

## INTRODUCTION

In the vinification process, malolactic fermentation (MLF) is carried out by lactic acid bacteria (LAB) mainly *Oenococcus oeni*. This LAB can express a proteolytic system with activity on wine proteins and grape juice, releasing peptides and free amino acids necessary to satisfy their nutritional requirements. Other wine LAB species, such as some *Lactobacillus* species, can produce detrimental effects in the final product, modifying the organoleptic properties or health of the consumers. The wine is a complex environment where several bacterial interactions occur. In this way, in a microorganism co-culture, the growth, viability and metabolism of participant species are different from that take place in a pure culture. *Lactobacillus paracasei* AT45 isolated from wines of Tucumán province (Argentina) at concentrations ranging from  $10^2$  to  $10^3$ , produces histamine and tyramine, compounds that can produce allergic disorders in consumers. *Oenococcus oeni* RAM10 isolated from Tucumán wine, was studied and selected by their optimal technological properties to carry out malolactic fermentation. The study of interactions of both species in co-culture could predict their metabolic behavior in wine environment.

## OBJECTIVE

The objective of this work was to evaluate the interaction between *O. oeni* RAM10 and *Lb. paracasei* AT45 in mixed culture in order to establish a relationship with the production of biogenic amines under winemaking conditions.

## MATERIALS AND METHODS

### Microorganisms and growth conditions

*O. oeni* RAM10 and *Lb. paracasei* AT45 were isolated from a winery located in the district of Colalao del Valle, Tucumán, Argentina. The microorganisms were pre-cultured in MLO pH 4.8 and supplemented with histidine and tyramine (0.1%) in order to promote decarboxylase enzyme induction. The cells were collected at exponential growth phase, washed 3 times with isotonic saline solution and inoculated in red wine sterilized by filtration. The wine was inoculated individually with pure cultures of *Lb. paracasei* AT45 or *O. oeni* RAM10 at a final concentration of  $10^3$  cfu/mL and  $10^7$  cfu/mL, respectively. A mixed culture was carried out by inoculation of both microorganisms at the  $10^3$  cfu/mL of *Lb. paracasei* AT45 and  $10^7$  cfu/mL of *O. oeni* RAM10. The trials were incubated at 23 °C for 96 h. Bacterial viability was evaluated by count of colonies in agarized MLO pH 5.5 medium.

### Quantification of histamine

The presence of histamine was evaluated by a colorimetric method developed in our laboratory (Ledesma et al., 2018). Appropriate dilutions of histamine standard in commercial red wine were used to obtain a standard calibration curve. The first step is the extraction of histamine with n-butanol. Then the colorimetric reaction involves the reaction between the imidazole ring of histamine with *p*-phenyldiazonium sulfonate. Results are expressed in mg/L of histamine.

### Quantification of tyramine

The presence of tyramine was evaluated by a colorimetric method developed in our laboratory. Appropriate dilutions of tyramine standard in commercial red wine were used to obtain a standard calibration curve. The first step is the extraction of histamine with ethyl acetate. Then the colorimetric reaction that involves the reaction of tyramine and 1-nitroso-2-naphthol. Results are expressed in mg/L of tyramine.

## RESULTS

Microorganisms	Time	Log cfu/mL		Histamine [mg/L]	Tyramine [mg/L]
		<i>O. oeni</i> RAM10	<i>Lb. paracasei</i> AT45		
<i>O. oeni</i> RAM10	0	7.54±0.75		ND	ND
	48	7.34±0.73		ND	ND
	96	7.13±0.71		ND	ND
<i>Lb. paracasei</i> AT45	0	3.72±0.37		ND	ND
	48	3.41±0.34		2.64±0.23	0.31±0.03
	96	3.34±0.34		6.52±0.65	3.57±0.06
Mixed culture	0	7.50±0.75	3.30±0.30	ND	ND
	48	7.32±0.72	1.85±0.19	ND	ND
	96	7.15±0.72	ND	ND	ND

In pure cultures, the viability of both microorganisms remained constant for all incubation period. In mixed culture, *O. oeni* RAM10 maintain their viability until 96 h, while *Lb. paracasei* AT45 decrease the growth to undetectable values. In pure culture, *O. oeni* RAM10 did not produce histamine or tyramine, while *Lb. paracasei* AT45 produced a concentration of 6.52 mg/L histamine and 3.57 mg/L tyramine at 96 h. In mixed culture no production of any of the amines were observed.

## CONCLUSION

In wine conditions, the co-inoculation of *O. oeni* RAM10 with *Lb. paracasei* AT45 a spoilage wine bacterium, cause a total inhibition of the production of biogenic amines.

## REFERENCES

Ledesma SC, Rubio MC, Aredes Fernández PA (2019). Novel and Rapid Methods for Histamine Quantification in Wine and Culture Medium. *Am J Enol Vitic.* 70: 435-439.