

ESEAC2004

NUI, Galway



10th International Conference on Electroanalysis

European Society for ElectroAnalytical Chemistry

National University of Ireland, Galway

6-10 June 2004

BOOK OF ABSTRACTS

ESEAC2004 Sponsoring Agencies



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Science Foundation Ireland, funded under the National
Development Plan 2000-2006.



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A study of heavy metal complexation in wines

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This work is concerned with the loss of colour observed in some of the red wines of Navarra. Polyphenolic compounds are responsible for the structure and wine colour and can be found in the skin, seeds and pulp of the grapes. The colour and stability of these pigments depend on the pH and the presence of several metallic cations.

The present work deals with the identification and quantification of the metallic complexes responsible for the colour of wine and the study of their evolution with time throughout the fermentation of grapes. A method has been optimised to perform a chromatographic separation of the different polyphenolic fractions of wine, keeping constant chemical parameters like pH, alcoholic degree ... After separation, samples were digested under microwave and strong acidic media and their total metal concentration in Zn, Cu, Fe and Mn determined by atomic absorption spectroscopy.

The studied samples were must and wine, both fermented at different times ranging from 0 to 360 days, being 0 the time when grapes were introduced in the vessel where fermentation will take place. The evolution with time of the total metal content for each sample, shows a sharp decrease during the period 0-10 days, reaching the metal concentration a constant value from that day on. A parallel behaviour was observed between certain metals and the main anthocyanins present in wine, i.e., cyanidine-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside.

Differential pulse voltammetry was used to study complexation processes of Zn in must samples, at their natural pH. Titration curves allowed the calculation of ligand concentration and stability constants, following the suitable linearization methods.

Present work concerns the study, by differential pulse and square wave voltammetry, of the complexation behaviour of both Zn and Cu with model ligands resembling the main antocyanins present in must and wine samples.

Authors would like to acknowledge the "Viticulture and Enology Station of Navarre" (EVENA) for kind supply of grape, must and wine samples.



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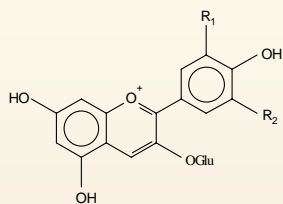
A study of heavy metal complexation in wines

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INTRODUCTION

The colour of red wines is one of the most valued organoleptic parameters in terms of wine quality, both for enologists and consumers¹. It is well known that phenolic compounds are responsible for the colour of wine² and more specifically anthocyanins (a kind of polyphenolic compounds) are the red pigments located in the skin of grapes³⁻⁴. The chemical structure of the anthocyanins identified in grapes and wine is shown below:



R ₁	R ₂	Name
OH	H	Cyanidin
OH	OH	Delfinidin
OH	OCH ₃	Petunidin
OCH ₃	H	Peonidina
OCH ₃	OCH ₃	Malvidin

Malvidin and cyanidin are the most abundant anthocyanins and the *Tempranillo* variety presents the 3-glucoside substituent for both anthocyanins. From their chemical structure they can behave as chelating agents for metal cations such as Al, Fe, Cu, Zn, Mg, Mn ... which are also present in grapes and wine.

Electrochemical techniques have successfully been applied to the study of metal speciation in seawater⁵, allowing the determination of several parameters like stability constant of the metallic complex, ligand and labile metal concentrations and complexes stoichiometry.

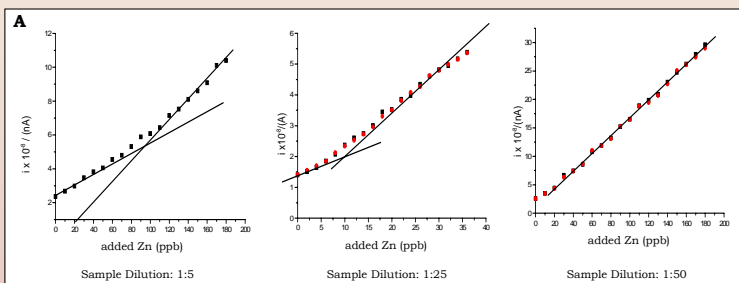
The present work deals with the electrochemical study of must samples in order to find whether it is possible or not the direct speciation of must and wine samples by electrochemical techniques. Alternative routes are also explored.

EXPERIMENTAL

The studied samples were fresh must obtained smashing red grapes and filtering the juice to avoid the presence of any piece of skin or seeds. Must samples were diluted with just water. Electrochemical technique used was DPASV (differential pulse anodic stripping voltammetry).

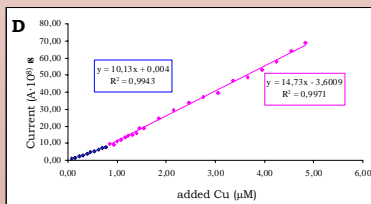
(+)-Catechin was commercially available from Fluka. Zn and Cu were 1000ppm acidic solutions from Merck.

RESULTS AND DISCUSSION



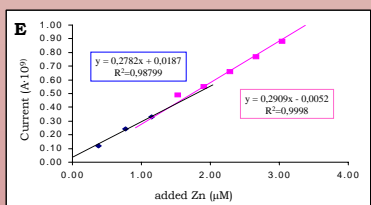
DPASV was successfully used to study fresh must samples at different dilutions. These plots show the titration curves obtained after additions of aliquots of a Zn solution. Dilutions 1:5 and 1:25 behave as expected for a matrix containing non-saturated ligands able to chelate the increasing amounts of metal added: two slopes are recorded, the lowest one corresponding to complexed metal and the highest one to free Zn. The intersection of both straight lines gives the concentration of the ligands. The third sample, dilution 1:50, was too diluted and the two sloped calibration curve could not be registered.

However, when the mathematical algorithms usually applied to seawater analysis (Scatchard and Langmuir linearization methods⁶⁻⁷) were used in our must samples (1:5 and 1:25), correlations did not follow the pattern described in literature⁶⁻⁷. It was concluded that must samples were too difficult to be measured directly, possibly due to the high number of organic molecules able to participate as ligands for the studied metals.



Must and wine samples were collected at different times of vinification. The evolution of the total polyphenol and trace metal contents with fermentation time was evaluated as described elsewhere⁸. Selected samples were fractionated through a Sephadex LH-20 opened column following the procedure previously described⁸ in order to separate and identify the main polyphenolic compounds present in every fraction and find a correlation with the trace metals eluted in the same fractions.

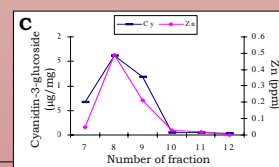
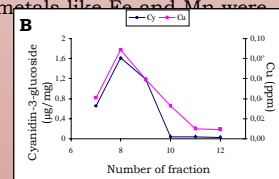
Cyanidin-3-glucoside and malvidin-3-glucoside were the main anthocyanins quantified and it was found that they eluted at the same time as Cu and Zn (figures B and C). Other trace metals like Fe and Mn were not present in these fractions.



From these results, a different strategy was utilised: model compounds resembling the structures of the main anthocyanins found in the fractions (with no 3-glucoside substituent) were studied electrochemically at the same pH of must and wine samples (pH = 4).

Figures D and E show the preliminary calibration curves obtained by DPASV when solutions of catechin (non ionic model compound with R₁=OH and R₂=H, 3-flavonol) were spiked with Cu and Zn.

A 1:1 molar correlation was found for both metals with catechin (for D a catechin concentration was 0,6µM and for E catechin was 1,4µM).



FUTURE WORK

Electrochemical studies will continue with different model compounds in order to optimise the conditions for quantification of Zn and Cu, but other metals like Fe and Mn will also be studied. Must and wine fractionated samples will be directly studied by DPASV. Theoretical studies, using Hyperchem software will be carried out in order to confirm the stoichiometry found electrochemically, for the same model compounds.

ACKNOWLEDGEMENTS

This work has been fully funded by the PIUNA Research Program of the University of Navarra.

Authors would like to thank EVENA (Estación de Viticultura y Enología de Navarra) for providing us the samples used in this project. I.E., I.S. and S.G. would like to thank the local Government of Navarra and the Friends of the University of Navarra, Incorporated, for funding support.

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