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# Elaboration of Tempranillo wines at two different pHs. Influence on biogenic amine contents

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ABSTRACT

The aim of this work was to study the effect of pH on biogenic amine formation during the elaboration and conservation (7 months) of Tempranillo wines. Grapes at two pHs (3.4 and 3.7) were vinified to achieve this purpose. After alcoholic fermentation the wines were either inoculated with a commercial malolactic starter or not inoculated. Identification and clonal distribution of lactic acid bacteria and amino acid concentration were determined in an attempt to explain the biogenic amine generation. The results showed that the pH of the must influenced the clonal distribution of the *Oenococcus oeni* strains which conducted the malolactic fermentation and also the concentration of amino acids in the wines after alcoholic fermentation. These aspects could account for the higher biogenic amine formation in wines with the lowest pH during malolactic fermentation. In these wines, inoculation with a malolactic starter was favourable since it produced a lower biogenic amine concentration after malolactic fermentation. Furthermore, the pH also influenced the concentration of amino acids after malolactic fermentation and the lactic acid bacteria distribution during the conservation of the wines. These aspects allow explaining the greater formation of histamine, tyramine and putrescine in the wines with the lowest pH vinified via lactic acid bacteria inoculation after seven months of conservation.



## 1. Introduction

Biogenic amines (BA) are low molecular weight organic compounds that are found in cases of food poisoning, in fermented foods like cheese, meat, fish products and wine (Silla Santos, 1996). Some amines are normal constituents of grapes and the amounts varying with grape variety, region, soil type and composition, fertilisation and climatic conditions during grape ripening and degree of maturation (García-Villar, Hernández-Cassou, & Saurina, 2007; Gloria, Watson, Simon-Sarkadi, & Daeschel, 1998; Leitão, Marques, & San Romão, 2005; Soleas, Carey, & Goldberg, 1999). Besides the amines already present in grapes, several others can be formed and accumulate during winemaking.

The main BA found in wines are histamine, tyramine, putrescine, cadaverine and phenylethylamine (Beneduce et al., 2010; Coton, Rollan, Bertrand, & Lonvaud-Funel, 1998; Lonvaud-Funel, 2001; Soufleros, Barrios, & Bertrand, 1998). These amines can affect human health and lead to low quality wines. The formation of more or less quantity of these toxic compounds during winemaking basically depends on three aspects: availability of free precursor amino acids, presence of micro-organisms with decarboxylase activity and conditions which permit both microbial growth and the expression of the decarboxylase activity (ten Brink, Damink, Joosten, Huis in't, & Veld, 1990).

Wine fermentation is a complex process driven by micro-organisms such as yeasts and lactic acid bacteria (LAB). There are conflicting views regarding the possible formation of BA during AF, although authors who report the production of these compounds by the yeasts conclude that their concentration as a consequence of this reason is small (Granchi, Romano, Mangani, Guerrini, & Vincenzini, 2005; Torrea & Ancín, 2001). Malolactic fermentation (MLF) is generally considered a desirable transformation in red wines and usually takes place after alcoholic fermentation (AF), mainly produced by the action of the *Oenococcus oeni* (*O. oeni*) species (López et al., 2007). Other genera, *Lactobacillus*, *Leuconostoc* and *Pediococcus* may also be present and occasionally cause undesirable spoilage in wine. Evidence of amine formation during MLF has been described (Coton et al., 1998; Pramateftaki, Metaza,

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Kallithraka, & Lanaridis, 2006; Soufleros et al., 1998). BA formation was considered the result of wine spoilage from strains belonging to the *Pediococcus* genera (Delfini, 1989). Nevertheless, certain authors proved that some strains of the main malolactic species *O. oeni*, as well as *Lactobacillus* and *Leuconostoc* genera, were capable of performing decarboxylation of amino acids to produce BA (Coton et al., 1998; Moreno-Arribas, Polo, Jorganes, & Muñoz, 2003). Spontaneous MLF in wine, which is conducted by indigenous lactic acid bacteria originating from the vines and grape skins and surviving on winery equipment, is highly unpredictable. It implies several risks, such as a considerable increase in volatile acidity, microbiological problems associated with delayed MLF (Murat, Gindreau, & Augustin, 2007) and formation of undesirable metabolites such as BA (Liu, 2002; Marcobal, Martin-Alvarez, Polo, Muñoz, & Moreno-Arribas, 2006; Polo, Ferrer, Peña-Gallego, Hernández-Orte, & Pardo, 2011). Inoculation with starter cultures reduces the potential risks of spoilage ensuring the rapid development of MLF and avoiding the accumulation of BA (Manfroi, Silva, Rizzon, Sabaini, & Glória, 2009).

The quality impact of bacterial activity can be variable depending on factors which can influence the survival and growth of LAB, including chemical and physical composition of the wine and winery practices (Delaquis et al., 2000). Among these aspects, the pH of the wine is one of the essential factors in biological stability, since it influences the species which participate in the wines microbiota, in their growth rate, malolactic activity and in the nature of the substrates formed (Ribéreau-Gayon, Glories, Maujeau, & Dubourdieu, 1998). In general, wines with a pH below 3.3 may find difficulty in undergoing MLF, but a high pH can increase the susceptibility of the wine to microbial spoilage. Some authors have established a critical pH level between 3.5 and 3.6, above which it is more difficult to control the microorganism population, with the possibility of problems arising due to the production of BA (Bauer & Dicks, 2004; Renouf, Lonvaud-Funel, Walling, & Coulon, 2006). Many studies correlate the formation of BA with high values of pH in wine (Landete, Ferrer, Polo, & Pardo, 2005; Martín-Alvarez, Marcobal, Polo, & Moreno-Arribas, 2006; Moreno-Arribas, Marcobal, & Muñoz, 2003; Vázquez-Lasa, Iñíguez-Crespo, González-Larraina, & González-Guerrero, 1998).

Due to the health concerns of amines in wine, efforts should be made to understand their formation in wine to optimise processing technology so as to ensure low amine content.

Currently we are witnessing a worldwide increase in the pH of wines due to a series of reasons: climate change, changes in growing techniques, the demand for riper grapes, etc. And this is also happening in the case of wines made from the Tempranillo variety. Tempranillo is one of the most important red grape cultivars and the principal variety of the Appellation of Origin Rioja in Spain. The aim of this study was to examine the effect of the pH of the Tempranillo grape on BA formation during elaboration and conservation of wines. Identification and clonal distribution of LAB and amino acid concentration were determined in an attempt to explain the amine generation. MLF took place either by allowing the natural microflora to act or by inoculation of wine with a commercial malolactic starter.

## 2. Materials and methods

### 2.1. Wine elaboration

The elaboration was carried out at the experimental winery of CIDA (Research Centre of the Spanish northern region of La Rioja) from c.v. Tempranillo local red grapes (probable alcohol 14.8% v/v; pH 3.4 and malic acid 3.30 g/L). A diagram of the experiment is shown in Fig. 1. After destemming, crushing and sulfiting (50 mg/L $SO_2$) operations, the grapes were placed homogeneously into four 200-L fermentation vats. In two of these, the pH was not modified. In the others, 2 g/L of calcium carbonate was added, sufficient amount to achieve a pH of 3.7. AF was induced with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand S.A.S., St. Simon-France) and conducted following traditional procedures (in the presence of grape skins and seeds until the residual reducing sugar content was <2 g/L), at 22 °C. At the end of AF, each wine was drawn off from the lees and placed homogeneously into two 50 L vats. In this way eight vats were prepared for MLF, with four duplicate tests performed. Four vats contained wines elaborated from grape with a pH of 3.4 and the other four contained wines elaborated from grape with a pH of 3.7. Two vats for each pH were inoculated using the commercial *O. oeni* strain Uvaferm Alpha "U" (Lallemand S.A.S.). The other two were not inoculated and performed MLF with the indigenous microbiota. Temperature was maintained at around 20 °C and MLF was followed by measuring wine L-malic acid content using the L-malic acid Enzymatic Bio-Analysis (Boehringer-Manheim/R-Biopharm, Darmstadt, Germany). When MLF had finished (residual malic acid < 0.2 g/L), the wines were racked off into 25 L vats and sulfited with 30 mg/L of $SO_2$. After 1 month at 10 °C they were bottled, with the bottles kept at 16 °C during 6 months.

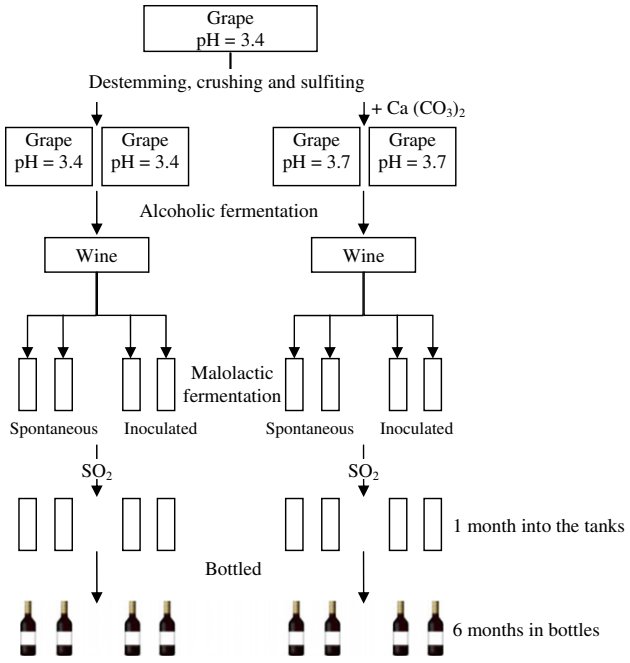


**Fig. 1.** Diagram of experiment.

Wine samples were taken for bacterial analysis at different times: consumption of 60% of the initial malic acid (tumultuous MLF), one month (before bottling) and seven months after MLF (six months in the bottle). Chemical analysis of the wines was performed after AF, after MLF and one month and seven months after MLF. Results reported here are the average values of two independent experiments.

### 2.2. Bacterial enumeration, isolation and identification

Bacterial count and isolation were carried out according to the method of serial decimal dilutions in sterile saline solution (0.9% NaCl). 100 μL of the appropriate dilutions of wine samples were spread in duplicate onto modified MRS-agar medium (Sharlau CHEMIE S.A., Barcelona, Spain) plates with added L-cysteine