LALLEMAND WINE EXPERT

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# BIOGENIC AMINES IN WINE

## What are biogenic amines?

Biogenic amines are a class of small nitrogen containing organic compounds found in fermented foods, such as cheese, fermented sausage, pickled vegetables and fermented beverages (beer, cider and wine). In these products, ten different biogenic amines can be formed by the enzymatic decarboxylation of naturally occurring amino acids. In wine, the main biogenic amines are histamine, tyramine, cadaverine and putrescine, respectively obtained from the decarboxylation of histidine, tyrosine, lysine and ornithine or agmatine (Figure 1). Other biogenic amines occasionally found in wine include ethylamine, ethanolamine, phenylethylamine, dimethylamine, spermine and spermidine.

It is important to remember that biogenic amines are also naturally occurring compounds in animals, including humans, where they can act as hormones and neurotransmitters influencing various metabolic functions including blood pressure and body temperature.  Histamine can be released by the body in response to allergens and tissue damage.

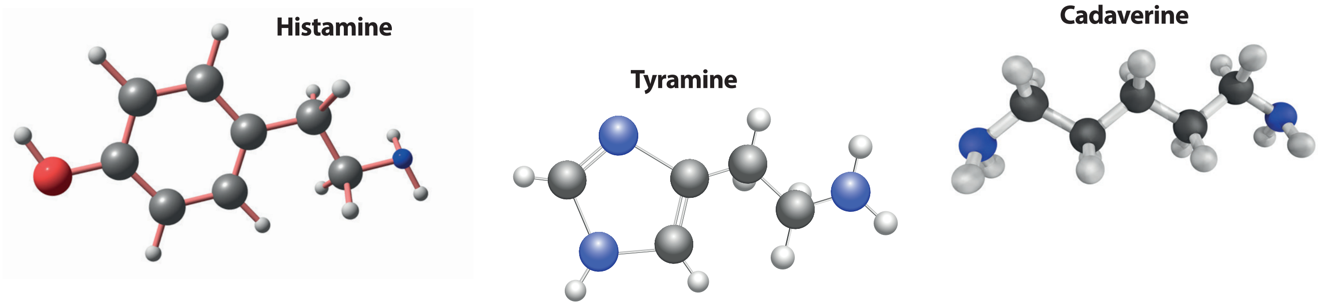



Figure 1. Three of the most important biogenic amines found in wine

## What are the impacts of biogenic amines in wines?

The presence of biogenic amines (BA) in wine is becoming increasingly important to consumers and producers alike, due to the potential threats of allergic reaction,  toxicity to humans and consequent trade implications. A significant amount of research has been published regarding the negative physiological impact from excess histamine. Symptoms can include nausea, hot flashes, headaches, facial flushing and respiratory distress. Generally, the levels of histamine in wine are not enough to cause problems with most individuals. Some people, however, may be unable to adequately metabolize even low levels of histamine. In such cases, the cumulative amount of histamine (also coming from other food sources) could conceivably cause concern. If a low concentration of biogenic amines is ingested, they are normally quickly metabolised by amine oxidases to produce aldehyde, hydrogen peroxide and ammonia, although the presence of ethanol and acetaldehyde may inhibit this normal metabolism in humans. Therefore, the presence of high BA concentrations is related to wine safety (European Food Safety Authority 2011), but they may also be indicators of wine quality and hygienic conditions.

Biogenic amines may cause a reduction in overall wine aroma, resulting in wines that have lost varietal character. High levels of biogenic amines can result in the formation of metallic, meaty or putrid aromas. This was demonstrated by research conducted at the Universidad de La Rioja, Spain (Palacios *et al*., 2004), where putrescine and cadaverine were added to red wine. Increasing concentrations of putrescine did not result in detection of the compound itself, but such descriptors as rotten fruit, rancid and dirty were used to describe the overall flavour impact. Cadaverine was not only readily identified as the compound involved, but at higher concentrations wine was described as causing meaty, vinegary and dirty aromas.

## What are the legal limits for biogenic amines?

There are no legal limits for biogenic amines in wines even though some countries have recommended upper limits of histamine in wine (Costantini *et al*., 2019; Smit *et al*., 2008). The OIV advocates the use of viticultural and winemaking practices that reduce the risk of elevated histamine concentrations in grapes and wine.

## Where do biogenic amines originate from?

Even though yeast may contribute to amine production, it is the wine bacteria that significantly contribute to the wine biogenic amine content. Biogenic amines are mainly formed by lactic acid bacteria (LAB) belonging to the species within *Lactobacillus, Pediococcus* and *Oenococcus*. Not all the strains within these species have the capacity to produce biogenic amines. It depends on the presence or absence in their genomes of genes coding for amino acid decarboxylases, hence the importance of a rigorous wine bacteria selection process to avoid the production of BA. Also, in the context of global warming, higher musts pH, low $SO_2$ strategies, water stress in the vineyards, are all conditions promoting the development of spoilage microorganisms that produce biogenic amines. There is a resurgence of high contents of BA in wines and it could results in demands to limit their content more severely.

## What are the mechanisms of formation of biogenic amines by wine bacteria?

The first biogenic amine decarboxylase to be studied in detail was the histidine decarboxylase (HDC) enzyme isolated from a histamine producing wine LAB strain by Lonvaud-Funel & Joyeux (1994) and has since been studied by various authors. Coton *et al*. (1998b) purified this HDC enzyme to homogeneity and characterization provided valuable structural and biochemical pathway (Constantini et al 2019) (Figure 2). 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). Today selected bacteria strains are checked via sequencing so it is easy to detect the absence of the amino acid decarboxylase genes.

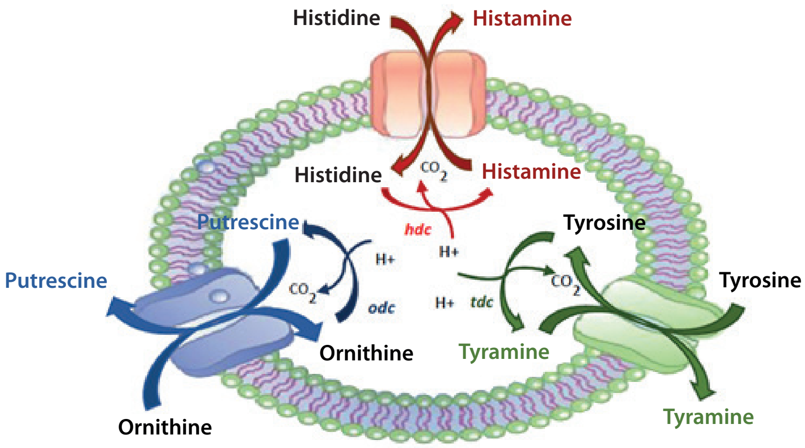



Figure 2. Biogenic amine formation from their precursor amino acids and their intake/uptake by the transmembrane antiporter

## What conditions influence their concentrations?

Nitrogenous fertilization of the soil, the poor state of health of the grapes combined with mold, a high must pH and the development of certain yeasts during alcoholic fermentation can all favor a moderate level of biogenic amines; thereafter, certain lactic acid bacteria can, significantly increase the presence of biogenic amines in wines. Post-fermentative maceration and prior to bottling can also favour the formation of biogenic amines. They are also more prevalent in red wines compared to white wines.

In general, biogenic amines levels are higher in wines with high pH due to the presence of various contaminating microflora. It can be deduced that climate change could result in higher biogenic amine levels. However, even at lower pH, **spontaneous malolactic fermentation** can be associated with the production of biogenic amines as there is always a risk that MLF is conducted by LAB having the capacity to produce biogenic amines. It is possible to find musts which already contain significant BA content depending on the vintage, the grape variety, the terroir and climate. It is important to control the increase of BA in these compromised wines as quickly as possible by inoculating for MLF with a selected bacteria unable to produce BAs.

# RESULTS

## How to minimise biogenic amine production in wine?

Different strategies can be used to reduce biogenic amines in the wine. $SO_2$ addition or the use of Bactiless™ (chitosan based natural product from *Aspergillus niger*) can help reduce the number of indigeneous LAB producing biogenic amines, often found in high numbers in high pH must. Proper management of MLF is critical to controlling biogenic amines. This can be achieved by complete and thorough cellar sanitation, good wine pH management, stabilize wine with sulfur dioxide, and by inoculation with only known, selected and characterized wine LAB strains.

An experiment testing LAB selected strains and indigenous microflora for their ability to produce histamine and tyramine in a Pinot noir wine highlighted the importance of using known selected LAB strains for conducting MLF (IFV – Vincent Gerbaux, 2015). Selected strains are screened specifically for their inability to produce biogenic amines.

| Analysis after MLF (mg/L) | Pinot noir | | Pinot noir + histidine + tyrosine | |
|---|---|---|---|---|
| | Histamine | Tyramine | Histamine | Tyramine |
| Selected LAB | < 1 | | < 1 | |
| Indigenous flora | 13.8 | 2.7 | 50.3 | 8.3 |

Table 1. Biogenic amine production in Pinot Noir with selected wine bacteria and during spontaneous malolactic fermentation.

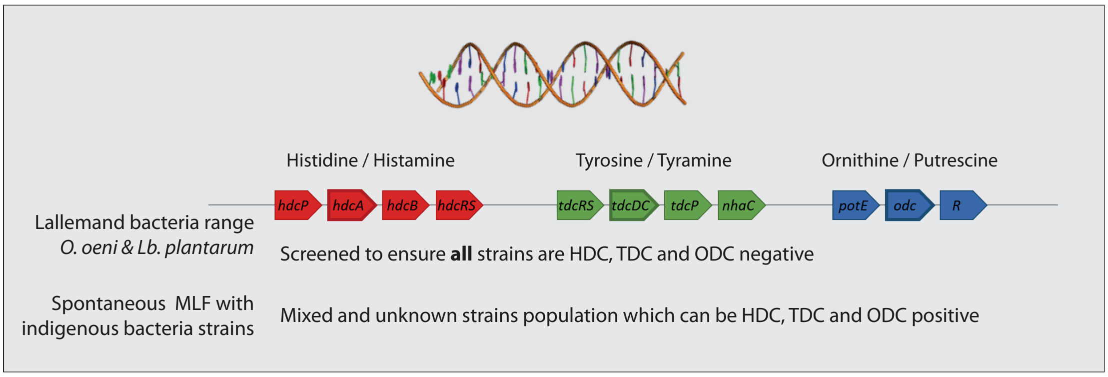



Figure 3. Summary of the screening mechanisms to test for the ability of LAB to produce biogenic amines.

Co-inoculation with selected wine bacteria not able to produce biogenic amines to perform MLF is beneficial to minimize the risk of indigeneous microflora growth and over-production of biogenic amines.

In the example, different wine trials demonstrate that inoculation with selected bacteria strains reduces the biogenic content of wine (Figure 4); control is a spontaneous MLF.

Co-inoculation also ensures that the wine can be stabilized quickly and will also reduce the risk of production of BA during the steps prior to bottling. Moreover, as malolactic bacteria are compatible with organic winemaking, they are even more important in order to control the levels of BA in those wines.

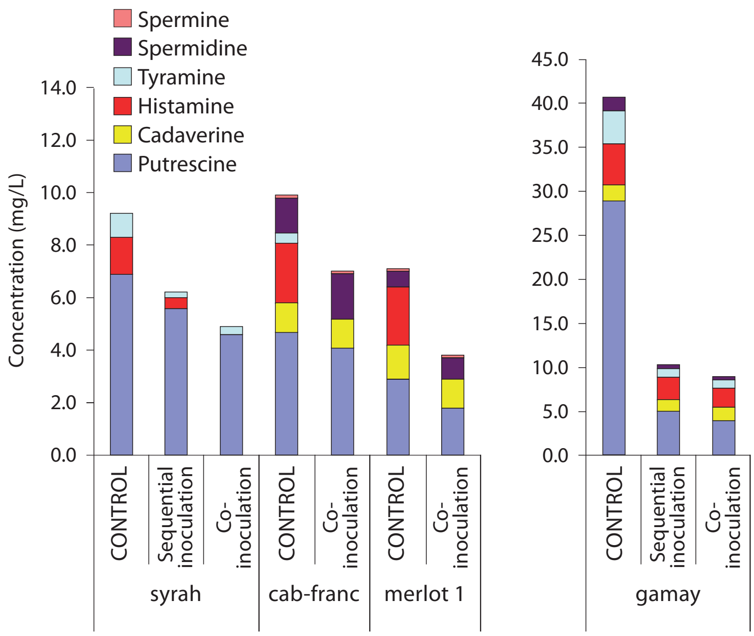



Figure 4. Biogenic amine levels after malolactic fermentation. Comparison between different timing of inoculation

With higher pH wines where the molecular $SO_2$ will be < 0.5 mg/L , the use of Bactiless™ following MLF is recommended, providing added security to the wine.

THE WINE EXPERT

# A word from our expert

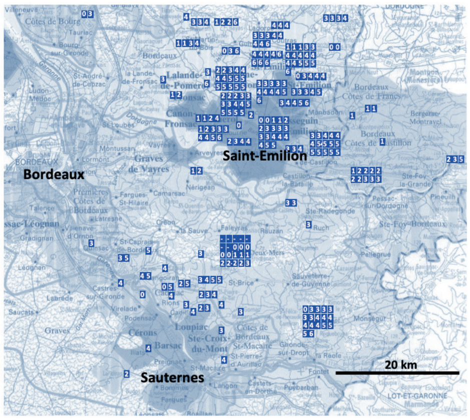
*Patrick LUCAS is professor of wine microbiology and deputy director of the Oenology Research Unit at ISVV of the University of Bordeaux. After a doctorate in biochemistry and a post-doctorate in Canada, he joined the Oenology Research Unit in 2003 to undertake research on lactic acid bacteria in wine. He specialized in the study of bacteria producing biogenic amines, notably during two European projects from 2003 to 2012, and in the study of the biodiversity of the bacterium* Oenococcus oeni *and its role during malolactic fermentation. He has directed the work of 11 doctoral and post-doctoral students, carried out numerous national and international collaborations, and is widely published with over 70 articles.*

Biogenic amines are a matter of concern in all food production. The earliest scientific work related to it dates back to the 1940s, and now there are over 11,000 scientific publications. For wine alone, there are around 400 publications, which underlines the importance of this theme for the wine industry.

In the early 2000s, a great deal of research was undertaken to identify the bacteria that produce the main biogenic amines in wines: histamine, putrescine, tyramine and cadaverine. Some strains of *Oenococcus oeni*, *Lactobacillus* spp. and *Pediococcus* spp., but only those that have the required genes are able to produce biogenic amines. Using this knowledge, analytical tests have been developed to detect bacteria strains that produce one or more biogenic amines. They are now offered by laboratories to analyze the quality of wines, or that of selected bacteria.

In 2005, we used these tests to look for histamine-producing bacteria in 264 wines undergoing spontaneous malolactic fermentation. We detected histamine-producing bacteria in 70% of wines at significant population levels. In some wines, the histamine content was already above 10 mg/L before the end of MLF. All the bacteria responsible were *Oenococcus oeni*, which are native bacteria capable of producing MLF at the same time as they produce histamine. The risk of the appearance of histamine or other biogenic amines is therefore permanent and significant in the case of spontaneous MLF.

In 2011, on the basis of all the scientific studies published, the OIV issued a code of good viticulture and vinification practices aimed at limiting the content of biogenic amines in wines. The main recommendations are to avoid the presence of native bacteria during MLF, by carrying out fermentation using selected bacteria, and to avoid the presence of native bacteria during the aging and storage periods of the wines.




Numbers represent the population levels of histamine-producing bacteria in 264 wine during MLF vintage 2004: 6= above $10^{E}6$ cells/ml, 5= above $10^{E}5$ cells/ml, etc.

# Summary

Biogenic amines are found in fermented food and beverages, including wine. Of the many biogenic amines, histamine, tyramine and putrescine are the most important in wine. It is the metabolism of amino acids by lactic acid bacteria that produce the biogenic amines found in wine. Red wines tend to have higher biogenic amines content than white wines, as this wine type all under go the bacteria driven malolactic fermentation.

The direct decarboxylation of amino acids results in the formation of biogenic amines. Extensive biochemical and genomic characterisation has led to simple tests for the identification of biogenic amine genes in LAB strains. Wines produced using native microflora can have high biogenic amine content. Consumer safety justifies taking extra precautions to avoid the production of biogenic amines.

Good winemaking practices should be used to avoid the production of biogenic amines; management of must and wine pH to minimise the proliferation of native microflora, stabilise musts or wines for antimicrobial protection with $SO_2$ or new biological solutions (such as Bactiless™) and use malolactic bacteria strains (and particularly in co-inoculation) that have been screened for the absence of biogenic amine genes. Compatible with organic winemaking or in a strategy to reduce chemical additions, the use of malolactic bacteria is a key step to achieve wines with low to no biogenic amines.