Proteolytic activity of strains of *Oenococcus oeni* during the malolactic fermentation. Bioactive peptides.

KRISTOF, Irina (1,2), VERA, Nancy R. (1) y **AREDES FERNÁNDEZ, Pedro A.*** (1,2). *1 Facultad de Bioquímica, Química y Farmacia – UNT. 2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Argentina.* pedroaredes@conicet.gov.ar

1 INTRODUCTION

In cider production, the malolactic fermentation (MLF) is usually carried out by *Oenococcus oeni*. This microorganism can develop adaptation mechanisms against nutritional stress, like the production of proteolytic enzymes that are released to extracellular medium to supply the demand for nitrogenous nutrient. During the MLF, the proteolytic activity allows the release of peptides and amino acids from proteins presents in apple juice and yeast lees (constituted mainly by yeasts and their autolysis products). These hydrolytic compounds have an important role for nutrition and viability of *O. oeni*. Likewise, the released peptides could have biological activities with beneficial effects on human health.

OBJECTIVES

The objectives of this study were to evaluate the proteolytic activity of three strains of *O. oeni* and the release of peptides with antihypertensive activity during malolactic fermentation in a conventional cider production.

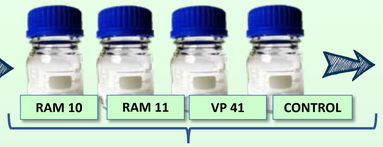
2 MATERIALS AND METHODS

▪ **FERMENTATIONS**

Pasteurized apple juice fermented with *S. cerevisiae EC 1118 (Lallemand)* ***at 18°C 16 days***

Batch 1 600 mL: rpm x 15min. filtered, pasteurized

Batch 2 600 mL : with yeast lees



Appropriate active cultures of three *O. oeni* strains: RAM10 - RAM 11 (isolated from argentinean wines) and VP41 (Lallemand) were separately inoculated at a cell concentration of 10^6 cfu /mL in flasks containing 100 mL of fermented juice. A control of fermented juice without MLF was carried out.

MLF was monitored by L-malic acid consumption

Samples (5ml) obtained each 24h

supernatants (supn.)

Analytical determinations

▪ **ANALYTICAL DETERMINATIONS**

❖ **Proteolytic activity**

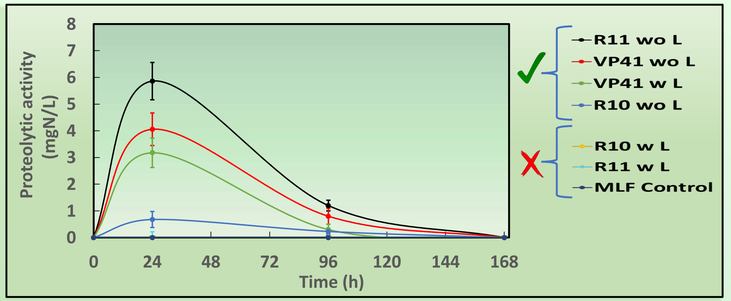
Proteolytic activity was determined in supernatants, that were used as enzyme source. Pasteurized apple juice was used as protein substrate. The low molecular weight nitrogenous compounds released after the enzymatic reaction were quantified by the colorimetric method of Doi. This method is based in the specific reaction of ninhydrin reagent with the amino nitrogen group of peptides and amino acids that develop a purple color. The absorbance values was measured in a spectrophotometer and were used to determine the proteolytic activity as the released milligrams of aminic nitrogen per liter (mgN/L). Dilutions of L-leucine were used to perform a calibration curve

❖ **Inhibitory activity against angiotensin-converting enzyme (ACE)**

To evaluate the antihypertensive activity in supernatants, the inhibition of angiotensin converting enzyme (ACE) "in vitro" was determined according to the method of Cushman and Cheung. The technique allows quantifying the hippuric acid formed by the reaction of the enzyme with their analogous substrate (Hipuril-histidyl-leucine) using a spectrophotometer at 228 nm (UV). The inhibition in hippuric acid formation was evaluated in presence of ACE inhibitors (supernatants from MLF containing low molecular weight nitrogenous compounds). The results were expressed in % of inhibition of ACE (% IACE) respect to the reaction of ACE with hipuril-histidyl-leucine in absence of enzyme inhibitors.

3 RESULTS

❖ **PROTEOLYTIC ACTIVITY of three strains of *O. oeni* against apple juice proteins**



Proteolytic activity*	MLF CONTROL	MLF supn. without Yeast lees (wo/L)			MLF supn. with Yeast lees (w/L)		
		R10	R11	VP41	R10	R11	VP41
24h	nd	0.68±0.30	5.86±0.70	4.06±0.61	nd	nd	3.18±0.55
96h	nd	0.23±0.10	1.2±0,25	0.8±0.30	nd	nd	0.3±0.10
7d	nd	nd	nd	nd	nd	nd	nd

nd= not detected

*concentration of peptides and amino acids released during the reaction of supernatants from MLF against apple juice proteins expressed in mgN / L

4 RESULTS

❖ **ANTIHIPERTENSIVE ACTIVITY.** Ability of supernatants after 7 days of MLF to inhibit the angiotensin-converting enzyme

		%IACE 7d MLF
MLF without Yeast lees	Control	14.3
	R10	18.8
	R11	33.8
	VP41	21.7
MLF with Yeast lees	Control	17.5
	R10	15.2
	R11CL	16.5
	VPCL	39.0

5 CONCLUSIONS

1. The expression of proteolytic enzyme of *O. oeni* in cider conditions was evidenced by a first time.
2. The antihypertensive activity "in vitro" of cider and their relationship with the *O. oeni* proteolytic activity was also demonstrated by a first time.
3. The maximum proteolytic activity of the studied strains was detected in the supernatants after 24 hours of MLF, in presence or absence of lees. In general, the proteolytic activity was higher in absence of lees, being highest in RAM11 and VP41 supernatants.
4. The maximum inhibitory activity on ECA was detected in supernatant without lees from RAM11 strain and the supernatant of strain VP41 obtained from medium with lees. The supernatant of the strain RAM10 present lower inhibitory activity against ECA.