besides the amines already present in grapes, several can be formed and accumulate during wine making among them putrescine, tyramine, histamine and phenylethylamine, whereas spermidine levels decrease (Hajós *et al*. 2000; Sass-Kiss *et al*. 2000). However, reports in this regard are contradictory.

Alcoholic fermentation

There were a couple of reports on the formation of tyramine and histamine during alcoholic fermentation (Buteau *et al*. 1984; Vidal-Carou *et al*. 1990b). However, in these studies there was no control of the microbial population present in the must, therefore, the formation of amines could not be attributed solely to yeasts.

Bover-Cid *et al*. (2006) observed that spermidine and spermine disappeared during alcoholic fermentation, which could be explained by the potential consumption by the alcoholic fermentative yeast. The levels of diamines also decreased: putrescine decreased linearly throughout time whereas cadaverine decreased significantly at the maceration stage. These results are in agreement with the generally accepted concept that yeasts are unable to liberate these amines in significant amounts. The aromatic amines usually

Table 6 Levels of free bioactive amines produced by yeast inoculated in sterilized musts.

Yeast	Amines levels (mg/L)					
	AGM	PUT	CAD	HIM	TRM	PHM
Saccharomyces cerevisiae	3.98	0.38	nd	0.01	0.80	0.39
Kloeckera apiculata	1.42	0.32	0.68	0.15	0.10	2.88
Candida stellata	4.11	nd	nd	nd	0.48	nd
Metschnikowia pulcherrima	1.12	0.83	0.19	nd	nd	6.56
Brettanomyces bruxellensis	2.27	1.18	0.31	0.20	nd	10.1

nd – not detected. AGM - agmatine, PUT - putrescine, CAD - cadaverine, HIM - histamine, TRM - tryptamine, PHM - phenylethylamine.
Source: Caruso *et al*. 2002.

found in retail wines did not appear in must or during the alcoholic fermentation.

Garde-Cerdán and Ancín-Azpilicueta (2007) investigated the evolution of bioactive amines during spontaneous alcoholic fermentation and also during vinification of sterilized must inoculated with a strain of *Saccharomyces cerevisiae*. During spontaneous alcoholic fermentation, putrescine was synthesized after consumption of the first 25% of sugars. However, the formation of spermidine + phenylethylamine took place in the last phase of the alcoholic fermentation. During vinification of sterilized must inoculated with *S. cerevisiae* in the presence or not of SO_2, there was formation and accumulation of putrescine with higher levels being formed after consumption of 25% of the sugars. Spermine and spermidine + phenylethylamine were formed after 50% of the sugar was consumed. The presence of SO_2 did not affect the formation of these amines. The formation of amines was higher in the inoculated than in the spontaneous fermentation. The formation of phenylethylamine by yeast during alcoholic fermentation was also observed by Torrea and Ancín (2002) and Marcobal *et al*. (2006), although quantitatively only very low concentrations were reached, less than 3 mg/L. Caruso *et al*. (2002) observed the formation of agmatine, phenylethylamine and low levels of tryptamine, putrescine, cadaverine and histamine by 50 strains of the yeast species *Saccharomyces cerevisiae*, *Kloeckera apiculata*, *Candida stellata*, *Metschnikowia pulcherrima* and *Brettanomyces bruxellensis* (**Table 6**). All species produced very low amounts or non-detectable levels of histamine, whereas agmatine was produced by all the species. The highest levels of biogenic amines were produced by *B. bruxellensis* followed by *S. cerevisiae*.

Landete *et al*. (2007a) investigated the potential of 36 yeast strains isolated from wine to produce amines and observed no formation of biogenic amines. The yeasts used in the study were strains of *Aureobasidium pullulans*, *Candida boidinii*, *Hanseniaspora guilliermondii*, *H. uvarum*, *H. mrakii*, *Kloeckera apiculata*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, *P. membranaefaciens*, *P. pinus*, *Rhodotorula rubra*, *Saccharomyces cerevisiae*, *S. cerevisiae var. bayanus*, *S. cerevisiae* var. *chevalieri*, *S. cerevisiae* var. *Steiner*.

Therefore, the characterization of strains of yeasts for the production of biogenic amines is necessary for the selection of starter cultures, in order to warrant the safety of consumer health. Husnik *et al*. (2006) constructed a genetically stable industrial strain of *Saccharomyces cerevisiae* by integrating a linear cassette containing the *Schizosaccharomyces pombe* malate permease gene and the *Oenococcus oeni* malolactic gene under control of the *S. cerevisiae* PGK1 promoter and terminator sequences into the URA3 locus of industrial wine yeast. The application of this industrial wine yeast could prevent the formation of noxious biogenic amines produced by lactic acid bacteria in wine.

Malolactic fermentation

Evidences of amine formation during malolactic fermentation have been described in the literature. Most researchers attributed the formation of amines, especially tyramine and histamine, to the action of bacteria involved in malolactic fermentation (Buteau *et al*. 1984; Aerny 1985; Cilliers and van Wyk 1985; Vidal-Carou *et al*. 1990b; Soufleros *et al*. 1998; Lonvaud-Funel 2000; Marcobal *et al*. 2006; Pramateftaki *et al*. 2006). The rates of formation and the levels of amines varied widely according to the type of microorganisms involved.

The production of histamine, tyramine, phenylethylamine and putrescine by lactic acid bacteria (LAB) isolated from wine is well documented (Moreno-Arribas *et al*. 2003; Landete *et al*. 2005; Marcobal *et al*. 2006; Landete *et al*. 2007a, 2007b). According to Soufleros *et al*. (1998) and Marcobal *et al*. (2006), during fermentation carried out by indigenous LAB, amino acid concentrations decreased significantly while bioactive amines increased. Another evidence was the negative correlation with malic and citric acids content (Rollan *et al*. 1995).

Delfini (1989) compared the ability of several strains of *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp. to produce histamine, and observed that *Pediococcus damnosus* (*P. cerevisiae*) had the capability to produce significant amounts while *Leuconostoc oenos* (*Oenococcus oeni*) strains were poor producers. Lafon-Lafoucade (1975) suggested that histamine build up occurred mainly as a result of bacteria growth in poor media. Lonvaud-Funel and Joyeux (1994) showed that histamine production by *O. oeni* was stimulated in media without glucose or malic acid and depended particularly on the histidine concentration of the media. Under these conditions, histidine decarboxylation contributed to an additional energy source for the bacteria as already demonstrated for other microorganisms (Molenaar *et al*. 1993). *Pediococcus* was the genus with the higher histamine production. Although *Oenococcus* showed the highest percentage of histidine decarboxylase positive strains, very low levels of histamine were produced. Within the *Lactobacillus* genus, *L. hilgardii* produced higher levels of histamine whereas *L. mali* showed a low histamine production (Landete *et al*. 2007a). Therefore, *Pediococcus* spp. is the main organism responsible for histamine production although only some strains can produce at high concentrations (Delfini 1989; Landete *et al*. 2007a).

Coton *et al*. (1999) found that the activity of histidine decarboxylase from *O. oeni* was not affected by the concentration of ethanol remaining in wine and was stable over time, even after extinction of the viable bacteria cells. Furthermore, the activity of this enzyme was higher at room temperature than at extreme temperatures (4 and 35°C) during wine storage (González-Marco and Ancín-Azpilicueta 2006a).

The ability to produce tyramine appears to be a general characteristic of *Lactobacillus brevis*, whereas tyramine production by *L. hilgardii* had a strain dependent character (Landete *et al*. 2007a). Moreno-Arribas *et al*. (2003) observed that *Leuconostoc* strains were the most intensive tyramine formers. Moreno-Arribas *et al*. (2000) isolated *L. brevis* and *L. hilgardii* capable of tyramine and phenylethylamine formation from wines containing high levels of amines. They observed that the factors affecting tyramine formation were tyrosine levels in the must and also the presence of sugars, mainly glucose. Tyramine producer LAB strains were also able to produce phenylethylamine (Gonzáles del Llano *et al*. 1998; Moreno-Arribas *et al*. 2000; Liu 2002; Landete *et al*. 2007a, 2007b). This could be explained by the fact that phenylethylamine is also a substrate for tyrosine decarboxylase, producing phenylethylamine in a secondary reaction. However, the ability to form tyramine

and phenylethylamine is not widespread among LAB. They are held mainly by *L. brevis* and some *L. hilgardii* strains. According to Landete *et al*. (2007b), all strains of wine lactic acid bacteria possessing the *tdc* gene were shown to produce tyramine and phenylethylamine.

According to Moreno-Arribas *et al*. (2003), *Oenococcus oeni* strains were not able to produce biogenic amines *in vitro*. However, *O. oeni* T56 produced higher amounts of biogenic amines whereas high ethanol concentrations and low levels of pyridoxal-5-phosphate reduced their accumulation (Gardini *et al*. 2005). Some strains of *Lactobacillus buchneri* were associated with putrescine formation (Moreno-Arribas *et al*. 2003). Putrescine was also formed by *Oenococcus oeni* from ortnithine and arginine (Guerrini *et al*. 2002; Mangani *et al*. 2005). According to Landete *et al*. (2007a), strains of LAB were able to produce histamine, tyramine, phenylethylamine and putrescine. However, no LAB was able to produce cadaverine and tryptamine. *Pediococcus parvulus*, *L. mali* and *Leuconostoc mesenteroids* could only produce histamine; *L. brevis* produced tyramine and phenylethylamine; *O. oeni* produced histamine and putrescine while *L. hilgardii* was able to produce histamine, tyramine, phenylethylamine and putrescine. According to Pessione *et al*. (2005), the biosynthesis of histamine and putrescine forming enzymes (histidine and ornithine decarboxylases, respectively) are closely dependent on the presence of higher concentrations of free amino acids in the growth medium and to be modulated by the growth phase. Arena and Manca de Nadra (2001) reported that agmatine was formed by *L. hilgardii* X_1B isolated from wine. It was an intermediate in the formation of putrescine. Agmatine degradation increased the growth and survival of the microorganism. However, in the presence of phenolic compounds, there was a decrease on putrescine formation. Therefore, the phenolic compounds present in wine could be a natural way to decrease putrescine formation (Alberto *et al*. 2007).

However, malolactic fermentation does not necessarily result in the formation of biogenic amines (Vidal-Carou *et al*. 1990a, 1990b; Cavazza *et al*. 1995; Soufleros *et al*. 2007). *In vitro* studies have demonstrated that some commercial malolactic bacteria did not produce histamine, tyramine and putrescine (Moreno-Arribas *et al*. 2003). In fact, Manfroi *et al*. (2008) reported the presence of only grape amines (spermidine, putrescine, serotonin and cadaverine) in Merlot wines prepared under standard microvinification procedures in the presence of two strains of *Oenococcus*, one of *Lactobacillus plantarum* and a natural spontaneous LAB. Furthermore, inoculation of must with commercial strains of malolactic bacteria was useful to reduce the contents of histamine, tyramine and cadaverine compared with those not inoculated (Martín-Álvarez *et al*. 2006).

A 100% correlation was found between the presence of histidine decarboxylase, tyrosine decarboxylase and ornithine decarboxylase genes and the production of histamine, tyramine and putrescine, respectively. Therefore PCR primers and DNA probes could be a genetic tool useful for the screening of dangerous LAB in food (Lonvaud-Funel 2000; Costantini *et al*. 2006; Landete *et al*. 2007a).

Hygienic conditions during winemaking

There are reports indicating the possibility that amines are formed in wine by the action of contaminant microorganisms or by those not directly implicated in the fermentation process, for example enteric bacteria (Buteau *et al*. 1984). Hygienic conditions of the grapes affects the levels of some amines, e.g., rotten grape material gave higher amine levels, especially phenylethylamine (Eder *et al*. 2002), tyramine and putrescine (Kiss *et al*. 2006). Unsatisfactory hygienic conditions during winemaking have also been related to higher concentration of some amines in wine (Bauza *et al*. 1995). Therefore, histamine alone or together with other amines could be an indicator of the quality of raw materials employed or unsanitary conditions prevailing during wine production (Buteau *et al*. 1984; Vidal-Carou *et al*. 1990b; Soufleros *et al*. 1998; Kiss *et al*. 2006).

Marques *et al.* (2008) investigated the influence of antifungi products applied to grapes on the levels of biogenic amines in wines. After malolactic fermentation, control wines contained the highest levels of amines. Comparing treated samples, the wines from grapes treated with carbendazyme showed the higher levels and procymidone the lower levels of biogenic amines. According to these investigators, the use of fermentation activators did not affect the amounts of biogenic amines in the wines.

The lack of accumulation of biogenic amines during the winemaking process is in agreement with the proper hygienic and controlled conditions applied. Therefore, it is feasible to produce wine with extremely low levels of amines (Bover-Cid *et al*. 2006).

Other factors affecting amine build up during the vinification process

Besides the presence of microorganisms, other factors during the vinification process can be a source of amines in wines. They include must treatment, length of fermentation in the presence of pulp and skin, alcohol content, sulfur dioxide concentration, added nutrients, pH, temperature and quantity and type of finings and clarification agents (Zee *et al*. 1983; Buteau *et al*. 1984; Glória *et al*. 1998; Vazquez-Lasa *et al*. 1998; Hajós *et al*. 2000; Leitão *et al*. 2000; Sass-Kiss *et al*. 2000; Arena and Manca de Nadra 2001).

Length of skin maceration

The length of skin maceration affects extraction of some compounds present in the grape skin, such as phenolics, proteins, polysaccharides, and also amino acids. According to Martín-Álvarez *et al*. (2006), significantly lower concentrations of histamine, tyramine and putrescine were observed in wines manufactured with less than 10 days of skin maceration, whereas wine elaborated with longer macerations had 2 to 4 times higher mean levels of these amines. These results are in agreement with previous study (Bauza *et al*. 1995).

Use of pressing machine

The use of pressing machine in wines allows the extraction of more phenolic compounds. It is well known that most phenolic compounds possess an antimicrobial activity, which can change the microflora of the initial must (Yildirim *et al*. 2007).

pH

pH may influence growth and metabolic activity of LAB. According to Gerbaux and Monamy (2000), pH has been shown to be one of the most important enological factors influencing biogenic amines, particularly histamine, tyramine and putrescine production.

Use of sulfur dioxide

According to Garde-Cerdán and Ancín-Azpilicueta (2007), addition of SO_2 did not affect the formation of biogenic amines during alcoholic fermentation. However, it prevented the formation of biogenic amines during wine aging (Vidal-Carou *et al*. 1990b; Marcobal *et al*. 2006). Yildirim *et al*. (2007) observed high quantities of putrescine in wines made with sulfur dioxide and concluded that the amount used could not be sufficient to prevent the formation of this amine.

Addition of yeast autolysate

The addition of yeast autolysate to must provided amino acids, however it did not produce an increase of amines during alcoholic fermentation despite the fact that consump-

tion of amino nitrogen and some precursor amino acids was higher. However, after malolactic fermentation it was observed that the concentration of biogenic amines was higher in the wine from the supplemented must (Marco *et al*. 2006).

Use of enzymes

Commercial pectolytic enzymes are used in wine making to increase juice yields, facilitate pressing and filtering, and to provide a greater clarity to must and wines. However these enzymes were observed to affect the levels of cadaverine and phenylethylamine. Lower levels of these amines were found in wines with supplements of pectinases (Martín-Álvarez *et al*. 2006). Proteolytic enzymes can favor amine formation by liberation of amino acids, which are the precursors for bioactive amines (Souza *et al*. 2005).

Use of clarifying agents

Treatment of wines with bentonite (silicate mineral) may decrease the concentration of some amines, such as histamine and putrescine, while a combined application of isinglass and gelatin lowered biogenic amines of white wines (Ough 1971; Radler and Fath 1991). Some treatments like the addition of calcium carbonate, activated carbon or ascorbic acid caused a decrease of the levels of biogenic amines (Radler and Fath 1991). The use of clarifying agents can reduce amine levels (Ough 1971; Zee *et al*. 1983), however, it is preferable to avoid or prevent the formation of amines.

Aging of wines

Jimenez-Moreno *et al*. (2003) observed that during aging of wine in oak barrels, there was a significant increase on the levels of histamine, tyramine, putrescine and cadaverine, followed by a decrease on the levels of histamine and tyramine. There was no significant influence of the types of oak woods on amine evolution during aging of the wine. According to González-Marco and Ancín-Azpilicueta (2006b), spermine, which was present in the wine before aging, disappeared quickly as it was undetected after 45 days of aging. Aging time also affected the concentration of amines in Chardonnay wine: the concentration of amines increased at the beginning and at the end of the aging period. When comparing the effect of stirring weekly during aging, there was no significant difference on putrescine levels, however, histamine and tyramine levels in the wine with weekly stirring were significantly higher, 21.4 and 68%, respectively, compared with the sample without stirring.

Garcia-Villar *et al*. (2007) found a significant relationship between aging process and amines in Spanish wines using chemometric data analysis. Tyramine levels increased significantly whereas the concentrations of histamine, phenylethylamine, putrescine and tryptamine increased.

When comparing the levels of amines in wines aged or not on yeasts lees, the levels of tyramine and putrescine were significantly affected. Putrescine levels were higher and tyramine levels were lower in wines aged on yeast lees (Martín-Álvarez *et al*. 2006). However, Bauza *et al*. (1995) found a higher production of putrescine and tyramine in wines with the addition of lees. González-Marco and Ancín-Azpilicueta (2006b) also detected a significantly increased (200%) concentration of putrescine during the initial 45 days of wine aging on lees, and afterwards, the concentration remained constant. The levels of histamine and tyramine at the end of the aging period were significantly higher compared to white wines from the same must which were not aged on lees.

During yeast lees contact, the composition of the wine changes as a consequence of hydrolysis of various molecules within the yeast cell. One of the enzymatic processes is proteolysis, in which proteins are hydrolyzed to peptides and amino acids, and these compounds pass through the cell wall into the wine. When bacteria undergo scarcity of nutrients, which is typical of aging, they use amino acids for energy generation through decarboxylation reactions. Furthermore, yeast extracts are known to contain high levels of histamine and tyramine. Extended contact with the lees lead to a higher content of amines as yeasts autolysis results in the release of cellular amines into the wine (Buteau *et al*. 1984; González-Marco and Ancín-Azpilicueta 2006b; Martín-Álvarez *et al*. 2006; Alcaide-Hidalgo *et al*. 2007; Soufleros *et al*. 2007; Marques *et al*. 2008).

Storage of wines

Wine storage temperature has a decisive effect on the quality of the wine because a rise in temperature increases reactions within the wine. When Chardonnay wines were stored in bottles at 4, 20 and 35°C during 105 days, formation or degradation of amines was mainly observed during the first 45 days of storage for all the temperatures studied. The levels of histamine, putrescine, cadaverine and tyramine increased whereas the levels of spermine decreased throughout storage, independently of the temperature. The levels of histamine were higher in wines stored for 105 days at 20°C than at the two more extreme temperatures (González-Marco and Ancín-Azpilicueta 2006a).

Gerbaux and Monamy (2000) found an increase of histamine, tyramine and putrescine levels in Chardonnay and Pinot Noir wines when stored in bottles. The increase of the levels of these amines at the beginning of storage arises because microorganisms with decarboxylase activity remained in the product (Louvand-Funel 2000). These microorganisms decarboxylate the amino acids released at the end of the fermentation as a result of alteration of the plasmatic membrane of the yeasts in order to get energy (González-Marco and Ancín-Azpilicueta 2006a).

A decrease of the concentration of tyramine after 75 days of wine storage has been observed. This was probably due to the presence of tyramine oxidase in the wine (Leuchner *et al*. 1998). It has been found that this enzyme has activity in wine although the greatest activity takes place at neutral or basic pH.

PREVENTION OF AMINE FORMATION IN WINE

Based on the information described above, it is feasible to produce wine with extremely low levels of amines (Bover-Cid *et al*. 2006). In order to prevent amine formation and build up the length of the processes that incorporate amino acids to must or wines such as grape skin maceration and the contact with lees must be reduced to a minimum. However it is impossible when aged wines are intended. Another way to prevent biogenic amine formation could be the inhibition of the growth of indigenous LAB and the inoculation of commercial selected strains which are unable to produce biogenic amines (Landete *et al*. 2007a).

In addition, other factors such as wine pH and the characteristics of the vintage can also play a critical role in amine biogenesis and should also be taken into account (Martín-Álvarez *et al*. 2006). Some additives such as sulfur dioxide could be used as it prevents the formation of biogenic amines during wine aging (Marcobal *et al*. 2006).

CONCLUDING REMARKS

Bioactive amines are among the major factors determining the quality of fermented beverages such as wine, since they play important roles in grape production and wine quality. Polyamines are essential for grape productivity and quality. Their contents in grapes and grape leaves are good biochemical markers to distinguish optimum cultivation conditions prior to the appearance of the deficiency symptoms. Biogenic amines can also be used as an authenticity index for botrytized grapes and wines.

Wine, like other fermented foods, is an ideal substrate for biogenic amine production. However, high amine levels can make the product unfit for consumption due to toxicolo-

gical aspects. Biogenic amines can be significant in terms of aroma and flavor. They can also indicate poor hygienic conditions during winemaking. Furthermore, they could cause problems in commercial transactions.

The levels of biogenic amines are very low or non existent in grapes, unless they are infected with microorganisms. Alcoholic fermentation hardly contributes to biogenic amines formation; however, during malolactic fermentation there can be a significant increase in amine levels.

In order to prevent the formation and accumulation of biogenic amines in wines, several approaches can be used: use of good quality raw material, use of hygienic conditions throughout the winemaking process, selection of starter cultures (the ones with absence of amino acids decarboxylases), aging and storage should be performed at ideal conditions. By doing so, it is feasible to produce wines with low levels of biogenic amines.

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