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SELECTIVE BIOSENSORIAL ASSAY IN *LACTIC SOURING* WINES DISEASE PREVENTION

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Abstract

D-lactic acid resulted in case of an accidental stopping of the alcoholic fermentation process, by all sugars converting, changes the wines taste and the wines become sweet and sour. Adding yeasts it can prevent the white and red wines disease lactic souring. The technological parameter D-lactic acid was comparatively measured spectrophotometrically and successfully performed by a selective D-lactic acid biosensor, suitable for oenological applications. There were obtained significant performant functional features of the conceived biosensor (185 sec. as response time, 0.001 mM as detection threshold, 37 days as life time and a good sensibility) in order to detect and prevent earlier the beginning of the wine disease.
Keywords: *lactic souring, biosensorial assay, D-lactic parameter*

Introduction

During the wine-making process, especially in the alcoholic fermentation, the bacteria can convert the undegradated sugars by the yeasts, in D-lactic acid. The acid hardly influence the wines taste, remaining as such and stamping a sweet and sour taste in the same time (Garcia, 1993).

In current practice there is only measured the wine volatile acidity and the oenologists can only suspect the disease appearance when the volatile acidity is higher (Luong, 2003). The premise is false as the parallel formatted acetic acid is responsible for volatile acidity.

In order to detect and to early prevent the beginning of the wine disease, the present work concerns to analyse, by an easy assay, a new technological parameter that can be monitories, the D-lactic acid

concentration. There were comparatively used: spectrophotometry coupled with enzymatic reactions and amperometric detection by a selective D-lactic acid enzymatic biosensor. The compound monitoring was made on some cuves with red and white wines of different origin, on heath and ill wines samples. Measurement of the d-lactic acid concentration was successfully performed, by the selective bi-enzymatic D-lactic acid sensor, suitable and tried in previous studies, for oenological applications.

Experimental

A D-lactic amperometric biosensor conceived and optimised in previously experiments (Darie, 1998) for synthetic solutions, was adapted for white and red wines samples. The amperomeric biosensor used in the study, was immersed before-hand in buffered and stirred solution of hexacyanoferrate (III) contain also NAD^+-dextran. When the steady-state current is touched, the current difference corresponds to the D-lactic acid concentration in wine sample (Sheller, 1992).

The amperometric biosensor was firstly tested on some white, red and rose wines before stock age. For each wine type, the D-lactic acid concentration was estimated using the current intensity at the steady-state given by the enzymatic electrode. After the calibration curve making, the results were compared with those obtained by spectrophometric D-lactic acid assay (with Boehringer F-kits and after international knowing assays ISO 8069). The chemicals employed were analytical grade and the needed solutions were prepared with twice distilled and deionised water.

The Chardonnay, Sauvignon, Muscat and Pinot Gris white wine samples were analysed during alcoholic fermentation, using samples brought every day. In assay were used instruments as: a stabilised power supply, UV-VIS spectrophotometer coupled with a computer and HP plotter laser, D-lactic acid biosensor, a 300-500 mV stabilised current supply and multimeter Fluke 29-series.

Results and Discussions

The obtained values are the same in case of white wines (table 1) but are significantly different in the case of the red and rose wines.

Table 1. D-lactic amperometric and spectrophotometry measurements (in good health white wines)

Samples (white wines)	Spectrophotometric measurements (Boehringer kit, n_1=11)			Biosensorial assay (n_2=11)			t	F
	x_i	S_1^2	$x \pm \Delta x$	x_i	S_2^2	$x \pm \Delta x$		
Chardonnay	1.8	0	1.8±0.136	1.8	0	1.8±0.100	2.5	2.2
Pinot gris	1.5	0.1	1.5±0.197	1.5	0	1.5±0.132	2.5	2.1
Sauvignon	1.7	0.1	1.7±0.215	1.7	0.1	1.7±0.154	2.5	1.8
Muscat	2.2	0.1	2.2±0.232	2.3	0.1	2.3±0.148	2.5	2.0

The values deduced from the amperometric measurements are higher, showing the presence of a reducing interfering compound. The experimental study, the results show that the using of an active charcoal is the best method in order to eliminate the reducing interferents from the red and rose wines. There were present significant performant parameters (detection threshold, linear range, response time); all these justify its use in wine-making monitoring (table 2).

Table 2. D-lactic acid biosensor parameters in prevent and detecting wine *lactic souring* disease

D-lactic acid biosensor	Functional features					
	Response time (sec.)	Temp (^{0}C)	Detection threshold (mM)	Linear field (mM)	Life time (days)	Sensibility (nA/mM)
	185	25	0.001	0.02-5.5	37	651

The adjustment in the fermentative medium has successfully achievement. During wine-making, the beginning of wines lactic souring was detecting very early. After 37 function days, without a daily calibration, it remains 62.5% from response-current intensity of the sensor (figure 1). The most significant advantages of the biosensorial device in wines disease detecting are: threshold of 0.001 mM, short assay time of 185 seconds, a good sensibility of 651 nA/mM on a linear field of 0.02-5.5 mM. There were saved, by using

biosensorial assay in D-lactic measurements, more wines curves, when there was detected the beginning of wines lactic souring.

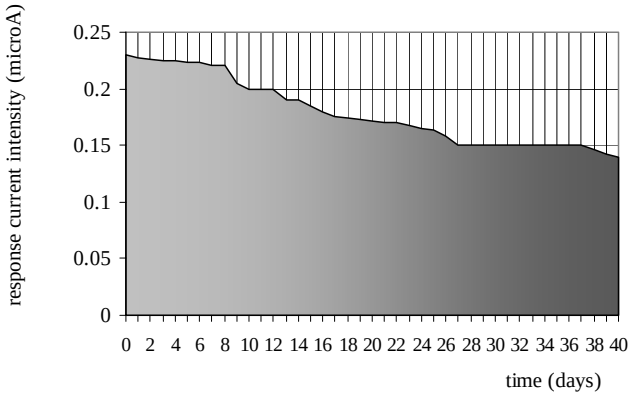

Figure 1. Evolution of D-lactic biosensor response in time

From the dispersion analysis of the obtained data, through comparative analytical methods, it can establish that for a 95% probability the confidence interval of the individual values are between 0.996 and 1.018 mM. The amperometric detection method is exactly and the results neither are nor affected by the systematic errors.

Conclusions

The performances obtained with the D-lactic acid biosensor recommend it among the sensitive and fast analytical tools for the detecting and preventing the wines diseases.

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