BIOGENIC AMINES: AVOID SPONTANEOUS MLF

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BIOGENIC AMINES: A PROBLEM RECOGNIZED BY THE EUROPEAN UNION

Although consequences are considered moderately dangerous, biogenic amines are the second cause of food poisoning in Europe. For sensitive consumers, they suffer headaches, flushing and nausea.

Biogenic amines are present in fish, sausages, sauerkraut and some mature cheeses, but also in fermented beverages: beer, cider and wine. In humans, enzymes which can lower their adverse effects are inhibited by ethanol and acetaldehyde, two compounds present in wine. This explains why, while it contains much less biogenic amine than other food products, wine is frequently highlighted.

There are 10 different biogenic amines:

- Heterocyclic amines: Histamine, Serotonine et Tryptamine
- Polyamines : Putrescine, Cadavérine, Agmatine, Spermidine, Spermine
- Monoamines : Tyramine and phenylethylamine.

In wine, histamine is by far the most common, followed by tyramine, putrescine and cadaverine.

Currently, the histamine content of wine is already regulated in countries like Switzerland and Canada, and some wine marketing companies apply their own rules. In general, the maximum histamine level tolerated is 10 milligrams per litter.

The EU is particularly attentive to health and economical problems represented by biogenic amines. It is currently funding an extensive research project involving a dozen laboratories in various universities and private companies, including the SARCO laboratory of the LAFFORT group, and the Faculty of Oenology in Bordeaux ISVV (European Project BIAMFOOD, www.biamfood.eu). This project aims to understand how these biogenic amines are produced in wine, to develop methods for determination of biogenic amines, and offer winemaking advice on how to avoid the formation of biogenic amines in wine.

SPONTANEOUS MLF: THE SOURCE OF BIOGENIC AMINES IN WINE.

For the organisms that produce them, biogenic amines are produced in response to stressful situations: acid stress, oxidative stress or osmotic stress. Thus, we can find biogenic amines in all living cells, including grape cells. However, it is only after uncontrolled microbial growth that levels become dangerous.

Even though some *Saccharomyces* yeasts can produce biogenic amines, like the spoilage yeast *Brettanomyces bruxellensis* (Caruso *et al.* 2002), lactic acid bacteria are the main indigenous microorganisms responsible for the production of biogenic amines in wine. The biggest producers are the *Lactobacilli*, but also *Pediococci* and many *Œnococcus œni* indigenous strains (Lonvaud-Funel and Joyeux 1994).

Bacteria that are able to produce biogenic amines have specific genes and enzymes (Lucas *et al.* 2005) that allow them to convert the amino acids present in wine into histamine, tyramine, putrescine, cadaverine and other biogenic amines which are harmful to wine quality.

The wines most sensitive to biogenic amine contamination are usually high pH wines, since the diversity of native microflora, and therefore the probability of bacteria producing biogenic amines being naturally present in the wine, is very strong. But even at classical wine pH (between 3.3 and 3.8), risks are also significant with spontaneous MLF, particularly if the ethanol concentration is high.

In 2005, Patrick Lucas, lecturer at the university of Bordeaux, conducted a large study of Bordeaux vineyards and wines. During spontaneous MLF, of the approximately 300 wines (spanning 100 different producers) 70% of the wines had more than 10^3 cells/mL of indigenous lactic acid bacteria capable of producing histamine (Lucas *et al.* 2008). In most cases, the organism present was indigenous *Œnococcus œni*. The study also revealed that this level of contamination was sufficient to produce more than 10 mg/L of histamine during spontaneous MLF.

In 2008, further monitoring showed that at the end of spontaneous MLF, the histamine content can reach more than 30 mg/L, more than three times the standard tolerated by certain regulatory bodies. Again, in most cases the cause was indigenous *Œnococcus œni*. This study also established that this population level was sufficient to produce more than 10 mg/L of histamine during spontaneous MLF.

This year the indigenous populations of lactic acid bacteria at the end of AF were sometimes high and, faced with relatively high alcohol levels, the ability to produce biogenic amines provided a selective advantage to strains that expressed these metabolites, so that the majority of the dominant indigenous bacteria were capable of producing biogenic amines during spontaneous MLF.

TOOLS TO CONTROL BIOGENIC AMINES IN WINE

The best way to control biogenic amines is without any doubt the use of a malolactic starter culture. Identification of genes involved in the production of biogenic amines has allowed the development of effective genetic tests (Nannelli et al. 2008) to ensure that commercial bacterial strains developed for use as malolactic starters are incapable of producing biogenic amines. In the LAFFORT LACTOENOS range, no bacteria are capable of producing histamine or any other biogenic amines. The addition of a massive population of a selected malolactic stater suppresses the harmful activities of the indigenous population, including the production of biogenic amines (Nannelli *et al.* 2008).

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The table below illustrates results obtained after a post-AF inoculation with **LACTOENOS 450 PreAc®** and **LACTOENOS 350 PreAc®** compared with spontaneous MLF.

	Wine 1		Wine 2	
	MLF with **LACTOENOS 450 PreAc®**	Indigenous MLF	MLF with **LACTOENOS 350 PreAc®**	Indigenous MLF
Histamine	None detected	36 mg/L	1,2 mg/L*	28 mg/L

** Produced by indigenous lactic acid bacteria prior to inoculation with Lactoenos 350 PreAc.*

The addition of **LACTOENOS PreAc® 450** or **LACTOENOS 350 PreAc®** after devatting is, therefore, a very effective way to prevent the production of histamine in wines.

Note also that adding **MALOSTART®**, designed to overcome the nutritional deficiencies of the environment, is a safe practice because the product has been specially formulated to provide essential amino acids to the growth of bacteria without providing to the bacteria biogenic amine precursors. Yeast cell walls **(BIOCELL®)** are also used to detoxify the medium (adsorption of medium chain fatty acids) and to facilitate the beginning of MLF, also do not provide biogenic amine precursors.

IN CONCLUSION

Although conditions (pH ...) and the presence of indigenous lactic acid bacteria after post-AF maceration appear able to favour a spontaneous onset of MLF, the risk of biogenic amine production is not avoided. Instead, this year for example, the conditions (alcohol, pH ...) and the proportion of indigenous lactic acid bacteria capable of producing biogenic amines are increased, and the contamination should be frequent if not managed.

The addition of a malolactic starter from the LAFFORT range is the best way to prevent the production of histamine during fermentation. Subsequently, since these bacteria prevent the growth of indigenous bacteria, stabilization and spoilage prevention are also facilitated during maturation.

Finally, it is important to note that the determination of histamine concentration is now possible at the SARCO laboratory like the detection and enumeration of lactic acid bacteria producing biogenic amines.

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