

Biogenic Amines in Wine: Understanding the Headache

A.Y. Smit¹, W.J. du Toit¹ and M. du Toit^{1,2*}

(1) Department of Viticulture and Oenology Stellenbosch University, Private Bag X1, Matieland (Stellenbosch), South Africa

(2) Institute for Wine Biotechnology, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch), South Africa

Submitted for publication: August 2008

Accepted for publication: September 2008

Key words: Biogenic amines; wine; bacteria; yeast; wine factors; decarboxylase enzymes; detection methods; toxicology

The presence of biogenic amines in wine is becoming increasingly important to consumers and producers alike, due to the potential threats of toxicity to humans and consequent trade implications. In the scientific field, biogenic amines have the potential to be applied as indicators of food spoilage and/or authenticity. Biogenic amines can be formed from their respective amino acid precursors by various microorganisms present in the wine, at any stage of production, ageing or storage. To understand the large number of factors that could influence the formation of biogenic amines, the chemical, biochemical, enzymatic and genetic properties relating to these compounds have to be considered. Analytical and molecular methods to detect biogenic amines in wine, as well as possibilities that could enable better control over their production levels in wine will also be explored in this review.

Biogenic amines are a group of organic nitrogenous compounds formed and degraded by the metabolisms of living organisms (microorganisms, plants and animals). The main biogenic amines associated with wine are putrescine, histamine, tyramine and cadaverine, followed by phenylethylamine, spermidine, spermine, agmatine and tryptamine. Biogenic amines in wine can be formed by various microorganisms associated with the different stages of wine production and storage.

The vinification of grapes consists of two main fermentation steps. Alcoholic fermentation of grape must, usually performed by the wine yeast *Saccharomyces cerevisiae*, involves the conversion of grape sugar to ethanol and CO₂. Malolactic fermentation is conducted in most red and some white wines by lactic acid bacteria of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Oenococcus*; principally to deacidify the medium by the conversion of L-malic acid to L-lactic acid. Malolactic fermentation also ensures a certain degree of microbial stability to the wine and modifies the sensory characteristics of the wine by the production of secondary bacterial metabolites (Lonvaud-Funel, 1999). Spoilage organisms such as acetic acid bacteria and the yeast *Brettanomyces bruxellensis* can grow during fermentations or storage and produce compounds that impart negative characteristics to the wine.

Apart from the primary metabolic products and many flavour compounds (both desirable and undesirable) released during the fermentations, some microorganisms produce secondary metabolic products that may affect the wholesomeness of the wine. Biogenic amines are one such group of compounds. Other secondary metabolites associated with winemaking that could pose a health risk to humans include sulphur dioxide (SO₂), ethyl carbamate and the mycotoxin, Ochratoxin A.

The focus of this review will be biogenic amines. Their formation by various wine microorganisms will be discussed with specific reference to the enzymes and genes involved. The chemical, biochemical and toxicological properties of biogenic amines are

considered. Factors influencing the formation of biogenic amines, covering the entire production chain, are critically reviewed. Analytical and molecular methods used in biogenic amine identification and quantification are also discussed.

MICROORGANISMS ASSOCIATED WITH BIOGENIC AMINE FORMATION IN GRAPES AND WINE

Lactic acid bacteria

Lactic acid bacteria are present on healthy grapes in low numbers and the population generally declines during alcoholic fermentation. Lactic acid bacteria are also transferred to winery equipment, where they can be present in significant numbers (Wibowo *et al.*, 1985). *Oenococcus oeni* is normally the dominant species that survives to the end of alcoholic fermentation and is predominantly responsible for malolactic fermentation. However, if the pH of the wine is above pH 3.5 species of *Pediococcus* and *Lactobacillus*, generally associated with spoilage, may survive and grow to levels of 10⁶ to 10⁸ cells/mL and have antagonistic interactions with *O. oeni* (Wibowo *et al.*, 1985; Lonvaud-Funel, 1999).

Extensive research has been done to correlate biogenic amine production in wine with species of lactic acid bacteria involved in the winemaking process. In the past, spoilage bacteria, mainly *Pediococcus* spp. such as *Pediococcus cerevisiae*, were held responsible for histamine production in wine (Delfini 1989). Recent results by Landete *et al.* (2007b) show an agreement that, although the percentage of *Pediococcus* spp. capable of producing histamine seems to be low, some strains (for example *Pediococcus parvulus* in this study) can be responsible for the highest concentrations of histamine. It is widely known that *Lactobacillus*, *Leuconostoc* and *Oenococcus* spp. are also implicated in biogenic amine production in wine. Different strains of *Lactobacillus hilgardii*, *Lactobacillus buchneri*, *Lactobacillus brevis* and *Lactobacillus mali* produce a variety of biogenic amines in wine (Moreno-Arribas & Lonvaud-Funel, 1999; Moreno-Arribas *et al.*, 2000; Downing, 2003; Moreno-Arribas *et al.*, 2003; Costantini *et al.*, 2006; Landete *et al.*, 2007b).

*Corresponding author: E-mail address: mdt@sun.ac.za

Until 2003 there had been no reports on the role of *Leuconostoc* strains in the formation of biogenic amines in wine. Moreno-Arribas *et al.* (2003) have shown that strains of *Leuconostoc mesenteroides* have a high potential to produce tyramine. Another strain of *Leuc. mesenteroides* isolated from wine, capable of producing relatively high levels of histamine, has been identified by Landete *et al.* (2007b).

Commercial *O. oeni* strains are selected for their oenological parameters, including the absence of amino acid decarboxylases. According to *in vitro* studies conducted by Moreno-Arribas *et al.* (2003), none of four commercial malolactic starter cultures examined could produce histamine, tyramine or putrescine. Martín-Álvarez *et al.* (2006) also compared inoculated with spontaneous malolactic fermentation in 224 samples of Spanish red wine. The authors found that inoculation with a commercial starter culture of lactic acid bacteria could reduce the incidence of biogenic amines compared to spontaneous malolactic fermentation in wines. Starter cultures could eliminate indigenous bacteria, or might be able to degrade biogenic amines produced by undesirable strains.

No biogenic amine production was observed by Moreno-Arribas *et al.* (2003), who tested 39 strains in a decarboxylase assay broth medium, using HPLC to quantify biogenic amine concentrations. Similar results were also obtained by Straub *et al.* (1995), who tested for histamine producers among 88 strains of *O. oeni*. Costantini *et al.* (2006) found no *O. oeni* among 92 strains able to produce biogenic amines in a broth medium. PCR screening was used by these authors to confirm the absence of the respective decarboxylase genes.

In contrast, Guerrini *et al.* (2002) found that *O. oeni* is able to significantly contribute to the overall biogenic amine content of wines and that the ability of *O. oeni* to produce biogenic amines varies among strains. Twenty six of 44 strains tested were able to produce up to 33 mg/L histamine under optimal growth conditions in a synthetic medium. Putrescine and cadaverine were also produced by some strains. The high frequency of histamine-producing strains found in this study is in accordance with a study done by Coton *et al.* (1998a), who reported that most *O. oeni* strains isolated from wine have histamine producing capability. The latter research group found that almost half of the 118 wines tested from the Southwest of France contained histamine in varying amounts and contained histidine decarboxylase positive bacteria belonging to *O. oeni*.

Thus, the ability of lactic acid bacteria to produce amines seems to be strain-dependent, and not a species specific characteristic. It may be that decarboxylase activities are randomly distributed within the different species of *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Oenococcus*. Some lactic acid bacteria strains have the ability to simultaneously produce different amines (Coton *et al.*, 1998a; Moreno-Arribas *et al.*, 2000; Guerrini *et al.*, 2002) supporting the suggestion by Gale (1946) that some strains might possess more than one amino acid decarboxylase activity under specific culture conditions.

According to literature (Lonvaud-Funel, 2001) only lactic acid bacterial strains with histidine-, tyrosine- and ornithine decarboxylase activities have been isolated from wine. The properties of each of the decarboxylase enzymes will be discussed briefly.

Histidine decarboxylase

Two different kinds of decarboxylase enzymes exist. The majority of amino acid decarboxylase enzymes - those belonging to animals and Gram-negative bacteria - require pyridoxal 5'-phosphate as a cofactor. However, some bacterial histidine decarboxylase enzymes use a covalently-bound pyruvoyl group as a cofactor in the reaction and are pyridoxal 5'-phosphate independent (Coton *et al.*, 1998b; Lonvaud-Funel, 2001). The latter group include well-studied Gram-positive bacteria: *Lactobacillus* 30a (Chang & Snell, 1968); *L. buchneri*, *Clostridium perfringens* (Recsei *et al.*, 1983); *Micrococcus* sp. (Prozorowski & Jörnvall, 1975) and *O. oeni* (Rollan *et al.*, 1995). Yet, Landete *et al.* (2006) demonstrated experimentally that pyridoxal 5'-phosphate does enhance amino acid decarboxylase activity in Gram-positive lactic acid bacteria and could therefore also be considered a decarboxylase cofactor for this group of bacteria.

The first histidine decarboxylase (HDC) enzyme of a wine lactic acid bacterium was isolated from a histamine producing strain (*O. oeni* 9204) by Lonvaud-Funel & Joyeux (1994) and has since been studied by various authors. Coton *et al.* (1998b) purified this HDC enzyme to homogeneity and provided valuable molecular data. This HDC enzyme is a single polypeptide of 315 amino acids, comprised of α and β subunits. The gene sequence aided researchers to develop rapid and specific detection systems based on polymerase chain reaction (PCR) to detect potential histamine-producing bacteria from wine (Le Jeune *et al.*, 1995; Coton *et al.*, 1998a).

The HDC enzyme has a high degree of cooperativity; at low histidine concentrations HDC has a low substrate affinity, but as histidine concentration increases, binding to the active site is favoured. The product, histamine, acts as a competitive inhibitor of the antiport histidine/histamine at the cell membrane and decreases the HDC activity (Rollan *et al.*, 1995). The pH optimum of HDC is 4.8. These findings are the same as described previously for HDC of *Lactobacillus* 30a (Chang & Snell, 1968).

Lucas *et al.* (2005) related HDC enzyme activity of *L. hilgardii* 0006 (isolated from wine), to the presence of an 80 kb plasmid on which the decarboxylase gene was located as part of a four-gene cluster. They proposed that this discovery is possibly the first described histamine-producing pathway. The four genes, *hdcP*, *hdcA*, *hisRS* and *hdcB* code for a histidine/histamine exchanger, a HDC, a histidyl-tRNA synthetase and an unknown product respectively. There is evidence to suggest that this same gene cluster may also be present in other histamine producers, such as *Lactobacillus* 30a and *O. oeni* 9204. Also, lactobacilli are able to transfer a conjugative plasmid to bacteria of the same or different genera (Gevers *et al.*, 2003). Lucas *et al.* (2005) suggest that a plasmid-encoded HDC system could be transferred horizontally; and that the location of the gene on an unstable plasmid may explain the random distribution of HDC positive bacteria.

Some authors reported the presence of histamine decarboxylase activity amongst a high proportion of wine lactic acid bacteria, even if the levels of histamine produced in wine are generally low (Lonvaud-Funel & Joyeux, 1994; Le Jeune *et al.*, 1995; Coton *et al.*, 1998a; Guerrini *et al.*, 2002; Landete *et al.*, 2005a, 2007b). Other authors found the frequency of histamine producers in wine to be very low (Moreno-Arribas *et al.*, 2003).

Landete *et al.* (2005a) screened 136 strains of wine lactic acid bacteria for the presence of the HDC gene and the ability to produce histamine in a synthetic medium. These included strains of *Lactobacillus* (54), *Oenococcus* (32), *Pediococcus* (34) and *Leuconostoc* (16). Their results showed that *Lactobacillus* (13%), *Oenococcus* (78%), *Pediococcus* (12%) and *Leuconostoc* (6%) were able to produce histamine. *O. oeni* had the highest frequency of histamine production, but produced the lowest concentrations. *L. hilgardii* and *P. parvulus* produced histamine at the highest concentrations (up to 200 mg/L) and can therefore be regarded as potential spoilage bacteria in wine. According to this study, other strains able to contribute to histamine in wine are *O. oeni*, *L. mali* and *Leuc. mesenteroides*.

Tyrosine decarboxylase

Tyrosine decarboxylase (TDC) in bacteria had only been investigated in *Enterococcus faecalis* (Allenmark & Servenius, 1978) until 1999, whereafter a tyramine-producing strain was isolated from wine and identified as *L. brevis* IOEB 9809 (Moreno-Arribas & Lonvaud-Funel, 1999).

In 2002 and 2003 the TDC gene of *L. brevis* IOEB 9809 was purified and sequenced (Lucas & Lonvaud-Funel, 2002; Lucas *et al.*, 2003). The sequence (7979 bp) contained four complete genes encoding a tyrosyl-tRNA synthetase, the tyrosine decarboxylase, a probable tyrosine permease and a Na⁺/H⁺ antiporter. The authors suggest that the TDC gene of *L. brevis* 9809 is the first well-characterised bacterial TDC gene. The TDC gene encodes for the 264 amino acids of the enzyme.

TDC was found to be active in a pH range of 3.0 to 7.0 with an optimum at pH 5.0. TDC enzyme activity was reported to be pyridoxal 5'-phosphate dependent (Moreno-Arribas & Lonvaud-Funel, 1999; Coton & Coton, 2005). Its activity is enhanced by the substrate (L-tyrosine) and the coenzyme (pyridoxal 5'-phosphate). These findings are similar to the properties described for TDC of *Ent. faecalis* (Allenmark & Servenius, 1978). As with histamine and HDC activity, tyramine acts as a competitive inhibitor of TDC and therefore the presence of the formed product will decrease the enzyme activity.

A rapid PCR assay was developed to detect *L. brevis* strains carrying the TDC gene (Lucas & Lonvaud-Funel, 2002). Downing (2003) determined that the described primers could be used to amplify a fragment of the TDC gene in tyrosine decarboxylating *L. brevis* as well as *L. hilgardii* strains from South African wines.

A number of wine lactic acid bacteria strains have since been identified as tyramine producers. Moreno-Arribas *et al.* (2000) isolated and identified a number of tyramine producing lactic acid bacteria in wine that had undergone malolactic fermentation. None of the strains in their study were identified as *O. oeni*, but all as *L. brevis* and *L. hilgardii*. Downing (2003) found tyramine to be the main amine formed in synthetic medium by *L. brevis* and *L. hilgardii* strains isolated from South African wines. Arena *et al.* (2007) found that *Lactobacillus plantarum* strains harbouring the TDC gene were able to produce tyramine in wine. As noted elsewhere in this review, *Leuc. mesenteroides* was also found to be a tyramine producer (Moreno-Arribas *et al.*, 2003). *O. oeni* seems to have a low distribution of the metabolic ability to produce tyramine (Moreno-Arribas *et al.*, 2000; Geurrini *et al.*, 2002). As far as literature suggested in 2003, no tyramine producing *O. oeni*

strain had yet been reported (Moreno-Arribas *et al.*, 2003), with the exception of a strain (*O. oeni* DSM 2025) that was shown to be able to produce tyramine in a laboratory medium (Choudhury *et al.*, 1990).

Ornithine decarboxylase

Marcobal *et al.* (2004) reported that a putrescine producing strain of *O. oeni* (IOEB 8419) was isolated from ropy red wine by Coton *et al.* (1999) and was suspected of having ornithine decarboxylase (ODC) activity. Later, seven strains of *O. oeni* (out of 44) were reported to be able to produce putrescine in culture media (Guerrini *et al.*, 2002). Then, a putrescine producing strain (*O. oeni* BIFI-83) was isolated from the fermentation lees of a Spanish wine which showed a high concentration of putrescine. This led to the first report of the ODC gene to be present in the genome *O. oeni* and being detectable by PCR (Marcobal *et al.*, 2004, 2005). The ODC gene encodes a 745 amino acid residue protein. ODC is also a pyridoxal 5'-phosphate dependent enzyme. The amino acid sequence of this ODC gene shares a 67% identity with that of *Lactobacillus* 30a (Marcobal *et al.*, 2004).

Putrescine is reported to be the most abundant biogenic amine in wine, both qualitatively and quantitatively. Putrescine could be observed in high concentrations (up to 200 mg/L), especially after malolactic fermentation (Glória *et al.*, 1998). Although putrescine has been found to be present in almost 100% of samples analysed by many authors (Glória *et al.*, 1998; Soufleros *et al.*, 1998; Vasquez-Lasa *et al.*, 1998; Soleas *et al.*, 1999; Moreno & Azpilicueta, 2004; Landete *et al.*, 2005b; Bover-Cid *et al.*, 2006; González Marco & Ancin Azpilicueta, 2006; Alcaide-Hidalgo *et al.*, 2007), only one strain of wine lactic acid bacteria has been reported in literature to possess the ODC gene: *O. oeni* BIFI-83 (Marcobal *et al.*, 2004). Marcobal *et al.* (2004) found that the ODC gene is rarely present in the genome of *O. oeni*. The authors found none of 42 strains tested to possess the gene. Also, ornithine is usually only present at low levels in wine and it would be expected that the levels of putrescine would be correspondingly low.

The arginine deiminase pathway

A possible explanation for the production of high levels of putrescine in wine has been proposed by Mangani *et al.* (2005). The authors proved that putrescine, the most abundant biogenic amine in wine, could be produced by *O. oeni* from ornithine and also from arginine at wine pH (3.2). Some strains that can produce putrescine possess the complete enzyme system to convert arginine, a major amino acid in wine, to putrescine. In this case arginine is catabolised via the arginine deiminase (ADI) pathway. For this to occur, all three enzymes of this pathway must be present in the bacterial strain and be active under wine conditions. These enzymes are arginine deiminase (ADI), ornithine transcarbamoylase (OTC), and carbamate kinase (CK). Some *O. oeni* strains can be deficient in one or two of these enzymes or arginine catabolism could be inhibited by low pH (Mangani *et al.*, 2005). However, metabiosis can occur among *O. oeni* species. This process is an association and exchange between strains able to metabolise arginine to ornithine, but unable to produce putrescine and strains that cannot degrade arginine but can produce putrescine from ornithine. Putrescine production by metabiotic association can occur slowly after the completion of malolactic fermentation, whereas conversion of ornithine to putrescine by the ODC enzyme of a single *O. oeni* strain was found to occur simultaneous with malic acid degradation and at a faster

rate (Mangani *et al.*, 2005). Thus, the authors concluded that despite commercial starter cultures being unable to use arginine or possess no ODC, metabiosis can occur with indigenous strains and may play an important role in putrescine accumulation in wine.

Similarly, Arena & Manca de Nadra (2001) showed previously that a *Lactobacillus* species (*L. hilgardii* X₁B), isolated from wine was able to degrade arginine via the arginine deiminase pathway and the arginine decarboxylase pathway. This enables the bacteria to produce putrescine from the intermediates ornithine and agmatine.

Other decarboxylases

De Las Rivas *et al.* (2006) described the first oligonucleotides for the PCR detection of lysine decarboxylase encoding genes in foodborne bacteria. Primers were designed to amplify the genes coding for lysine decarboxylase by alignment of lysine decarboxylase proteins in two groups: first for *Enterobacteriaceae* and second for bacteria from the genera *Bacillus*, *Clostridium*, *Listeria* and *Staphylococcus*. The possibility exists to extend this PCR method to detect cadaverine-producing bacteria in wine.

It is known that an association exists between phenylethylamine and tyramine production in lactic acid bacteria. This could be explained by the fact that phenylalanine is also a substrate for TDC; producing phenylethylamine in a secondary reaction (Landete *et al.*, 2007c). The ability of wine lactic acid bacteria to form phenylethylamine seems to be rare, with *L. brevis* and *L. hilgardii* being the only strains to date associated with high levels of phenylethylamine in wines (Moreno-Arribas *et al.*, 2000; Landete *et al.*, 2007c). According to Landete *et al.* (2007c) there have been no reports on studies regarding other phenylethylamine decarboxylase enzymes.

In general, lactic acid bacteria are the main microorganisms held responsible for biogenic amine production in wine. Soufleros *et al.* (1998) determined that the levels of biogenic amines (histamine, tyramine and putrescine) are low after alcoholic fermentations and increase in most wines during and after spontaneous malolactic fermentations with a corresponding decrease in the respective precursor amino acid concentration. Izquierdo Cañas *et al.* (2007) also determined that histamine, tyramine and putrescine concentrations increased by 106% to 174% in Spanish wines due to spontaneous MLF.

Granchi *et al.* (2005) examined biogenic amine production and the dynamics of microorganisms throughout the whole winemaking process of commercial vinifications. They found a decrease of biogenic amines (especially putrescine) during both spontaneous and induced alcoholic fermentations while yeast dominated. Biogenic amine concentrations (putrescine and histamine and/or cadaverine and tyramine) increased significantly during both spontaneous and inoculated malolactic fermentations conducted by *O. oeni* strains. Other authors who observed the most important increase of biogenic amines during malolactic fermentation, when compared to the contributions by alcoholic fermentation and/or ageing, include Cilliers & Van Wyk (1985), Landete *et al.* (2005b), Marcobal *et al.* (2006b) and Alcaide-Hidalgo *et al.* (2007). According to the results of Landete *et al.* (2007b), lactic acid bacteria can be considered the microorganisms responsible for the production of biogenic amines in wine, since in this study (involving 231 microorganisms), no yeasts or acetic acid bacteria were found capable of producing biogenic amines.

Yeasts

A large variety of indigenous yeast species can grow and perform the alcoholic fermentation in wine, along with commercial *S. cerevisiae* strains. Few studies have been conducted on the formation of biogenic amines by yeasts, of which most only compared different yeasts species and quantified only histamine (Torrea & Ancin, 2002).

A number of authors reported that no remarkable increase in the concentration of biogenic amines could be observed during alcoholic fermentation, with the conclusion that yeasts do not appear to be responsible for the production of most amines found in industrial commercial red wines (Herbert *et al.*, 2005; Marcobal *et al.*, 2006b). Granchi *et al.* (2005) even reported a decrease of biogenic amines (especially putrescine) during alcoholic fermentation while yeast dominates, for both spontaneous and induced commercial vinifications. In another study, no potential to produce biogenic amines in synthetic medium, grape must or wine was found among 36 strains of yeast isolated from grape must and wine. The yeasts tested included strains belonging to the genera *Aureobasidium*, *Candida*, *Hanseniaspora*, *Hansenula*, *Kloeckera*, *Metschnikowia*, *Pichia*, *Rhodotorula* and strains of the species *S. cerevisiae*, *S. cerevisiae* var. *bayanus*, *S. cerevisiae* var. *chevalieri* and *S. cerevisiae* var. *steiner* (Landete *et al.*, 2007b).

Any contribution of yeast to biogenic amine production could therefore be indirect: yeasts can alter the composition of grape musts by using some amino acids and secreting others during alcoholic fermentation and autolysis, thereby changing the concentration of precursor amino acids in the wine that can be used by other microorganisms in subsequent fermentation steps (Soufleros *et al.*, 1998).

In contrast, a number of studies show that an increase in biogenic amine concentration in wine by the direct action of yeast during both spontaneous and inoculated fermentations on experimental and commercial scale is possible. The results of a study performed by Buteau *et al.* (1984) disagree with the notion that biogenic amines (and particularly histamine) are formed by lactic acid bacteria during malolactic fermentation. These authors found that agmatine, cadaverine, ethanolamine, histamine, putrescine and tyramine were produced in the highest quantities during alcoholic fermentation. Moreover, some biogenic amines decreased during malolactic fermentation.

Goñi & Ancin Azpilicueta (2001) examined the concentration of biogenic amines produced by different *S. cerevisiae* strains in rosé wines. They found a slight increase in biogenic amines (putrescine, spermine, spermidine, phenylethylamine and tyramine) depending on the strain. Wines fermented by killer strains showed the highest concentration of biogenic amines, when compared to those with a killer neutral phenotype. The variation in the concentrations of the biogenic amines in this study was ascribed to differences in the extent of production or use of the amines by the yeasts as a source of nitrogen.

Torrea & Ancin (2002) proceeded with similar studies and found that inoculated musts produced wines with higher concentration of biogenic amines compared to spontaneous fermentations performed by indigenous yeasts. They attribute this to the greater consumption of precursor amino acids during fermentation by the commercial yeasts, and the lower yeast population in spontaneous fermentations.

In a study conducted by Caruso *et al.* (2002), 50 yeast strains of five different genera isolated from grapes and wine were screened for biogenic amine production. *B. bruxellensis* formed the highest concentration of total biogenic amines, followed by *S. cerevisiae*. The ability of *S. cerevisiae* to produce biogenic amines seems to be strain dependent and not a constant characteristic of the species. Some strains of *S. cerevisiae*, particularly those present during spontaneous alcoholic fermentations, can therefore be considered as wine spoilage microorganisms due to their ability to produce biogenic amines. *S. cerevisiae*, *Kloeckera apiculata*, *Candida stellata*, *Metschnikowia pulcherrima* and *B. bruxellensis* were also tested by Granchi *et al.* (2005) in another study. Table 1 gives an indication of the total biogenic amines produced by these yeasts, of which *B. bruxellensis* produced the highest concentration in both studies, followed by *S. cerevisiae*. Agmatine, followed by phenylethylamine and ethanolamine were produced in the highest amounts by most species.

It is clear that yeasts form different biogenic amines than lactic acid bacteria. Little is available in literature regarding the biochemistry, genetics and regulation of biogenic amine production of wine yeasts. The polyamines, spermine and spermidine, are formed from putrescine. Unlike putrescine itself, these polyamines have been found to be an absolute requirement for optimal growth in yeasts, in particular for meiotic sporulation and for maintenance of the double-stranded RNA killer plasmid (Cohn *et al.*, 1978). Yeast mutants with decreased ODC activity do not decarboxylate sufficient ornithine to putrescine and consequently grow slower because they synthesise fewer polyamines (Cohn *et al.*, 1980). Cohn *et al.* (1980) also suggest that putrescine in yeast serves primarily as precursor for spermidine and spermine biosynthesis. ODC in yeast is the key regulatory enzyme of the polyamine biosynthetic pathway (Gupta *et al.*, 2001). Yeast ODC activity requires a thiol and pyridoxal 5'-phosphate for activity and is rapidly decreased by the presence of spermine and spermidine (Tyagi *et al.*, 1981).

No mention could be found in literature of other decarboxylase enzymes studied in wine yeasts or yeasts from related fields.

Fungi

Biotic stresses to the grapevine, such as those caused by the fungus *Botrytis cinerea*, can lead to an increase of amine content of the grape berries (Hajós *et al.*, 2000). *B. cinerea* is responsible for the formation of aszu grapes, which are characteristic of the famous Tokaj wines from Hungary. The fungus penetrates the grape skin and significantly alters the composition and concentration of amino acids, carbohydrates and amines in the grape berries by increasing its concentrations while the water content of the grape decreases and the berry becomes raisin-like. Aszu wines are made by adding different quantities (butts) of these noble rot grapes to the ordinary wine grapes. Amine concentrations in aszu wines were found to be higher in three different cultivars studied when compared to corresponding normal wines from the same varieties. A positive correlation could be made between the biogenic amine content and the number of butts used for the aszu wine (Sass-Kiss *et al.*, 2000).

Marques *et al.* (2007) tested the potential of the fungicides carbendazyme, iprodione and procymidone (applied every three weeks to vines) to reduce the incidence of biogenic amines in grape musts and wine. It was found that control wine (that received no

fungicide treatments) presented the highest mean concentrations of biogenic amines at the end of malolactic fermentation. They concluded that fungal metabolic activity could directly contribute to biogenic amine formation (especially for isoamylamine) or lead to the activity of bacteria other than those normally present in healthy grapes.

Acetic acid bacteria and other bacteria

Forty strains of acetic acid bacteria isolated from grape must and wine were screened in synthetic medium and wine by Landete *et al.* (2007b) for their ability to form histamine, tyramine, phenylethylamine, putrescine, cadaverine and tryptamine, but no positive results were obtained. Histamine-producing *Bacillus* species (two strains) and acetic acid bacteria (one strain of *Acetobacter pasteurianus*) are reported to have been isolated from Taiwanese fruit wines (Chang *et al.*, 2008).

No further mention regarding the formation of biogenic amines by acetic acid bacteria in wine could be found in literature.

CHEMISTRY, BIOCHEMISTRY AND TOXICOLOGY OF BIOGENIC AMINES

Biogenic amines are compounds of low molecular weight, derived from aromatic or cationic amino acids and all have one or more positive charge and a hydrophobic skeleton (ten Brink *et al.*, 1990; Shalaby, 1996). The chemical structure of biogenic amines can be aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) (ten Brink *et al.*, 1990). Fig. 1 shows the chemical structures of some biogenic amines.

Putrescine, spermine and spermidine are present in plants, where they are important for physiological processes such as flowering and fruit development, cell division, stress responses and senescence (Halász *et al.*, 1994). A large number of volatile amines (for example ethylamine, methylamine, dimethylamine, pyrrolidine) are also present in grapes (Ouch *et al.*, 1981).

Biogenic amines are likely to be found in food and beverages that contain proteins or free amino acids; if conditions persist that favour microbial or biochemical activity. Such foodstuffs include fish, fish products, meat products (sausages), eggs, cheeses, nuts, fermented and fresh fruits and vegetables such as sauerkraut and soy bean products, beers and wines (Halász *et al.*, 1994; Silla Santos, 1996).

TABLE 1

Production of biogenic amines by different wine yeast species in sterile grape must under laboratory conditions in two different studies.

Yeast species	Average total biogenic amines (mg/L)	
	Caruso <i>et al.</i> (2002)	Granchi <i>et al.</i> (2005)
<i>Saccharomyces cerevisiae</i>	12.14	13.7
<i>Kloeckera apiculata</i>	6.21	9.7
<i>Candida stellata</i>	7.73	7.8
<i>Metschnikowia pulcherrima</i>	9.6	13.3
<i>Brettanomyces bruxellensis</i>	15.01	20

In fermented foods, the non-volatile or biogenic amines (histamine, putrescine, cadaverine, spermine, spermidine, agmatine, tyramine, tryptamine) and phenylethylamine (a volatile amine) are formed mainly by the microbial decarboxylation of the corresponding amino acids (Zee *et al.*, 1983; ten Brink *et al.*, 1990; Halász *et al.*, 1994). Volatile amines are believed to be formed by the reductive amination or transamination of the corresponding aldehyde or ketone (Smith, 1980; Ouch *et al.*, 1981).

Gram-positive bacteria are usually associated with spoilage of fermented foods (Silla Santos, 1996). In non-fermented foods, the presence of biogenic amines above a certain level can indicate microbial spoilage (ten Brink *et al.*, 1990). In raw fish and meat, Gram-negative bacteria are known to produce histamine when exposed to inappropriate temperatures.

Biogenic amines in wine can originate from the plant (grape berries) itself or be produced during the fermentation processes, ageing or storage when wine is exposed to the activity of decarboxylase positive microorganisms. Contamination may occur due to poor sanitary conditions of both grapes and processing equipment (Zee *et al.*, 1983; ten Brink *et al.*, 1990; Shalaby, 1996; Leitão *et al.*, 2005). Most biogenic amine contamination of wine is believed to take place during malolactic fermentation.

Many genera of bacteria are able to decarboxylate free amino acids by the action of decarboxylase enzymes. Because the amino acids are obtained by the bacterium from the extracellular medium (wine), it is believed that decarboxylase enzymes operate together with a transporter protein (Lucas *et al.*, 2005). During amino acid uptake, a membrane potential is generated by the

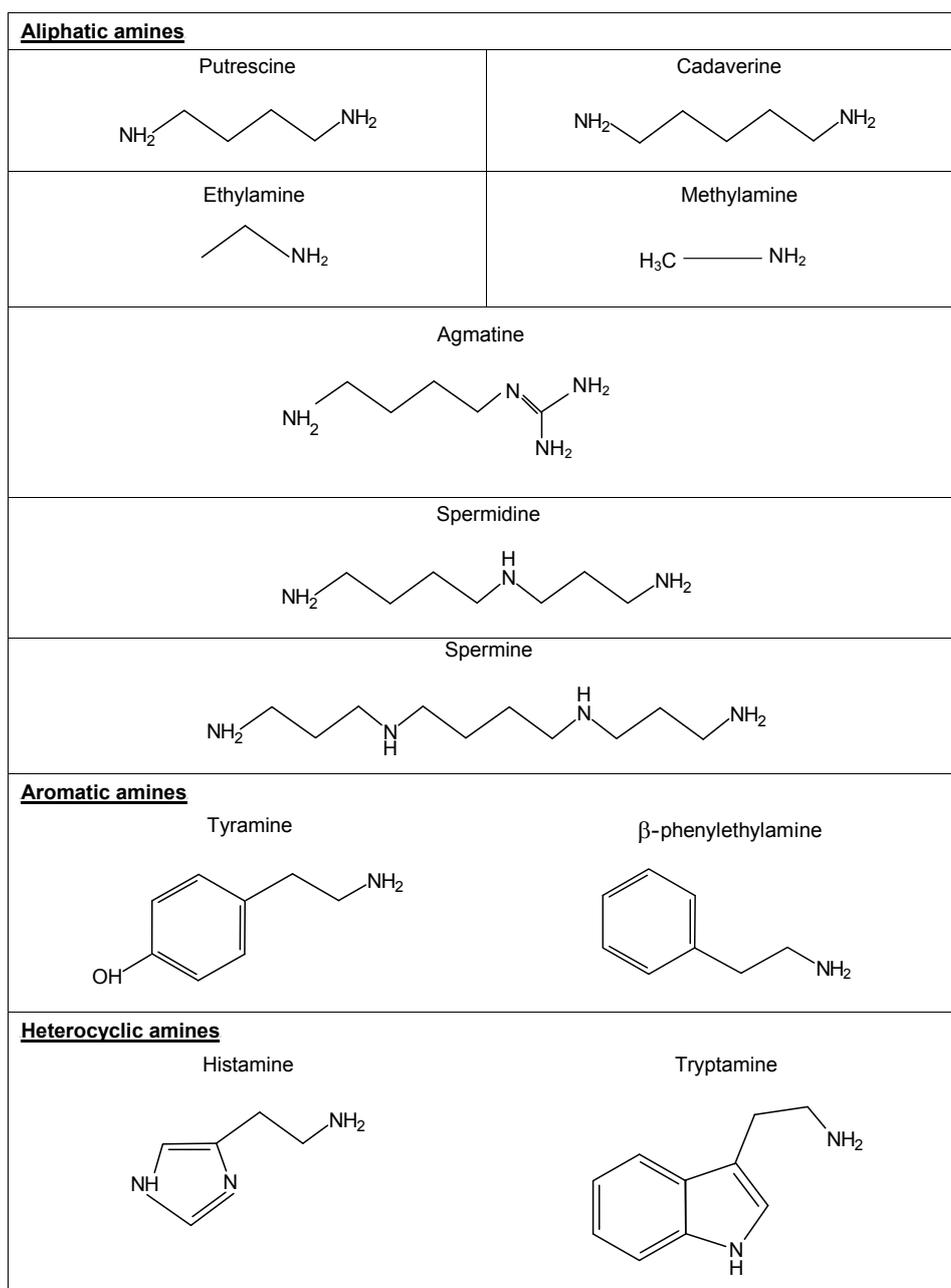


FIGURE 1

Chemical structures of some of the most oenologically important biogenic amines.

transporter because there is a net charge difference between the precursor (for example monovalent histidine) and the product (divalent histamine). A pH gradient is generated when a proton is consumed in the decarboxylation reaction. These two steps together generate a proton motive force. This reaction is thought to favour growth and survival in acidic media such as wine, since it produces metabolic energy by the described precursor/product antiport (Molenaar *et al.*, 1993), and regulates (increases) the pH - thereby extending the growth period by rendering the extracellular medium less toxic to the cell (Gale, 1946).

Histamine is present at low levels in the human body and is involved in key functions such as the allergic response, neurotransmission and vascular permeability (Ohtsu & Watanabe, 2003). Other normal physiological functions of biogenic amines in humans include the regulation of body temperature, stomach volume, stomach pH and brain activity (ten Brink *et al.*, 1990).

Normally, if a low concentration of biogenic amines is ingested, they are quickly detoxified in the human body by amine oxidases or through conjugation. Amine oxidases catalyse the oxidative deamination of biogenic amines to produce an aldehyde, hydrogen peroxide and ammonia (Gardini *et al.*, 2005). However, if an excessive amount of biogenic amines is ingested or if the normal catabolic routes are inhibited or genetically deficient, several physiological disorders can occur (ten Brink *et al.*, 1990). Histamine poisoning is sometimes referred to as “scombroid fish poisoning” due to illness resulting from consumption of fish such as tuna, mackerel and sardines; while high levels of tyramine in cheese causes a phenomenon known as the “cheese reaction” (Taylor, 1986; ten Brink *et al.*, 1990). These false food allergies are of particular importance in wine, because the presence of ethanol, acetaldehyde and other biogenic amines may promote the harmful effects of histamine and tyramine by inhibiting their normal metabolism in humans (Landete *et al.*, 2006). Histamine is often described as the most important biogenic amine since it is one of the most biologically active amines (Halász *et al.*, 1994). Histamine causes dilation of peripheral blood vessels, capillaries and arteries, thus resulting in hypotension, flushing and headache (Silla Santos, 1996). It also causes contraction of intestinal smooth muscle, resulting in abdominal cramps, diarrhoea and vomiting (Taylor, 1986).

Apart from allergic response, other serious human pathologies caused by biogenic amines include carcinogenesis and tumor invasion (ornithine-derived polyamines and histamine), immune response and neurological disorders (histamine), the formation of carcinogenic nitrosamines by reaction between nitrite and secondary amines (putrescine, cadaverine, agmatine), migraines and hypertension (tyramine and phenylethylamine) and Parkinson's disease, Schizophrenia and mood disorders (tyramine) (Smith, 1980; ten Brink *et al.*, 1990; Silla Santos, 1996; Medina *et al.*, 1999).

The toxic level of biogenic amines depends on the tolerance of the individual for the compound, the concentration of total biogenic amines and the consumption of ethanol and/or drugs. The toxicity of histamine and tyramine depends on the effectivity of the catabolic path which employs monoamine oxidase (MAO) and diamine oxidase (DAO) enzymes, which again varies in individuals (ten Brink *et al.*, 1990). Biogenic amines such as tyramine, putrescine and cadaverine that may also be present in the wine can inhibit

the metabolism of histamine. These amines favour the passage of histamine from the intestines into the systemic circulation by competing for binding sites in the gastrointestinal tract or by interfering with the catabolism of histamine by saturating the activity of mono- or diamine oxidases (Kanny *et al.*, 2001). The amine oxidase enzymes are not very specific and alcohol, acetaldehyde and anti-depressive drugs can also cause interference (ten Brink *et al.*, 1990; Straub *et al.*, 1995).

Generally the toxic dose in alcoholic beverages is considered to be between 8 and 20 mg/L for histamine, 25 and 40 mg/L for tyramine, while as little as 3 mg/L phenylethylamine can cause negative physiological effects (Soufleros *et al.*, 1998). Kanny *et al.* (2001) reports that a normal individual can tolerate 120 mg/L of histamine taken orally before symptoms occur, but only 7 µg administered intravenously.

However, there are studies that conclude that no relationship exists between the oral ingestion of biogenic amines and wine intolerance (Kanny *et al.*, 1999, 2001; Jansen *et al.*, 2003). Rather, these authors propose that wine may contain compounds (ethanol and acetaldehyde) that stimulate the release of histamine within the body.

Other than toxic effects, some biogenic amines also have other negative consequences, particularly regarding the sensory characteristics of the wine and economic implications.

A study by Rohn *et al.* (2005) suggests that histamine can be identified at high concentrations in commercial wines by well trained wine assessors. The study employed mouthfeel descriptors such as “irritation at the deep throat” and “crawling of the tongue”. No specific taste could be attributed to histamine. Putrescine, the most abundant biogenic amine in wine can reduce sensorial quality at 15 to 20 mg/L and 20 to 30 mg/L in white and red wines respectively (Arena & Manca de Nadra, 2001). In contrast, Wantke *et al.* (2008) determined that sensory wine quality is unrelated to histamine levels. During their study, 100 Austrian red wines and 26 sparkling wines judged by a professional wine taster were also analysed for histamine content.

In wine at a low pH, volatile amines occur as odourless salts, but in the mouth they are partially liberated and their flavours become apparent (Lehtonen, 1996). Volatile amines could therefore also influence wine aroma.

Biogenic amines in wine could also cause commercial import and export difficulties. Certain countries will reject wines that contain more than a certain concentration of histamine. The upper limits for histamine in wine in some European countries are (mg/L histamine): Germany (2), Holland (3), Finland (5), Belgium (5 to 6), France (8), Switzerland and Austria (10) (Lehtonen, 1996).

FACTORS AFFECTING THE FORMATION OF BIOGENIC AMINES IN GRAPES AND WINE

The levels of biogenic amines produced in wine largely depend on the abundance of amino acid precursors in the medium, since on the whole, biogenic amines increase with an increase in amino acids. Amino acid content may be influenced by vinification methods, grape variety, geographical region and vintage (Lonvaud-Funel & Joyeux, 1994; Soufleros *et al.*, 1998; Moreno Arribas *et al.*, 2000).

While some factors increase the precursor amino acid concentration, other factors influence the growth and enzyme activity of microorganisms that can form the biogenic amines.

Viticultural influences

Some amines, such as putrescine and other polyamines, may already be present in grape berries (Halász *et al.*, 1994; Bover-Cid *et al.*, 2006). Cabernet Sauvignon, for example, was found to have high concentrations of putrescine, cadaverine and spermidine in the pericarp of the berries (Glória *et al.*, 1998). Putrescine, cadaverine and spermidine have also been found present in high concentrations in the seeds of grape berries (Kiss *et al.*, 2006). Therefore, putrescine concentration in wine may be influenced more by geographical region and grape variety than by winemaking practices (Landete *et al.*, 2005). Potassium deficiencies in the soil have been linked to an increase in putrescine concentration in plants (Adams, 1991); while water deficiencies do not seem to influence the content of biogenic amines in grape berries and wines (Bover-Cid *et al.*, 2006). The degree of maturation of the grape and the soil type can also influence amine levels in the final product (Glória *et al.*, 1998).

Biogenic amines are also dependent on grape variety and vine nutrition, which will determine the concentration and composition of precursor amino acids in grape must and finally, together with yeast metabolisms, in the wine (Soufleros *et al.*, 1998). Nitrogen fertilization treatments can cause an increase in grape amino acids and amine concentrations (Spayd *et al.*, 1994; Soufleros *et al.*, 2007). Grape varieties with higher levels of assimilable amino acids have been found to yield the highest final concentrations of biogenic amines (Herbert *et al.*, 2005).

An experiment was conducted by Cecchini *et al.* (2005) to determine whether red grape cultivars have an effect on the content of biogenic amines in wines. Merlot, Syrah, Sangiovese, Cesanese d'Affile and Cabernet Franc were studied. Analysis of variance showed a significant difference between individual and total amine contents in wines obtained from musts of the different cultivars. Cabernet Franc was found to have significantly higher total amine content than any of the other cultivars studied.

More than one study has reported that the grape variety Pinot noir contains higher levels of biogenic amines when compared to Cabernet Sauvignon. Ough (1971) observed that significantly higher histamine concentrations were present in Pinot noir in California compared to Cabernet Sauvignon, while Glória *et al.* (1998) found that Pinot noir (Oregon, USA) contained significantly more total biogenic amines compared to Cabernet Sauvignon. Soleas *et al.* (1999) also observed higher amine concentrations in Pinot noir from Ontario (Canada) compared to other red wines. Other authors have observed cultivar related differences in biogenic amine content in Hungarian (Hajós *et al.*, 2000), Greek (Soufleros *et al.*, 2007) and Italian grapes and wines (Del Prete *et al.*, 2009).

The vintage and the region of production can also affect the free amino acid and amine content in must and wine (Herbert *et al.*, 2005). The concentrations of precursor amino acids can vary significantly over years. The influence of vintage on biogenic amine levels could also be attributed to the diversity of wine microorganisms that are naturally selected - their growth could again be correlated to the pH of the grapes and wines that can differ accordingly with the vintage (Martín-Álvarez *et al.*, 2006). These authors found Spanish Tempranillo wines from a specific region had significantly more biogenic amines in 2001 than in 2002. Amine levels of aszu wines were also found to be influenced

by vintage and were significantly different for the 1993, 1997 and 1998 vintages (Sass-Kiss *et al.*, 2000). On the contrary, Glória *et al.* (1998), found no difference in amine concentrations from two vintages (1991 and 1992).

Grape skin maceration practices

Grape skin maceration promotes extraction of grape components such as phenolic compounds, proteins, amino acids and polysaccharides. During cold maceration, grape must is left in contact with the grape skins at a cold temperature prior to alcoholic fermentation. Most red wines undergo alcoholic fermentation in contact with grape skins. Extended maceration after alcoholic fermentation can also be applied at cool temperature to extend the extraction period. Pectolytic enzymes are added to grape musts to increase the yield of juice, to clarify the must or wine, to extract more grape derived compounds such as phenols and to facilitate pressing and filtration.

Some authors could find no connection between grape skin maceration practices and the levels of biogenic amines in wines. Soleas *et al.*, (1999) found no correlation between length of skin contact and concentration of biogenic amines, while Martín-Álvarez *et al.*, (2006) concluded that the addition of pectolytic enzymes to the grapes at 2 g/100 kg did not promote biogenic amine accumulation in wine in their study.

However, others found that the duration of skin maceration to be a very important variable that affects biogenic amine content in wines, and that longer maceration time could favour increased production of biogenic amines (Bauza *et al.*, 1995; Martín-Álvarez *et al.*, 2006). Results from the latter research group are represented in Table 2.

An investigation on musts and wines of elderberry fruit showed that histamine content was dependent on the method of pulp treatment prior to processing, namely hot maceration, hot maceration and depectinisation and fermentation on the pulp. Their results show that histamine is formed by the action of native HDC of the berries, as well as by enzymes present in the Polish pectolytic preparation (Pectopol PT), and during fermentation by the action of decarboxylases produced by yeasts. The highest decarboxylase enzyme activity was found in Bordeaux yeast, followed by Burgundy, Malga, Tokay and Syrena yeasts. (These yeasts seem to be unspecified *S. cerevisiae* wine yeast starter cultures.) None of the wines had undergone any malolactic fermentation, thus excluding the possibility of biogenic amine formation by the action of bacterial decarboxylases (Pogorzelski, 1992). The presence of and possible contribution by indigenous lactic acid bacteria on the elderberry fruit was not reported.

Phenolic compounds

Grape phenolics are also extracted from the grape skins (and seeds) during maceration and fermentation. These compounds are responsible for many chemical reactions in grape must and wine. They are involved in oxidation reactions (they are strong antioxidants), and influence the flavour and colour of wine. Even though it has been known for some time that phenolic compounds may have an effect (inhibiting or stimulatory) on the growth, metabolism and malolactic activity of lactic acid bacteria (Vivas *et al.*, 1997; Alberto *et al.*, 2001, 2007), the first study to ascertain whether phenolic compounds can affect biogenic amine production by wine lactic acid bacteria was done

in 2007 by Alberto *et al.* with specific focus on the influence of phenolic compounds on agmatine metabolism of *L. hilgardii* X₁B. The formation of putrescine from agmatine does not involve amino acid decarboxylase. Alberto *et al.* (2007) observed the conversion of agmatine into putrescine was lower in the presence of all phenolic compounds, with the exception of gallic acid and quercetin. Gallic acid and quercetin also had no influence on bacterial growth or survival in the presence of agmatine and therefore had no interactions with agmatine metabolism.

Putrescine, the end product of this pathway, is able to protect DNA damaged by reactive oxygen species and increase cell survival of *Escherichia coli* under conditions of oxidative stress (Tkachenko, 2004). Phenolics are powerful antioxidants and the reduction of putrescine formation in the presence of phenolics is attributed to the ability of the phenolics to protect the cells against oxidative stress themselves (Alberto *et al.*, 2007). Phenolic decarboxylation could also compete with the enzyme involved in the conversion of agmatine into putrescine, *N*-carbamoylputrescine decarboxylase, and thus inhibits the formation of putrescine. According to this study, the presence of phenolic compounds could pose a natural solution to reduce putrescine formation in red wine.

Wine physiochemical composition

Wine physiochemical factors such as pH, temperature, SO₂ and a variety of substrates and products of fermentation can influence the concentration and diversity of microorganisms in the wine, but can also affect decarboxylase enzyme activity and gene expression.

Influence of wine composition on enzyme activity and decarboxylase gene expression

In wine, the presence of the HDC gene does not mean that the enzyme is functional and that histamine will necessarily be formed (Coton *et al.*, 1998a). As discussed earlier in this review, histidine decarboxylation can be used by bacterial cells to generate additional energy under poor growth conditions (Konings *et al.*, 1997). It seems as if though the production of histamine by lactic acid bacteria is always enhanced under poor growth conditions, for example when the medium has a shortage of fermentable substrates such as L-malic acid and glucose (Lonvaud-Funel & Joyeux, 1994). However, the presence of glucose and L-malic acid can have a stimulating effect on decarboxylation. Moreno-Arribas *et al.* (2000) reported that more tyramine was produced by a TDC positive *Lactobacillus* strain in the presence of glucose, possibly due to the energy provided to aid the enzyme activity. In contrast, Arena *et al.* (2007) showed that tyramine formation was decreased by increasing concentrations of glucose, fructose and L-malic acid. Glucose was found to have no effect on the HDC enzyme of *O. oeni* (Rollan *et al.*, 1995). Malic acid was found to play a key role in activating arginine catabolism by *O. oeni*, increasing the production of putrescine (Mangani *et al.*, 2005).

The product of malolactic fermentation, lactic acid, was found to inhibit HDC activity (Rollan *et al.*, 1995; Lonvaud-Funel, 2001); while on the contrary, lactic acid does not appear to inhibit ODC activity (Mangani *et al.*, 2005). Citric acid may also inhibit HDC and TDC activity to a small extent at levels normally present in

TABLE 2

Average biogenic amine concentrations (mg/L) for different levels of vintage, pectolytic enzymes, ageing on lees, maceration time and bacteria inoculation (Martín-Álvarez *et al.*, 2006).

	Number of samples	Histamine	Methylamine	Ethylamine	Tyramine	Phenylethylamine	Putrescine	Cadaverine
<i>Vintage</i>		**	**	**	**	*	**	
2001	117	4.87±0.67	1.36±0.12	3.07±0.20	2.12±0.55	3.36±0.50	9.69±1.24	1.16±0.44
2002	107	1.44±0.69	0.39±0.12	0.44±0.20	0.62±0.57	2.29±0.52	4.70±1.29	1.60±0.46
<i>Pectolytic enzymes</i>						**		*
No	162	2.76±0.59	1.01±0.11	1.66±0.17	0.96±0.49	3.86±0.44	8.08±1.10	1.93±0.39
Yes	62	3.55±0.81	0.74±0.14	1.85±0.24	1.77±0.67	1.79±0.61	10.46±1.45	1.55±0.51
<i>Ageing with lees</i>			*		**		**	
No	159	3.82±0.63	0.71±0.11	1.92±0.19	2.30±0.52	2.68±0.47	3.93±1.1	1.21±0.42
Yes	65	2.49±0.78	1.05±0.14	1.58±0.23	0.43±0.64	2.97±0.58	10.46±1.45	1.55±0.51
<i>Skin maceration (time)</i>		**	*		**		**	
<10 days	123	1.83±0.58	0.71±0.10	1.83±0.17	0.51±0.47	2.70±0.43	3.32±1.07	1.02±0.38
>10 days	101	4.89±0.40	1.04±0.14	1.68±0.23	2.22±0.65	2.95±0.59	11.07±1.46	1.74±0.52
<i>MLF inoculation</i>		**			*			*
No	193	4.89±0.40	1.01±0.07	2.00±0.12	2.37±0.33	2.67±0.30	7.34±0.74	2.09±0.26
Yes	31	1.42±1.04	0.75±0.19	1.50±0.3	0.37±0.86	2.99±0.78	7.05±1.94	0.67±0.69
	224							

*The factor has a statistically significant effect on the variable, P<0.05.

**The factor has a statistically significant effect on the variable, P<0.01.

wines after malolactic fermentation (Rollan *et al.*, 1995; Moreno-Arribas & Lonvaud-Funel, 1999). Other compounds found to inhibit HDC activity to different extents include glycerol, β -mercaptoethanol, lactic acid and ethanol. However, Moreno-Arribas & Lonvaud-Funel (1999) conclude that even the highest concentrations of these compounds likely to be present in wine will not be sufficient to prevent the formation of tyramine.

pH and ethanol at levels found in wine could inhibit decarboxylase enzyme activity (Leitão *et al.*, 2000). HDC activity and consequent histamine production is enhanced at pH 3.5 and by ethanol concentrations up to 10%, where the conditions for histidine transport inside the cells are more favourable due to the fluidification of the cell membrane by ethanol (Lonvaud-Funel & Joyeux, 1994). A high ethanol concentration (12% or more), as most often found in wine, reduces the HDC activity by altering the physicochemical properties of the membrane and slowing down histidine transport (Rollan *et al.*, 1995).

Histamine production was further found to be regulated by the presence of histidine, histamine, pyridoxal 5'-phosphate and the bacterial growth phase (Landete *et al.*, 2006). It was observed that HDC gene expression is induced by histidine (at 1 or 2 g/L) and decreased by histamine (at 1 or 2 g/L), while pyridoxal 5'-phosphate enhanced HDC activity (at 0.5 g/L).

During a study performed by Lucas *et al.* (2005) it was discovered that the HDC positive phenotype disappeared under certain culture conditions in *L. hilgardii* 0006, isolated from wine. In a poor, acidic medium the number of HDC positive cells increased, presumable due to the energetic and growth advantage of these cells. In a rich medium with a higher pH, the number of mutant cells lacking HDC activity increased. Loss of enzyme activity was found to correspond to the loss of a large plasmid (80 kb) on which the HDC gene was located.

In addition to decarboxylase activity, a small number of *O. oeni* strains (six of 220 strains tested) were found to also have proteolytic activity – the ability to release amino acids from peptides and proteins. The proteolytic activity is also dependent on nutritional and energetic composition of the medium, and generally increases when high-energy nutrients are exhausted in the late exponential growth phase (Leitão *et al.*, 2000). Decarboxylase activity is also expressed when the cells need the additional energy produced during amino acid transport. Northern blot analysis confirmed that HDC expression appeared during the early growth phase, reached a peak during the exponential growth phase (because the decarboxylation generates metabolic energy for this time of growth and cell division) and decreased significantly during the stationary phase when growth and cell division decrease (Molenaar *et al.*, 1993; Landete *et al.*, 2006). Other researchers also found that decarboxylase activity increases or could be biosynthesised towards the end of the active growth phase and during the exponential phase of bacterial growth under experimental and industrial winemaking conditions (Gale, 1946; Moreno-Arribas *et al.*, 2000; Marcobal *et al.*, 2006b). *O. oeni* expresses proteolytic and decarboxylase activities only when there is no easier strategy for cell survival (Molenaar *et al.*, 1993).

Influence of wine composition on bacterial growth and survival

A study was performed to analyse the effects of five physiochemical factors (incubation temperature, incubation time, environmental pH, added tyrosine concentration and pyridoxal 5'-phosphate

supplementation) on cell growth and tyramine production of *L. brevis* CECT 4669 and *Ent. faecium* BIFI-58 (isolated from grape must) under anaerobic and aerobic conditions. A multiple linear regression model was used to predict that the optimum conditions for growth and tyramine production was anaerobic incubation at acidic pH (4.4) in the presence of a high tyrosine concentration (Marcobal *et al.*, 2006a).

Studies of the complete vinification of industrial wines indicated that SO₂ does prevent the formation of biogenic amines by reducing lactic acid bacterial numbers in the wine (Marcobal *et al.*, 2006b). Vidal-Carou *et al.* (1990) found that the highest levels of biogenic amines are found in red wines with low SO₂ levels and that increased levels of SO₂ was correlated with a decrease in the concentration of histamine and tyramine. The effect of SO₂ on tyramine accumulation was also found to be dependent on pH. At a higher pH, an increase of SO₂ was found to cause a decrease in tyramine concentration, while at a lower pH tyramine increased with an increase of SO₂ (Gardini *et al.*, 2005). This response to pH is contrary to what is usually encountered during biogenic amine production. Normally, at a higher wine pH, the bacterial microflora is more diverse and the growth and survival of decarboxylase positive bacteria becomes more likely. Hence, at higher pH, higher levels of biogenic amines are produced in most cases (Wibowo *et al.*, 1985; Lonvaud-Funel & Joyeux, 1994; Gardini *et al.*, 2005; Landete *et al.*, 2005b; Martín-Álvarez *et al.*, 2006). Landete *et al.* (2005b) observed that wines from La Rioja with a high histamine content had a pH of 3.6 or higher. Cilliers & Van Wyk (1985) also noted that the pH of all the red wines containing large amounts of histamine (>10 mg/L) in their study exceeded 3.7.

Lower levels of biogenic amines were produced in conditions of high ethanol concentrations and low pyridoxal 5'-phosphate concentrations (Gardini *et al.*, 2005). The decrease in tyramine production by *O. oeni* T56 (a tyramine producer) under conditions of high ethanol and low pH was attributed to reduced metabolic activity and cellular viability and not to the specific decarboxylase activity in this study.

Acetic acid (volatile acidity) has been correlated with high levels of histamine in white and rosé wines in one study. However, the reason for this correlation was not determined (Vidal-Carou *et al.*, 1990).

Conditions during ageing and storage of wine

After malolactic fermentation Landete *et al.* (2005b) noticed a further increase in histamine during the first six months of storage in bottles. Gerbaux & Monamy (2000) also found that the concentration of histamine increases between four and eight months after malolactic fermentation in Pinot Noir and Chardonnay. A third study (Herbert *et al.*, 2005) showed a consistent increase of histamine in red and white wines 18 months after the completion of malolactic fermentation, whilst tyramine and putrescine seemed to increase immediately following malolactic fermentation in red wines in this study.

The reason for the initial increase following the completion of malolactic fermentation could be that SO₂ added to the wine after malolactic fermentation does not completely stop all biochemical reactions and enzyme activity. Also, due to the high pH of many wines, SO₂ is less effective and hence biogenic amines can increase in sulphated wines during ageing (Gerbaux & Monamy, 2000).

Another reason for increase in biogenic amines following fermentations is derived from the winemaking practice of ageing wine in contact with yeast lees. Yeast autolysis favours the growth and activity of lactic acid bacteria due to the release of vitamins and nitrogenous compounds. Lactic acid bacteria are able to hydrolyse and metabolise proteins and peptides and use the released amino acids as nutrients or energy sources. These amino acids may include the precursors of biogenic amines (Lonvaud-Funel & Joyeux, 1994). Yeast and bacterial lees can also be the source of decarboxylase positive lactic acid bacteria. Bauza *et al.* (1995) observed an increased production of tyramine and putrescine when wines are inoculated with bacteria through the addition of lees. Incidentally, the first ODC gene from a putrescine producing wine lactic acid bacterium was isolated from wine lees (Marcobal *et al.*, 2004).

Martín-Álvarez *et al.* (2006) left their wines in contact with lees for two months after alcoholic fermentation, before ageing in barrels. The mean concentrations of methylamine and putrescine were higher in wines aged on lees. In contrast, tyramine concentrations were significantly lower (Table 2). The authors postulate that tyramine could be consumed by residual microorganisms from lees for the production of carbon skeletons or amino groups. Coton *et al.* (1998a) also noted that even when no more culturable cells were detectable, HDC could still be active – thus biogenic amines can be produced during ageing. Moreover, most amines as well as decarboxylase enzymes are heat stable and will not be reduced during processing (such as pasteurisation), and can for this reason even increase during storage (ten Brink *et al.*, 1990). The influence of lees on the presence of biogenic amines in wine was recently reviewed by Pérez-Serradilla & Luque de Castro (2008).

After the initial increase of biogenic amines during storage, a general decrease or stabilisation in concentration could be observed by various research groups (Gerbaux & Monamy, 2000; Landete *et al.*, 2005b; Marcobal *et al.*, 2006b). Biogenic amines can be degraded by oxidase enzymes present in some bacteria towards the end of the ageing period, even at wine pH (Vidal-Carou *et al.*, 1991; Moreno & Azpilicueta, 2004). The general decrease of biogenic amines during ageing could explain why the highest histamine content (average 8.72 mg/L) was found by Vasquez-Lasa *et al.* (1998) in young red wines compared to red wines subjected to different traditional ageing prescriptions in Rioja, Spain. No statistically significant differences were found between histamine levels in crianza red wines (6.67 mg/L), reserva red wines (6.92 mg/L) or gran reserva red wines (5.12 mg/L).

Other factors present during ageing may or may not play a role in the accumulation of biogenic amines. Hernández-Orte *et al.* (2008) examined a number of factors that influence biogenic amine evolution, particularly during storage of wine in oak barrels. Moreno & Azpilicueta (2004) compared the biogenic amine concentrations of filtered and unfiltered wines aged in barrels for 243 days. Diatomaceous earth can adsorb cationic amino acids and proteins on its surface; which can influence the evolution of biogenic amines during ageing. Unfiltered wine can contain skin residues which can be rich in amino acid precursors. However, it was found that the degree of turbidity did not influence the accumulation of biogenic amines during ageing. Also, the type of barrel (American, French Allier and French Nevers oak) did not influence the content of biogenic amines. In another study,

the highest mean values for histamine were acquired in wines where malolactic fermentation was performed in tanks (not barrels), followed by ageing in the presence of lees stirred weekly or monthly. In this study, putrescine increased during ageing in wines aged in presence of yeast lees, but remained stable in wines without lees (Alcaide-Hidalgo *et al.*, 2007).

Normally, the storage of wine at elevated or fluctuating temperatures can cause unwanted chemical, microbial or enzymatic reactions of wine components and seriously decrease product quality. However, it was found that wine storage temperature only has a small effect on amine concentration. Histamine concentration was found to increase slightly when wines were stored for 105 days, more so at 20°C than at the more extreme temperatures of 4°C or 35°C. The formation or degradation of amines in wine mainly took place during the first 45 days of storage for all temperatures studied, due to the presence of residual decarboxylase activity after alcoholic and malolactic fermentations (González Marco & Ancín Azpilicueta, 2006). Vidal-Carou *et al.* (1991) found no formation or increase in biogenic amines (histamine or tyramine) at various temperatures ranging from 4°C to 35°C in wines stored for 93 to 125 days under spoilage conditions. The only changes observed in biogenic amine content were the decrease in histamine and tyramine, independent of temperature. The authors could not explain this phenomenon.

Ancín-Azpilicueta *et al.* (2008) reviewed some of the factors that influence biogenic amine concentration in wine, including their evolution at different winemaking stages and during storage of the product.

Wine style and type

Red wines generally show a higher concentration of biogenic amines than white and rosé wines. The higher values are attributed to the presence of lactic acid bacteria and malolactic fermentation (Landete *et al.*, 2005b); since in the case where white and rosé wines did undergo malolactic fermentation in this study, the amine values were close to those observed in red wines after malolactic fermentation. White wines, in general, also contain fewer amino acid precursors and have lower pH due to the absence of skin contact during fermentation and the absence of malolactic fermentation, and may consequently have lower biogenic amine concentrations than red wines (Zee *et al.*, 1983; Cilliers & Van Wyk, 1985; Vasquez-Lasa *et al.*, 1998; Leitão *et al.*, 2005). Buteau *et al.* (1984) attributed the higher levels found in red wines due to the lack of bentonite treatments (which adsorbs amines) and the release of cellular amines by autolysing yeast cells in lees during malolactic fermentation.

Rupasinghe & Clegg (2007) analysed and compared the biogenic amine content of ten different types of fruit wines (apple, black currant, blueberry, cherry, cranberry, elderberry, peach, pear, plum, raspberry) to wines made from grapes (Cabernet Sauvignon, Chardonnay, Riesling and icewine made from Vidal Blanc). The results indicate that Cabernet Sauvignon grapes contain significantly higher levels of biogenic amines (11 143 µg/L histamine) than all other fruit wines. Again, this was attributed to the fact that this red wine was the only wine that had undergone malolactic fermentation.

In Chinese rice wines, the average amount of total biogenic amines was found to be 107 mg/L, which is higher than average

levels reported in wine made from grapes. Interestingly, no putrescine was detected in any of the 42 samples of rice wines tested, while histamine was detected in all samples in the range of 5.02 to 78.50 mg/L, followed by spermine (present in 93% of samples), cadaverine (87%), tyramine (79%) and spermidine (79%). The highest levels of total biogenic amines (100 to 241 mg/L) were observed in Shaoxing rice wine. The vinification procedure followed in Shaoxing includes a soaking step where the rice is soaked in a fermentation substrate (soaking water) containing a large amount of free amino acids and active decarboxylase positive lactic acid bacteria. No significant correlation was found between the pH of rice wines (4.04 to 4.33) and biogenic amines in this work (Yongmei *et al.*, 2007).

More studies on the levels of biogenic amines in traditional alcoholic beverages (Lasekan & Lasekan, 2000), natural ciders (Garai *et al.*, 2006), beer and biologically aged sherry-type wines (Moreno-Arribas & Polo, 2008) have also been published. For the purpose of this review it is only important to note that the growth and activity of lactic acid bacteria are implicated in biogenic amine production in all these products.

ANALYTICAL AND MOLECULAR METHODS USED IN BIOGENIC AMINE IDENTIFICATION

Analysis of biogenic amines, individually or simultaneously, is important because of their toxicity potential and their potential to be applied as indicators of food spoilage or authenticity. Biogenic amines can be quantified by a variety of analytical methods that require sophisticated equipment. Qualitative measurements can also indicate the presence of amines in wine. For review papers on analytical methods used in the determination of biogenic amines, refer to Lehtonen (1996) and Önal (2007). The potential of biogenic amines to appear in wine can also be determined by using molecular tools which can detect the presence of decarboxylase positive microorganisms.

Qualitative and semi-quantitative methods

Screening methods using selective media

Some of the first methods developed for qualitative biogenic amine detection (initially for histamine) are microbiological screening methods that involve the use of a differential agar medium with a pH indicator (bromocresol purple), where an increase in pH as a result of amine formation can be easily observed by a change in colour. Amino acid precursors are contained in the decarboxylase assay medium. Modifications of the screening plate medium have been made by various researchers to make it more suitable for the growth of lactic acid bacteria and activity of decarboxylase enzymes. Improvements have also led to greater sensitivity and reliability of the screening plate method, and it has shown good correlation with other chemical analytical methods (Choudhury, 1990; Maijala, 1993; Bover-Cid & Holzapfel, 1999). Yet, it is still advisable to confirm the results of simple screening methods by another analytical method (such as HPLC), because false positive results can occur due to the formation of other alkaline compounds (such as ammonia), and failure to detect amine production has also been reported (Moreno-Arribas *et al.*, 2003).

Enzymatic methods

Enzymatic methods to quantify histamine were first reported for use in fish. When these enzymatic methods were applied to musts and wines, many false positive results were recorded. A direct

enzymatic test for the use in wine was developed by Landete *et al.* (2004). This test is performed by the sequential activity of the two enzymes, diamine oxidase (to break down histamine) and peroxidase (to produce a colour change). A linear correlation between optical density and histamine concentration is used for quantification. A very good correlation ($r^2=0.9984$) could also be established between biogenic amine quantification by this enzymatic method versus HPLC analysis. The advantage of this method is its limited sample preparation and time-consumption, and it does not require expensive or sophisticated equipment or training.

Enzyme-linked immunosorbent assays (ELISA) are commonly used for the quantitative analysis of histamine in scombroid fish. Such a test was applied to wine samples for the first time by Marcobal *et al.*, (2005b). No false negative results were obtained by the ELISA test, although there was a slight overestimation of histamine in a few samples when correlated to the results obtained by reverse phase-HPLC ($r=0.91$). This rapid, easy method could be used for screening in laboratories that are not equipped with an HPLC, in order to distinguish between wines with a histamine content of more or less than 10 mg/L.

Thin-layer chromatography

Thin-layer chromatography (TLC) was one of the first techniques used for the determination of biogenic amines in foods (Halász *et al.*, 1994). Despite the advantage of not requiring special or expensive equipment, TLC is known to be time consuming and only semi-quantitative.

A simple and rapid qualitative TLC method for the determination of the ability of bacteria to produce biogenic amines in liquid culture media containing the amino acid precursor is described by García-Moruno *et al.* (2005). This method improves on previous TLC methods due to the omission of an extraction step from the bacterial supernatants. The fluorescent dansyl derivatives of histamine, tyramine, putrescine and phenylethylamine can be separated and identified using TLC. This method is not known to give false positive results. The only equipment involved is a photo camera and a UV transilluminator.

Quantitative methods

Wine is a complex matrix and biogenic amines are present at low concentrations, hindering the ease of determination. Biogenic amines are non-volatile, polar compounds, which makes their isolation from wine (an aqueous matrix) complicated. In addition, the aliphatic biogenic amines are difficult to detect as they exhibit no characteristic ultraviolet (UV) absorption, fluorescent properties or electrochemical activity. These properties prohibit the direct detection of biogenic amines, for example by spectrophotometric or fluorimetric methods. To solve this problem, biogenic amines have to be derivatised to enable their detection by higher absorbance wavelengths. Samples usually also require a clean-up and pre-concentration step. Solid phase extraction (SPE) is widely used and has even been automated. Liquid-liquid extraction of the analyte with organic solvent can also be exploited. Both these methods have their disadvantages such as losses of amines and low recoveries (García-Villar *et al.*, 2006).

Liquid chromatography

Presently, high-performance liquid chromatography (HPLC) methods seem to still be the most widely used analytical approach to test for the presence of biogenic amines. These methods usually

include pre- or post-column derivatisation and fluorimetric detection of the corresponding derivatives. Various HPLC methods have been described. Modifications and improvements are consistently being made to pre- and post column treatments and reagents in order to reduce preparation and analysis time and to improve resolution of biogenic amine peaks in the chromatogram (Soleas *et al.*, 1999; Marcobal *et al.*, 2005b).

O-phthalaldehyde (OPA) is one of the preferred derivatising agents, giving rise to highly fluorescent derivatives. Vidal-Carou *et al.* (2003) optimised a method using OPA for the determination of 12 biogenic amines (both primary amines and polyamines) in wine and other alcoholic beverages. Dansyl-chloride, the other popular derivatising reagent, can react with primary, secondary and tertiary amino groups under selected conditions. These derivatives are stable and also fluorescent and therefore detectable in the UV region.

Fluorenylmethylchloroformate (FMOC) can be employed as derivatisation reagent to determine spermine and spermidine concentrations in addition to other biogenic amines, along with the simultaneous detection of their precursor amino acids in wine (Bauza *et al.*, 1995). Another derivatisation reagent that can react with primary, secondary and aromatic primary amino groups is 1,2-naphthoquinone-4-sulfonate (NQS). García-Villar *et al.* (2006) used liquid-liquid extraction with chloroform to preconcentrate biogenic amines and to remove polar compounds such as amino acid derivatives which interfere with subsequent separation steps.

8-phenyl-(4-oxy-acetic acid *N*-hydroxysuccinimide ester)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (TMPAB-OSu) is another recently synthesised fluorescent reagent that can be used for rapid pre-column derivatisation for HPLC analysis of primary as well as secondary biogenic amine concentrations in wine (Li *et al.*, 2006).

Another HPLC method proposes the direct injection of samples derivatised with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). No other pre-treatment (extraction) is required. Histamine, putrescine, cadaverine and tyramine in musts and wines can be analysed with this method (Hernández-Orte *et al.*, 2006).

Gómez-Alonso *et al.* (2007) use diethyl ethoxymethylmalonate (DEEMM) as derivatising agent to form aminoenones which can be detected in the UV-visible region. By this method, nine biogenic amines, 24 amino acids and the ammonium ion can be analysed simultaneously in a single injection by reverse phase-HPLC. The authors claim that this method is an improvement to previous HPLC methods that can simultaneously quantify amines and amino acids.

Loukou & Zotou (2003) presented the first HPLC-fluorescence method (as opposed to HPLC-UV) to simultaneously assay 11 oenologically important biogenic amines. An HPLC-diode array detection-atmospheric pressure chemical ionisation mass spectrometry system (HPLC-DAD-APCI-MS) was used to characterise dansylamides for the first time in wine and other alcoholic beverages after pretreatment with polyvinylpyrrolidone (PVP). Pretreatment is used to remove substances from the matrix that can interfere in the derivatisation and quantification. A similar method (reverse phase HPLC-DAD) was described by mo Dugo *et al.* (2006) that does not require any sample pretreatment before derivatisation with dansyl chloride.

Alberto *et al.* (2002) described two reverse phase HPLC methods, one of which uses pre-column derivatisation with OPA to separate and quantify both biogenic amines and amino acids in a single run.

Micellar liquid chromatography (MLC) uses a surfactant solution instead of aqueous-organic solvents as is used by HPLC. The method described by Gil-Agustí *et al.* (2007) does not require pre-treatment of samples (other than filtration) or an extraction procedure prior to analysis, and can be performed with a single direct injection. With this method, tryptamine and tyramine and their precursors concentrations, tryptophane and tyrosine, can be determined simultaneously in wine samples. Micellar electrokinetic chromatography (MECC) separation with laser-induced fluorescence (LIF) detection is another method that can be applied for the quantification of a large number of biogenic amines and amino acids in wine (Nouadje *et al.*, 1997).

A more recent liquid chromatography method to simultaneously analyse ten oenologically important biogenic amines has been proposed (Hernández-Borges *et al.*, 2007). Nano-liquid chromatography with UV detection was used to quantify the biogenic amines with dansyl-chloride as derivatising agent and employing SPE for post-derivatisation clean-up and to extract the analytes from the wine. Other liquid chromatography techniques that are applicable to biogenic amine analysis in wine have been described by Ibe *et al.* (1991); Hlabangana *et al.* (2006) (ion-pair liquid chromatography) and Millán *et al.* (2007) (liquid chromatography-electrospray ionisation ion trap mass spectrometry).

Capillary electrophoresis

Capillary electrophoresis (CE) methods are attractive due to their short analysis time and high resolution. One problem is their lack of sensitivity, which can be overcome by coupling the CE to mass spectrometry (MS) detection, instead of UV detection. Other detection systems are also available. For example, a CE method using conductometric detection and which requires no derivatisation or sample cleaning steps was developed recently by Kvasnicka & Voldrich (2006). This direct method is sensitive and can detect biogenic amines in foods and wine in less than 15 minutes. A high-performance capillary electrophoresis (HPCE) method exists to determine biogenic amines in wine, amongst other foodstuffs (Kovács *et al.*, 1999).

An automated method for the determination of biogenic amines in wine has been described in order to exclude manual pretreatment of the sample (Acre *et al.*, 1998). This method employs the use of a minicolumn for solid-phase extraction (SPE) to simultaneously clean-up and concentrate the sample prior to analysis by capillary electrophoresis (CE) with indirect UV detection, in a flow system. This method allows for the separation and determination of a wide range of biogenic amines present in wine in less than 15 minutes, compared to 25 minutes by HPLC. Another advantage over HPLC is the direct detection. As noted, HPLC determination requires derivatisation of the amine due to aliphatic biogenic amines containing no chromophores that absorb significantly in the UV-visual region.

Another automated method is described by Santos *et al.* (2004) for biogenic amine determination in white and red wines. They proposed the use of capillary electrophoresis-electrospray mass spectrometry (CE-ESI-MS) for the separation and quantification of

nine biogenic amines. Simó *et al.* (2008) compared the suitability of capillary electrophoresis–ion-trap mass spectrometry (CE–IT-MS) and capillary electrophoresis–time-of-flight mass spectrometry (CE–TOF-MS) to analyse biogenic amines in wine. Biogenic amines could be quantified in wines using both methods, without any previous treatment or derivatisation of wine samples. CE–TOF-MS showed the better capability to analyse ten oenological biogenic amines in a single analysis. Concentrations as low as 10 ng/mL could be detected and the analysis time of this method is fast (8 minutes by CE–TOF-MS compared to 40 minutes by HPLC).

Gas chromatography

Volatile amines have been measured in the past by gas chromatography. A very sensitive and accurate gas-chromatography/mass spectrometry method was developed by Fernandes & Ferreira (2000), with a run time of only 18 minutes. This method is the first GC-MS method that allows for the simultaneous determination of diamines, polyamines and aromatic amines in grape juice and various wine styles tested. The conversion of biogenic amines to their corresponding volatile (*o*-heptafluorobutryl) derivatives allows for determination by gas chromatography.

Polymerase chain reaction

The use of methods that can rapidly detect the presence of biogenic amines and biogenic amine producing lactic acid bacteria at an early stage is important for preventing the accumulation of biogenic amines in wine and other food products. PCR techniques have been developed to detect bacterial amino acid decarboxylases in a rapid, sensitive and accurate manner. Thus, PCR cannot determine quantitative (or qualitative) amounts of biogenic amines; but it can be used to estimate the potential risk of amine formation (Coton *et al.*, 1998a). Since molecular methods are independent of the culture conditions, it can be used to detect biogenic amine producers even under circumstances where the lactic acid bacteria had lost the ability to produce biogenic amines. Such instances have been reported after prolonged storage or cultivation in synthetic media (Lonvaud-Funel & Joyeux, 1994; Leitão *et al.*, 2000).

In principle, some DNA sequences of some genes are highly conserved, so PCR can be applied to detect specific genes in various organisms. Specific oligonucleotides for the amplification of histidine-, tyrosine-, ornithine- and lysine decarboxylase genes have been designed to detect the presence of bacteria which can possibly produce the corresponding biogenic amine in wine.

Le Jeune *et al.* (1995) developed degenerate primers to detect HDC positive lactic acid bacteria. These primers were based on nucleotide and amino acid sequences of *Lactobacillus* 30a and *C. perfringens* and the amino acid sequences of *L. buchneri* and *Micrococcus* sp. The primers allowed for amplification of a HDC gene fragment using DNA of these bacterial strains. The HDC gene of *O. oeni* has also been described (Coton *et al.*, 1998b). Another set of universal primers for the detection of HDC genes in Gram-positive bacteria was developed by Coton & Coton (2005). These primers are based on HDC gene nucleotide sequences from a variety of lactic acid bacteria such as *O. oeni* IOEB 9204, *Lactobacillus* 30a and *C. perfringens*.

By a similar approach, universal primers were designed by Coton *et al.* (2004) for the early detection of potential tyramine-producing bacteria in wine. Marcobal *et al.* (2005) designed the first complete set of primers to amplify the gene coding for ODC by aligning

amino acid sequences of ODCs from Gram-positive and Gram-negative bacteria. De las Rivas *et al.* (2006) described the first PCR assay to determine the presence of lysine decarboxylase positive, cadaverine producing microorganisms in food.

Multiplex amplification methods were developed to reduce the quantity of reagent, labour costs and time, because more than one gene species (target amines) can be detected simultaneously using multiplex PCR, as opposed to uniplex PCR (Coton & Coton, 2005; De las Rivas *et al.*, 2005; Marcobal *et al.*, 2005a). Molecular methods for the detection of biogenic amine producing bacteria have been reviewed recently by Landete *et al.* (2007a).

Using biogenic amines as an analytic tool

Different chemometric procedures have been applied to establish criteria for differentiation in wines (Rius & Massart, 1991). Principal Component Analysis (PCA) has proved to be a useful tool for pattern recognition, classification, modelling and data evaluation. In a number of recent studies, wines have been successfully classified on the basis of biogenic amines.

Souffleros *et al.* (1998) used PCA to classify French wines of four regions according to wine style and origin by analyses of amino acids, biogenic amines, volatile compounds, and organic acids. Wines made from botrytised grapes formed a separate group.

Csomós *et al.* (2002) distinguished Hungarian red and white wines from the same geographic origin and vintage using PCA, according to their biogenic amine and polyphenol content. A further study concerning Hungarian wines concluded that, on the basis of the results of chemometric analyses (PCA and linear discriminant analysis), free amino acid and biogenic amine contents seem to be useful to differentiate wines according to winemaking technology, geographic origin, grape variety, and year of vintage (Héberger *et al.*, 2003). García-Villar *et al.* (2007) also propose the use of simple chemometric techniques such as PCA and partial least-squares regression to characterise wines. Their results indicate that biogenic amines could be used as descriptors of enological practices and ageing patterns in Spanish wines.

Kiss *et al.* (2006) performed a study with the objective to compare the amine composition of Aszu grapes (noble rotten grapes) with that of gray rotten berries (infected mainly with *B. cinerea*) and of berries infected with other local green molds (mainly *Penicillium*). Using multivariate statistical analyses (principal component analysis and linear discriminant analysis) intact grapes, Aszu grapes, and grape berries infected mainly with *Penicillium* species could be separated from each other in grape samples with the same origin. The composition and concentration of biogenic amines might also provide useful information on the authentication of the Tokaj aszu wines (Hajós *et al.*, 2000) and could be used in grapes, wines and aszu wines for quality control purposes (Sass-Kiss *et al.*, 2000; Kiss & Sass-Kiss, 2005).

CONTROL OF BIOGENIC AMINE PRODUCTION

Due to indigenous lactic acid bacteria, including *O. oeni*, being able to produce biogenic amines during malolactic fermentation, the application of a genetically engineered malolactic wine yeast strain, capable of the complete degradation of L-malic acid in wine, has been proposed (Husnik *et al.*, 2006). According to the authors, this yeast could prevent the formation of biogenic amines in wine by omitting the need for lactic acid bacteria to perform malolactic fermentation. Currently, consumer rejection

of genetically modified organisms in wine producing countries still renders this option unavailable in most countries.

It is known that some microorganisms, among them lactic acid bacteria, are capable of degrading biogenic amines by amine oxidase enzyme activity. Consequently, Leuschner *et al.* (1998) studied a large number of food fermenting organisms to screen for the potential to degrade histamine and tyramine. Unfortunately, at this stage, amine degradation seems to be restricted to aerobic microorganisms which are of limited use in fermented foods such as wine which harbours an anaerobic environment.

Presently, the only realistic option to control the potential problem of biogenic amines is by inhibiting the growth of decarboxylase positive indigenous bacteria and other spoilage microorganisms. Sulphur dioxide is known to have antimicrobial properties. Molecular SO₂ can act to inhibit lactic acid bacteria in wine. The antibacterial activity of SO₂ is pH-dependent and will decrease with an increase in pH. Lysozyme is an enzyme that can cause lysis of the cell walls of Gram-positive bacteria, including wine lactic acid bacteria. Lysozyme retains its activity in higher pH wines. It can be successfully used to delay or inhibit the growth of most lactic acid bacteria, especially when used in combination with SO₂ (Delfini *et al.*, 2004; Ribéreau-Gayon *et al.*, 2006). Bacteriocins are antimicrobial peptides produced by some strains of lactic acid bacteria. Nisin is a commercially available bacteriocin that acts on the cytoplasmic membrane of Gram-positive bacteria. The possibility exists to exploit the synergistic effect of nisin and metabisulphite (SO₂) on growth inhibition of wine spoilage lactic acid bacteria for wine preservation (Rojo-Bezares *et al.*, 2007) but it has not been authorised for application in wine. The potential use of natural phenolic compounds as antimicrobial agents to control the growth of lactic acid bacteria in wine has also been proposed (García-Ruiz *et al.*, 2007).

Inoculation with *O. oeni* starter cultures that are unable to produce biogenic amines is a viable option for the control of these compounds in wine (Martín-Álvarez *et al.*, 2006). It seems that co-inoculation of *O. oeni* starter cultures together with alcoholic fermentation has the potential to curb biogenic amine formation even more than conventional inoculation for malolactic fermentation after the completion of alcoholic fermentation (Van der Merwe, 2007).

While regulating the soil and vine nutritional status are the only viticultural manipulations that could be made to control the accumulation of biogenic amines in grapes, their occurrence in musts or wine could possibly also be controlled by reducing practices that increase amino acid extraction such as grape skin maceration and lees contact.

CONCLUSION

The distribution of biogenic amine producers amongst wine microorganisms seems to be random and not a species specific quality. Lactic acid bacteria are the wine microorganisms that are, for the most part, associated with amino acid decarboxylation and biogenic amine formation. However, *O. oeni* seems to have a low distribution of tyrosine-, histidine-, and ODC genes in its genome. The most important increase of biogenic amines takes place during malolactic fermentation, when compared to the contributions by alcoholic fermentation and ageing respectively. The contribution of yeast to biogenic amine production could be indirect (by amino acid secretion and autolysis) or direct, where killer positive strains

produce the highest concentration of biogenic amines. Strains of *B. bruxellensis*, followed by strains of *S. cerevisiae* were found to produce significant concentrations of biogenic amines.

Biogenic amine concentrations in wine may be influenced by their presence in grape berries; dictated by factors such as soil potassium deficiencies, grape variety, geographical region and vintage. The concentrations of precursor amino acids are influenced by winemaking practices such as grape skin maceration. Biogenic amine formation is also determined by wine parameters and components of which pH, ethanol, SO₂ and pyridoxal 5'-phosphate have the most important effect on the diversity of microorganisms, decarboxylase enzyme activity and decarboxylase gene expression. The concentration of biogenic amines is dependent on wine type and style, but the presence of biogenic amines seems to be attributed to the presence of lactic acid bacteria in all cases.

PCR reactions can detect the presence of biogenic amine producing lactic acid bacteria and thereby estimate the potential risk of formation of histamine, tyrosine, cadaverine and putrescine in wine. Biogenic amines can be measured qualitatively (with screening and enzymatic methods), semi-quantitatively (by thin-layer chromatography) or quantitatively (using liquid chromatography, capillary electrophoresis or gas chromatography).

Biogenic amine formation in wine is most likely to be prevented by inhibition of indigenous lactic acid bacteria and other spoilage microorganisms that could possess decarboxylase activity.

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