N. Darie, et all. Journal of Agroalimentary Processes and Technologies,
Volume XII, No. 2 (2006), 365-370
Full Paper - Food Control Section

CLASSICAL AND UP-DATE MONITORING IN RED WINE-MAKING FERMENTATIVE PROCESSES

Neli Darie, Claudia Felicia Ognean, M. Ognean, O.S. Petrenciu
"L.Blaga" University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, 7-9 Dr.I.Ratiu Street, 550012 Sibiu, România,
neli.darie@ulbsibiu.ro

Abstract

The red wine-making fermentative processes evolution was estimated by some parameters measurements (pH, temperature, density, potential variation) using classical assays and by L-malic concentration measurements by selective biosensorial assay. The results recommend the L-malic concentration as a sensitive parameter and the amperometric biosensorial assay as a fast and faithful method in red wine-making monitoring.
Keywords: *L-malic acid biosensor, malolactic fermentation, density, potential variation, red wine-making*

Introduction

The red wine quality mainly depends on manner of alcoholic (FA) and malolactic fermentation (FML) processes, which could be stimulated, interrupted, activated or even avoided (figure 1). A fast and accurate malolactic fermentation monitoring assure the red wines quality and biological stability.

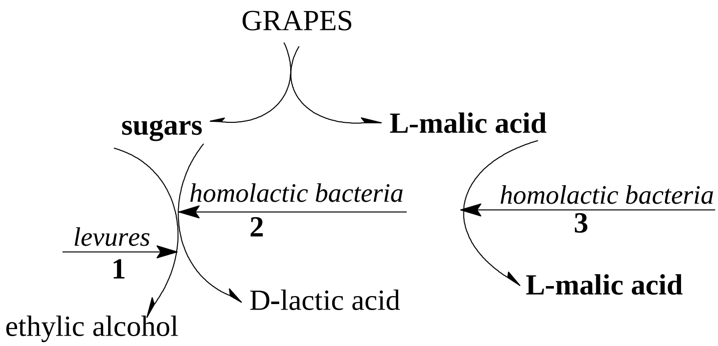

Fig. 1. Red wine-making diagram (1- FA, 2 - FML, 3 - piqure lactic)

FML accompanies the FA sugars grape, taking into account as a primary fermentation, and produces the degradation of L-malic acid into lactic acid, under the bacterial control. The wine quality improvement assumes the acidity decreasing and the malic acid replacement by lactic one, which acid impresses more pleasantly the gustative papillas (Whitaker, 1994). The FML finish point must be known by far precision, as the oenologists pass at the wine sulphitation and they have to be sure that, before beginning this operation, all L-malic acid is disappeared.

Some parameters are estimated in wine-making laboratories, as pH, temperature and sometimes density and L-malic acid concentration (but only by paper chromatography). There have obtained only informations on the fermentations development, by classical assays. The wine-makers resort to the specialised analytical laboratories, to measure ethylic alcohol and L-malic acid concentration by flow enzymic spectrophotometry (Batelle, 1996) or by HPLC (Garcia Romero, 1993) in wine samples. All this classical methods have became too expensive and laboriously, needing long execution time, performant equipment, specialised staff, expensive reagents and dissonant with the automatic fermentative processes.

The objective of this study was to estimate the variation of some parameters (pH, temperature, density, potential) by classical assays and L-malic acid concentration by amperomeric biosensorial assay, in order to monitoring faster, better and cheaper the malolactic fermentative processes in red wine-making.

Experimental

An amperometric L-malic biosensor has achieved using enzymes all supplied by Sigma: diaphorase D (5540), the glutamate oxaloacetate transaminase GOT (7005), L-malate dehydrogenase (9004) and NAD^+-dextran (3383). The other chemicals employed were analytical grade and the needed solutions were prepared with twice distilled and deionised water. For the enzymic spectrophotometer L-malate measurements were used F-kits (Boehringer). The Merlot and Cabernet Sauvignon red wine samples were analysed during wine-making, utilizing samples brought every day.

The following instruments have employed in the assays: 1.2 A stabilised power supply, spectrophotometer UV-VIS associated with a computer and plotter HP laser, L-malic acid biosensor, 300-500 mV stabilised current supply, a combined electrode of Pt and saturated calomel (ECS) Tacussel, multimeter Fluke 29-Series, pH-meter Methrom, densimeter Prolabo SR 100.

The amperometric measurements have made with a selective L-malic acid biosensor described previously (Darie, 1998). The sensor was immersed before-hand in 2 ml buffered and stirred solution of hexacyanoferrate (III) 4.5 mM wich also has contained glutamate 10 mM and NAD^+-dextran (Darie, 1998a). The electrolysis current intensity grows until a steady state current I_{max} and when this state is touched, the current difference corresponds to the L-malate concentration in sample. The biosensor response time is required to obtain the steady state current (Bergon, 1998).

Results and Discussions

There have estimated the parameters variation of the parameters: temperature, pH, density, potential and L-malic concentration.

In FA and FML processes the red wine density has decreased and arrived on a constant values plate (figure 2).

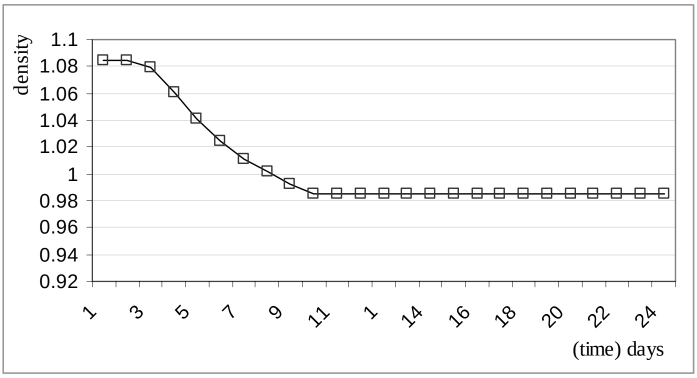

Fig. 2.Density evolution in FA and FML red wines

The last fermentative four days, having modified parameters, show a FML beginning. In the 8th day the temperature has decreased from 30 to 25 degrees centigrade (figure 3) and has remained constantly until the fermentation process has finished.

In the 18th day of wine-making, pH has registered a sudden increasing (figure 4) and also a sudden decreasing of potential (from 0 to -180 mV) only in a few hours.

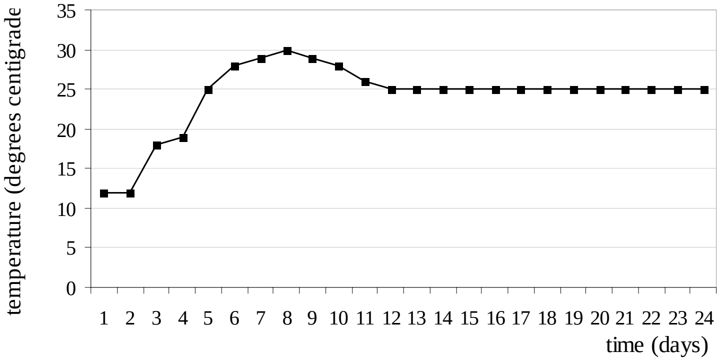

Fig. 3. Temperature evolution in red wine FA and FML

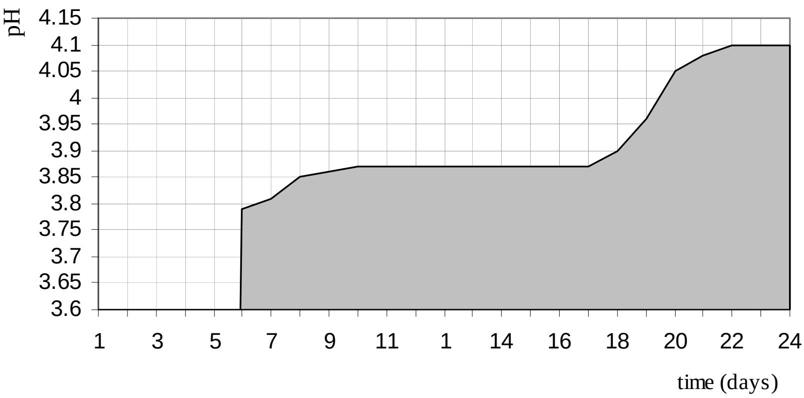

Fig. 4. pH variation in red wine fermentative processes

The potential high value (340 mV) from the FA beginning was explained by the oxygen presence in the grapes must (figure 5). After oxygen consuming by the levures, the potential has strongly decreased.

Changing the tub, the red wine has came in air contact, meaning a potential increasing, from 0 to 50 mV, as in the substrate absence the levures consume the oxygen. Since all L-malic acid has consumed, the potential has increased and fixed at -130 mV; the wine acidity has also decreased to the half of its value. The last decreasing of the potential corresponds at the sulphitation process, by adding of a reducing compound that involves a potential decreasing in wine.

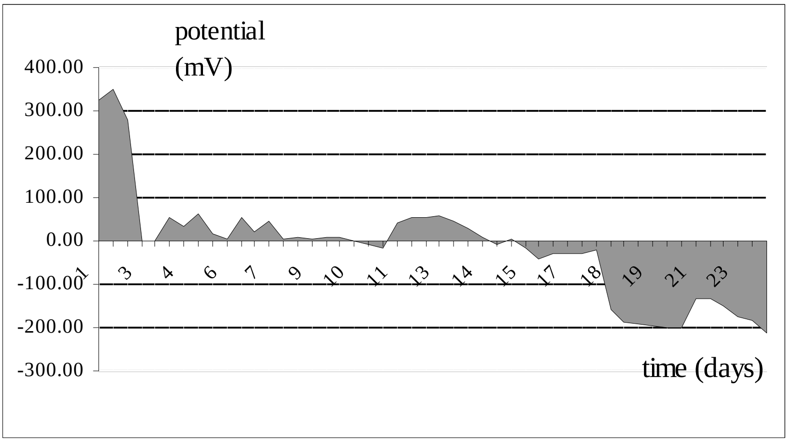

Fig. 5. Potential variation in red wine FA and FML

The red wine and also the must grapes are complex environments and the measured potential couldn't be in good agreement with the species concentrations in solution, as Nernst relation involves the establishment of equilibrium in solution and at the electrode, which isn't true for a rapid system. Thus, the potential evolution only informs of oxygen presence, being only an indicator of the micro-organisms activity.

Thus, from the experimental data there were observed a L-malic acid concentration decreasing, beginning with the 12th day of the fermentation. That moment corresponds to the slow step, when the lactic bacteria multiply and start the FML in the 18th day. The results of the measurements (figure 6) are in harmony with the data obtained by spectrophotometry method. The selective biosensor can be used about a month (28 days) with the same enzymatic solution (with the enzymes combination: [GOT] = 20 mg/ml; [L-MDH] = 0.2 mg/ml and [D] = 3 mg/ml, under the membrane from the electrode tip.

The precision and the exactness of the elaborated oenological method, based on amperometric biosensors, recommend the using of these modern instruments in the supervision of the red wines fermentative processes.

Conclusions

Red wine-making development can be supervised by modern methods that use modern analytical instruments, as selective biosensors. Through the analysed parameters during red wines fermentative processes: density, pH, temperature, potential and L-malic acid concentration, the last have been the most faithful parameter.

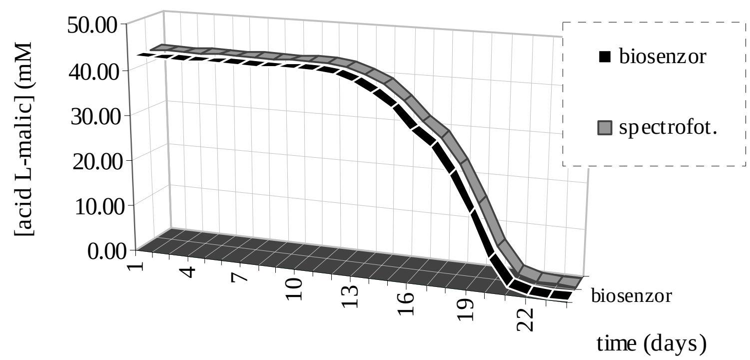

Fig. 6. L-malic acid concentration measurements by biosensor and spectrophotometry assay

References

Batelle, J.L., Joubert, R., Collon, Y., Jouret, C. (1996). Continuous flow enzymic assay of L-malate and L-lactate in grape musts and wines. *Annales des Falsifications et de l' Expertise Chimique*, 42, 151-156.

Bergon, P., Darie, N., Rivot, F. (1998). Specificity improvement of L-malate electrochemical biosensors by the enzyme amplification phenomenon. *Chem. Bull. "Politechnica"Univ.*, 43 (57), 1, 160-165.

Darie, N. (1998). *Electrochemical biosensors for wines lactic and malic acids determination. Doctoral thesis*. Cluj-Napoca: Babes-Bolyai University.

Darie, N. (1998a). Biochemical parameters effects on biosensors with NAD-dependent dehydrogenases applied in food field. *Studia Univ.Babes-Bolyai. Chemia*, XLIII (1), 147-156.

Garcia Romero, E., Sanchez Munoz, G., Martin Alvarez, P.J., Cubezudo Ibanez, M.D. (1993). Determination of organic acids in grape musts wines and vinegars by high-performance liquid chromatography. *J. Chromatogr*., 655 (1), 111-117.

Whitaker, R.J. (1994). *Principles of Enzymology for the Food Sciences*, New York: M.Dekker Inc.