DOI : https://doi.org/10.58233/yd90Ml8h

Ozone to improve the implantation of *Lachancea thermotolerans* for improving pH in warm areas in wines with low SO_2 levels

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Abstract. One of the most powerful biotechnologies for lowering pH in wines from warm zones and in high pH varieties is the use of the non-*Saccharomyces Lachancea thermotolerans* yeast. This species is capable of producing lactic acid from sugars while slightly reducing the alcohol content, which therefore ameliorates two of the main problems of wines from regions affected by global warming. Lactic acid is an organic acid with good sensory integration in wine flavor, and also chemically and biologically stable during wine aging. The use of *L. thermotolerans* can help reduce the pH from 4.0, a value that is common in the Tempranillo variety in many regions of Spain, to 3.4-3.6 which is a safe value to keep wines fresher and more stable during barrel aging. In addition, depending on the strain, the use of *L. thermotolerans* can improve the sensory profile of the wine by producing esters and other aromatic compounds. The use of ozonated water to clean and sanitize grapes is a very interesting strategy to eliminate competitive wild yeasts while reducing the use of SO_2. The objective of this research is to show the effect of ozonated water in the use of sequential fermentations with *L. thermotolerans* to acidify Tempranillo wines in the absence of SO_2.

1. Introduction

High sugar content and low acidity or high pH is a typical situation in grapes from warm areas. This scenario is going even worst in the current context of global warming. *Lachancea thermotolerans* (Lt) is a very useful non-*Saccharomyces* yeast for lowering pH due to its ability to produce lactic acid from sugars during fermentation. [1,2] (Fig.1). Therefore, bioacidification with Lt is a powerful tool to improve acidity and wine freshness [3]. The main problem with Lt is implantation, especially in red wines, where wild yeasts on the skins can be strongly competitive preventing good development and acidification. In addition, Lt is very sensitive to SO_2 [1,2].

Ozone is one strong oxidant with high antimicrobial activity. Its effectivity has been reported to control wild yeast (apiculate) and to decrease volatile acidity [4]. Moreover, O_3 and ozonated water are innocuous additives with a rapid evolution (minutes) to oxygen.

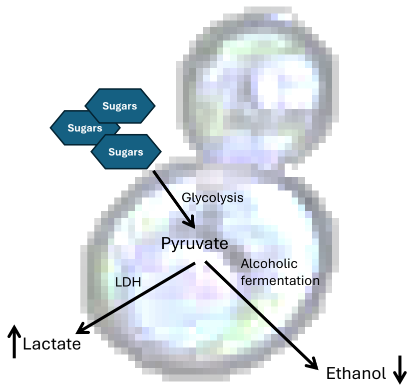

Figure 1. Bioacidification with Lachancea *thermotolerans*.

Ozone is usually produced in either corona discharge (Fig.2) or UV generators. Later ozone is mixed with water to produce ozone solutions with concentrations of 2-4 ppm.

The main objective of this research is to show the effect of ozonated water in grapes to reduce wild yeast populations and to facilitate the bioacidification by Lt.

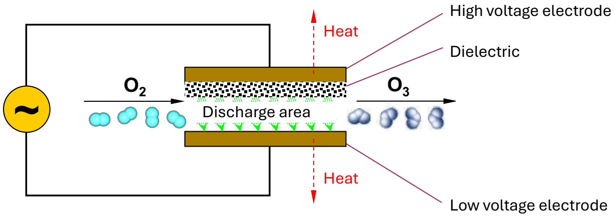

Figure 2. Ozone production by Corona discharge generators.

2. Materials and methods

Tempranillo red grapes (*Vitis vinifera* L.) were sprayed with ozonated water after destemming (4 mg/L) in the sorting table of berries, crushed and fermented in triplicate in barrels with 200 kg of grape each one (Fig.3). at room temperature after inoculation with *Lachancea thermotolerans* Blizz™ (Lallemand, Montreal, Canada). Crushed grapes without ozone treatment and under the same conditions were also fermented in triplicate and used as controls. Fermentations were performed without sulfites.

Figure 3. Barrel fermentations of ozonated grapes and controls.

Lt populations were determined on chromogenic media (CHROMagar™ Candida). General enological parameters were determined in wines after fermentation by FTIR. Lactic acid was analyzed by enzymatic techniques. Pigments were determined by LC-DAD and fermentative aromas were analyzed by GC-FID.

Wines were sensory evaluated by a tasting panel formed by eight experienced tasters.

3. Results and Discussion

3.1. Global metabolic balance

Wines at the end of fermentation showed an alcoholic degree in the range 14.6-14.8. The production of lactic acid was 13% higher in ozonated grapes and with lower sd what means a more repetitive effect for better implantation (Table 1). The production of volatile acidity was quite moderate in both treatments. All fermentations were finished properly with less than 2 g/L of residual sugars.

Table 1. General oenological parameters. Values are means ± SD of three independent fermentations.

Treatment	Control	O3
Ethanol (% v/v)	14.6±0.3	14.8±0.2
Lactic acid (g/L)	5.4±1.0	6.1±0.4
Volatile acidity (g/L)	0.34±0.04	0.34±0.02
Residual sugars (g/L)	<2 g/L	<2 g/L

3.2. Color and Anthocyanin pigments

The color of the wines at the end of fermentation was determined by CieLab method and all the wines showed similar parameters typical from red wines with intense extraction and without oxidative effects by ozone (Fig.4). Therefore, the use of ozonated water does not produce the oxidation of anthocyanins, neither other phenolic compounds, when applied on the whole berries; just affects the microbial wild load they have on the surface.

The content of grape anthocyanin pigments by families: 3-glucosides, and acylated derivatives (acetyl and coumaroyl) does not showed significant differences between wines from ozonated grapes and controls, what also supports that Ozone is not affecting pigments by oxidation because of the absence of contact with them when berries were treated. Consequently, it can be considered a gentle treatment for grapes and with a protective effect on sensory quality.

Derived pigments formed from grape anthocyanins during fermentation as vitisins were also in similar concentrations on wines from ozonated grapes and controls. Therefore, the treatment did not affect the release of carbonyl (pyruvate and acetaldehyde) precursors by yeasts neither the anthocyanin precursors (Fig.5).

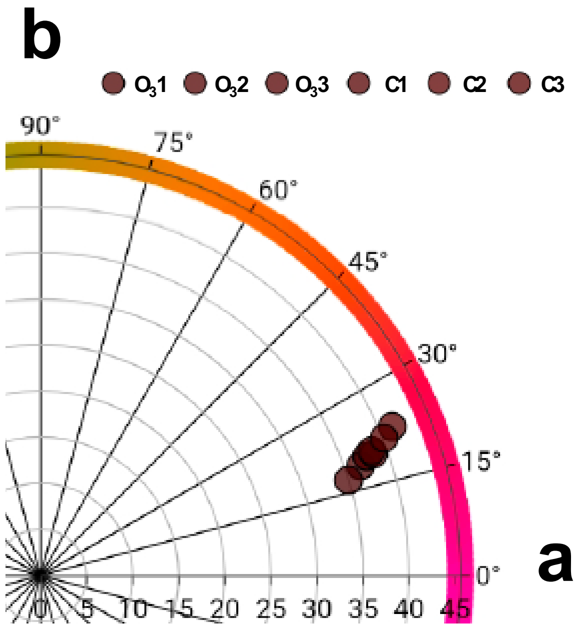

Figure 4. CieLab coordinates of the wines from ozonated grapes and controls.

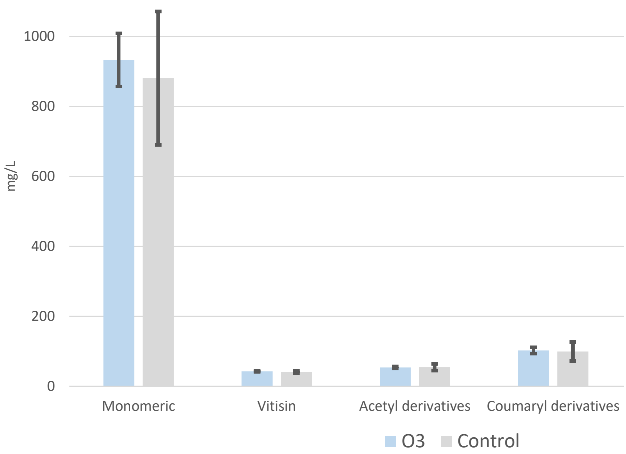

Figure 5. 3-glucosides, vitisins, acetyl and coumaroyl derivatives from anthocyanins. Values are the means and the error bars the SD of three independent fermentations.

3.3. Fermentative aroma compounds by GC-FID and sensory analysis

The analysis of volatile compounds showed also similar contents of higher alcohols and esters for the wines from ozonated grapes and controls. However, the controls had higher contents of acetaldehyde (x2) and ethyl acetate (x1.3). Probably due to the more heterogeneous fermentations with a higher contribution of wild yeasts. The use of ozonation produces safer and healthier fermentations with a more controlled initial microbiota and a better expression of the inoculated starters.

When a PCA is applied to the aroma compounds, wines from ozonated grapes and controls are perfectly separated in two clusters (Fig.6).

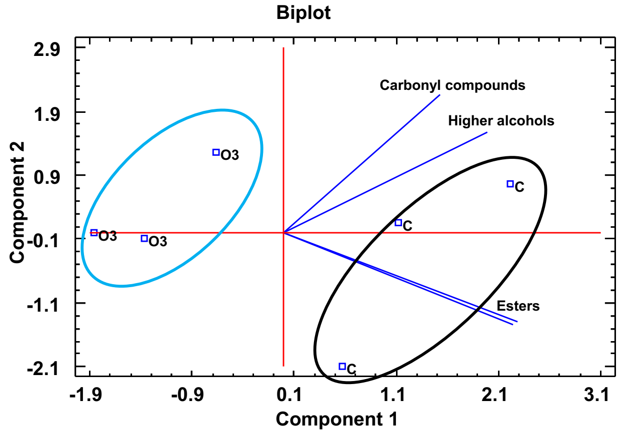

Figure 6. PCA biplot of aroma compounds showing the contribution of the main fermentative aroma families.

Sensory assessment showed similar profiles in both wines corresponding to young Tempranillo wines with intense maceration and defined by fruitiness, structure, intense color and powerful tannins.

4. Conclusions

The use of ozonated water is a clean, fast and cheap treatment to remove wild yeast and bacteria with low levels of SO_2. The fermentations and bioacidification by Lt on ozonated grapes were more reproducible and with higher formation of lactic acid. Ozonation is a very interesting technology to control microorganisms reducing SO_2 levels and can favour the pH control improving the bioacidification with Lt.

5. References

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