Annals of West University of Timisoara

Series of Chemistry **20 (3)** (2011) 19-26

ESTIMATION OF ARGININE DEGRADATION DURING MALOLACTIC FERMENTATION OF WINE

I. Popescu-Mitroi[a], Felicia Stoica[b]

[a]"Aurel Vlaicu" University of Arad, Faculty of Food Engineering, Tourism and Environmental Protection, Department of Chemical and Biological Sciences, 2-4 Elena Dragoi, Arad, 310330, ROMÂNIA.

[b]University of Craiova, Faculty of Horticulture, Department of Horticulture and Food Sciences, 13 Al.I. Cuza, Craiova, ROMÂNIA

Received: 15 September 2011 Modified: 21 Seprember 2011 Accepted: 30 September 2011

SUMMARY

The malolactic fermentation is a secondary fermentation leaded by lactic bacteria, which transforms L-malic acid in L-lactic acid and carbonic anhydride. The lactic bacteria are chemo-trophyc, needing, in order to obtain the metabolic energy, the degradation of some chemical compounds, being the apparition possibility of unwanted substances.The arginine degradation made by malolactic bacteria has some special oenologic implications: the ammonium production, increases the pH of wine and increases the development risk of rotten microorganisms and mould in aerobe conditions. Thus the citrulline secretion is worrying, toxicologically speaking, because the citrulline is a precursor for the wine urethane (carcinogenic). This paper follows the arginine metabolization dynamic during the malolactic fermentation in a comparative study with the degradation of the malic acid, as well as the forming risks of some compounds with a high toxicological potential, as a result of the degradations in wine.

Keywords: malolactic fermentation; arginine; acid malic; lactic bacteria.

INTRODUCTION

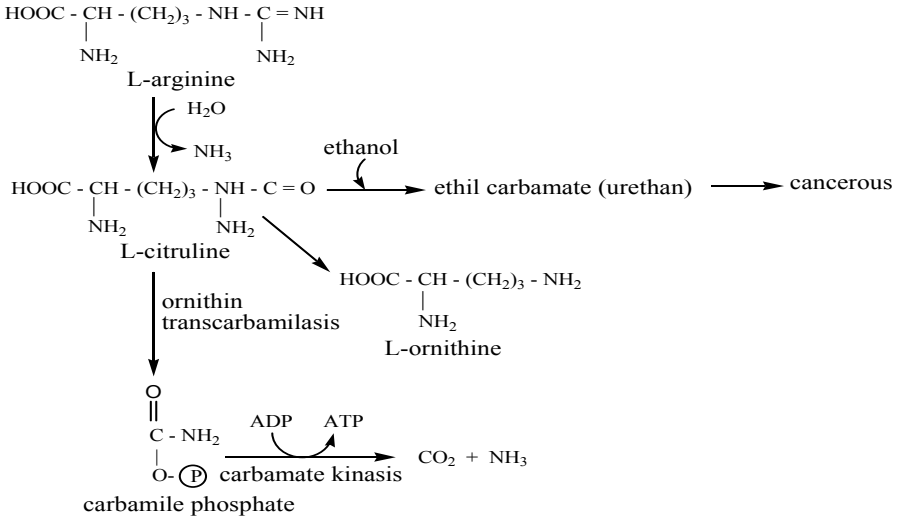
Amino acids with the largest weight in wine are: proline 570-720 mg/L,

arginine 180-450 mg/L, lysine 130-250 mg/L, phenylalanine 76-226 mg/L, glutamic acid 88-98 mg/L, histidine 69-85 mg/L and asparagine 50-56 mg/L [1].

Malolactic fermentation changes content in amino acid of wines. Lactic bacteria have enzymes that contribute to decarboxylation amino acids and formation biogenic amines in wine. Action of lactic bacteria is closely related to the structure of amino acids: arginine amino acid is most affected, followed by histidine, serine, glutamic acid, tyrosine and phenylalanine. Arginine, glutamic acid, isoleucine and tryptophan are indispensable amino acids (essential) for most species of lactic bacteria [2].

The complete degradation of arginine ensues the ADI (arginine deiminasis) way, leading to ammonia, ornithine, ATP and CO_2 production. During the arginine degradation, a certain amount of citruline is secreted [3].

The arginine degradation mechanism by ADI way made by malolactic bacteria, described by Mira de Orduna et al [4, 5, 6] is the following:



The arginine degradation during the malolactic fermentation is different, depending on the lactic bacteria type, who initiate the malolactic fermentation.

Arginine formed citrulline, by hydrolytic deamination, which in combination with alcohol in the wine gives rise to ethyl carbamate (carcinogenic). All of ornithine, can be formed citrulline, which may give rise by decarboxylation to putresceina (biogenic amines which compromises sensory attributes in wine).

Ethyl carbamate is formed by several mechanisms. First, yeasts during alcoholic fermentation can produce 3 μg/L ethyl carbamate. On the other hand, lactic bacteria produce more than 5-6 μg/L ethyl carbamate during malolactic fermentation. If there are metabolic

deviations from malolactic fermentation, heterofermentative lactic acid bacteria can produce abnormally high amounts of ethyl carbamate, without organoleptic characteristics of wine being affected [7, 8, 9].

Aging, primarily chemical process, leads to the formation of ethyl carbamate, especially if the wine contains at least 2 mg/L urea. Large amounts of ethyl carbamate appear in fortified wines (case of liqueur wines). Usually, large amounts of ethyl carbamate in wine are derived from plantations fertilized with excessive nitrogen, to those that were produced by maceration-fermentation at high temperatures, as well as those who had prolonged contact with yeast (malolactic fermentation was longer) [10].

In this paper follows dynamic degradation of arginine during spontaneous malolactic fermentation (with with inner micro flora) and conducted malolactic fermentation (using bacterial preparation INOFLORE R), in the comparative study with the degradation of malic acid.

MATERIALS AND METHODS

After finishing the alcoholic fermentation in wine cellar conditions, the Pinot noir wine was separated from lees, left to clarification in cold conditions and filtered through sterilized cellulose buffers. At the determination, the wine had the following physico-chemical characteristics: alcohol 11.5%, sugars 1.05 g/L, total acidity 4.40 g/L, volatile acidity 0.29 g/L, malic acid 1.5 g/L, lactic acid 0.28 g/L, free SO_2 5 mg/L, total SO_2 28 mg/L.

Arginine was detected in traces. The wine was adjusted at different values of the pH: 3.3, 3.6 and 3.9 with the help of a NaOH 1 M solution. Also, the arginine concentration was adjusted at the levels: 0; 0.5 g/L; and 1 g/L. After the sterile filtration of the wine (through Seitz filters of 0.45 μm) the wine was poured in bottles of 0.33 L, provided with a digestion-tank.

The malolactic fermentation was induced at 20ºC by inoculating in half of INOFLORE bacterial concoction assays which contain the *Leuconostoc oenos* species. The other half of assays remained inoculated, and the malolactic fermentation was initiated at 20ºC, in "spontaneous"conditions, by inner microflora.

The L-malic acid concentration from wine was spectrophotometricly determined, with the help of the enzymatic kits given by Diamedix Diagnostica firm.

Table I. The work protocol for the L-malic acid determination

Dripping	Standard	Sample
Solution 1	1.000 mL	1.000 mL
Solution 2	0.200 mL	0.200 mL
Suspension 3	0.010 mL	0.010 mL
Sample	-	0.100 mL
Bidistillated water	1.000 mL	0.900 mL
Mix, read absorbances of the solutions after 3 minutes (A_1). The reaction is started by adding:		
Solution 4	0.010 mL	0.010 mL
Mix, wait 10 minutes until the reaction is finished and are read the standard and sample absorbances (A_2)		

The kit composition:

- Bottle 1 with 30 mL solution formed by: glicylglycine , pH 10; L-glutamic acid 440 mg;
- Bottle 2 with 210 mg lyophilizate NAD;
- Bottle 3 with 0.4 mL GOT, 160 U;
- Bottle 4 with 0.4 mL L-malate-dehydrogenase, 2400 U;
- Control solution for L-malic acid analysis.

Concentration of arginine in wine was determined spectrophotometrically by color reaction with hidroxy-chinoleină in the presence of sodium hypobromites and urea.

Reagents:

- solution NaOH, concentration 10%
- solution of 8-hydroxy-chinoleină, concentration 0.02%
- solution of sodium hypobromites, concentration 1%
- solution of urea, concentration 40%
- standard solution of arginine, concentration 10 mg/L

For sample preparation, red wine was treated with charcoal and then filtered. To make the determination, in a vial with glass stopper were placed 0.5 mL of the filtrate, which were added 1 mL solution of 8-hydroxy-chinoleină 0.02% and 10 mL solution NaOH 10%; content of the vial was placed in a ice water bath for 2 minutes. Were quickly added to 0.2 mL of sodium hypobromites and after 15 seconds was added to 1 mL of urea 40%.; after stirring of vial for 1 minute were added 5 mL of cold distilled water. Absorbance of solution was measured spectrophotometrically at 500 nm, with reference to concentration using calibration curve.

RESULTS

Figure 1 shows the degradation in time of the malic acid and arginine at different values of pH, during the spontaneous malolactic fermentation (with inner flora). While the degradation of the malic acid took place only partially the arginine was completely degradated at a pH 3.9 and degradated in a proportion of 40% at a pH of 3.3. The malolactic fermentation recording was made in a periof of 21 days, and the determinations were made from 7 to 7 days.

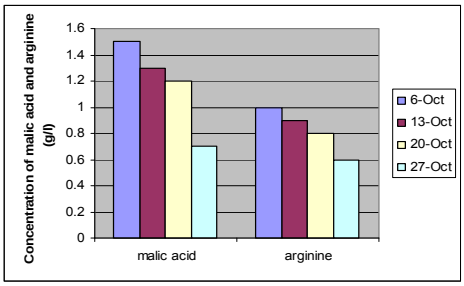


Figure 1a. The kinetic of the malic acid and arginine degradation at a pH of 3.3 for the nonce of spontaneous malolactic fermentation development

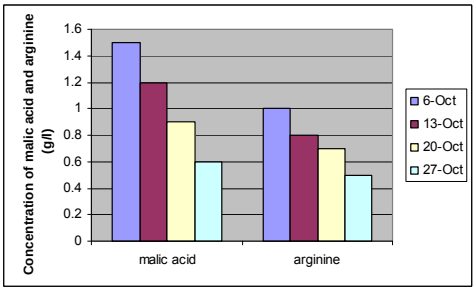


Figure 1b. The kinetic of the malic acid and arginine degradation at a pH of 3.6 for the nonce of spontaneous malolactic fermentation development

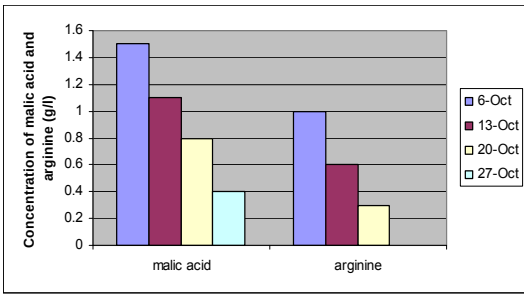


Figure 1c. The kinetic of the malic acid and arginine degradation at a pH of 3.9 for the nonce of spontaneous malolactic fermentation development

Figure 2 shows the malic acid and arginine degradation in time, at different values of pH, during the conducted malolactic fermentation (by using the INOFLORE R bacterial concoction). In contrast with the spontaneous malolactic fermentation, the *Leuconostoc oenos* degrades the malic acid at all pH values tested. Arginine was totally consumed only at a pH of 3.9, after 21 days of malolactic fermentation and degradated in proportion of 70% at a pH of 3.6. At all pH values, the malic acid degradation was complete, before eloquent degradation of arginine took place. At pH 3.3, where the malic acid degradation was delayed, arginine were not eloquently degradated.

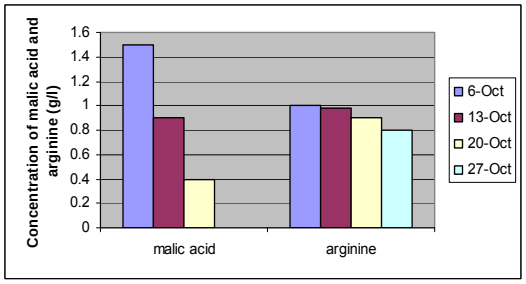


Figure 2a. The kinetic of the malic acid and arginine degradation at a pH of 3.3 for the nonce of conducted malolactic fermentation development

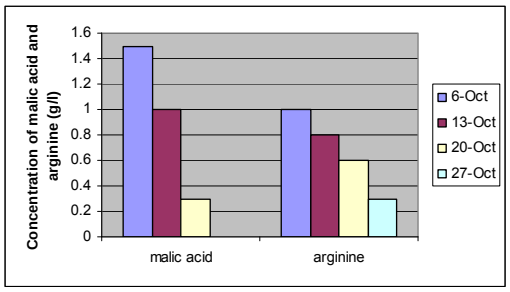


Figure 2b. The kinetic of the malic acid and arginine degradation at a pH of 3.6 for the nonce of conducted malolactic fermentation development

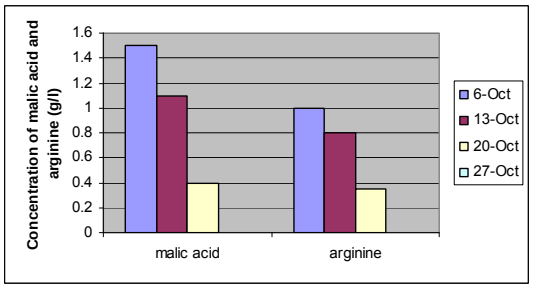


Figure 2c. The kinetic of the malic acid and arginine degradation at a pH of 3.9 for the nonce of conducted malolactic fermentation development

DISCUSSION

In this study, was investigated the catabolism of arginine for the nonce in spontaneous malolactic fermentation development (with inner micro flora) and conducted malolactic fermentation (by using INOFLORE R concoction which contains the *Leuconostoc oenos* species). In both cases, the lactic bacteria were able to degrade arginine and to segregate important amounts of citruline. Anyway, there are some differences in what concerns the minimum necessary pH value, to develop the arginine degradation. Therefore, the *Leuconostoc* species is able to completely degrade arginine at a pH of 3.9, partially at 3.6 and none at 3.3. In contrast, by spontaneous malolactic fermentation, the arginine degradation take place at all pH values tested.

CONCLUSION

From this study, we can conclude thet by conducting the malolactic fermentation, with the help of INOFLORE R bacterial concoction, the arginine degradation was delayed comparative with the malic acid degradation. In oenologic practice, this fact allows the wine makers to avoid the arginine degradation, by recording the malic acid degradation and inhibition of the malolactic bacteria, immediately after the malolactic fermentation is finished. From the presented results, is possible to reduce the risk of citruline formation, by conducting the malolactic fermentation with bacterial concoctions, superseding the possibility of undesirable substances appearance.

REFERENCES

1. Târdea C., "*Chimia și analiza vinului*", Ed. Ion Ionescu de la Brad, Iași, 2007, p. 754-770.
2. Makaga-Kabinda Massard E., "Etude du metabolisme de l'arginine chez l'espece Leuconostoc oenos", These de Doctorat 1994, Univ. de Reims-Champagne, France.
3. Stevens D., Ough C. S., "Ethyl carbamate formation: reaction of urea and citrulline with ethanol in wine under low to normal temperature conditions", *Am. J. Enol. Vitic.,* **44** (1993) 309-312.
4. Mira de Orduna R., Liu S. Q., Patchett M. L., Pilone G. H., "Etyl carbamate precursor citrulline formation from arginine degradation by malolactic wine lactic acid bacteria", *FEMS Microbiol. Lett.,* **183** (2000) 31-35.
5. Mira de Orduna R., Liu S. Q., Patchett M. L., Pilone G. H., "Kinetics of the arginine metabolism of malolactic wine lactic acid bacteria Lactobacillus buchneri CUC-3 and Oenococcus oeni Lo111", *J. Appl. Microbiol.,* **89** (2000) 547-552.
6. Mira de Orduna R., Patchett M. L., Liu S., Pilone G. J., "Growth and arginine metabolism of the wine lactic acid bacteria Lactobacillus buchneri and Oenococcus oeni at different pH values and arginine concentrations", *Applied and Environmental Microbiology*, **67**(4) (2001) 1657-1662.
7. Liu S. Q., "Arginine metabolism in malolactic wine lactic acid bacteria and its oenological implications", Doctorate Thesis 1993, Massey University, Palmerston North, New Zealand.
8. Liu S. Q., Pritchard G., Hardman M. J., Pilone G. J., "Citrulline production and ethyl carbamate (urethane) precursor formation from arginine degradation by wine lactic acid bacteria Leuconostoc oenos and Lactobacillus buchneri", *Am. J. Enol. Vitic.,* **45** (1994) 235-242.
9. Liu S. Q., Pritchard G., Hardman M. J., Pilone G. J., "Arginine catabolism in wine lactic acid bacteria: is it via the arginine deiminase pathway or the arginine-urease pathway?" *J. Appl. Bacteriol*. **81** (1996) 486-492.
10. Cotea D. V., Zănoagă C. V., Cotea V. V., "*Tratat de oenochimie*", vol. 1, Editura Academiei Române, Bucure□ti, 2009, p.137-139.