

# euroanalysisXIII

European Conference on Analytical Chemistry



5-10 September 2004. Salamanca, Spain

"The Role of Analytical Chemistry in the Protection of the Citizens"

Organized by:

Sociedad Española de Química Analítica (SEQA)



In cooperation with:

Departamento de Química Analítica,  
Nutrición y Bromatología. University of Salamanca (USAL)



Federation of European Chemical Societies

Division of Analytical Chemistry

FECS Event: 281



PROGRAMME



**Chairman:** Dr. J. Hernández Méndez  
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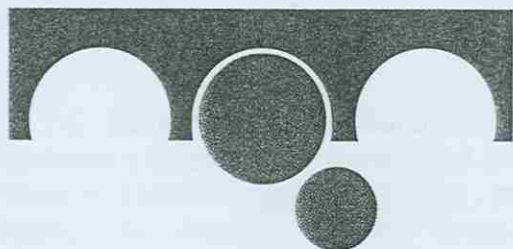
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- PS1-277 Dehydrogenase based electrochemical biosensors for wine quality control**  
Lupu, Alina; Iorgulescu, Emilia; Compagnone, Dario; Palleschi, Giuseppe.
- PS1-278 Evolution of Anthocyanin pigments and colour during vinification of Txakoli wines**  
Gallo, Blanca; López-Márquez, Diana M.; Abad-García, Beatriz; Crespo-Ferrer, Inés; Berrueta, Luis A.; Vicente, Francisca.
- PS1-279 Combined GPC/SPE clean-up in an HRGC-LRMS method for determination of polycyclic aromatic hydrocarbons (PAH) in olive oil**  
Fromberg, Arvid.
- PS1-280 Electrochemical and theoretical complexation studies for Zn, Cu and individual anthocyanins in wine samples**  
Esparza, Irene; Salinas, Iñigo; Santamaria, Carolina; García-Mina, José María; Fernández, José María.
- PS1-281 Characterization of Red Fruit Juices by their Anthocyanin Profile**  
Berrueta, Luis A.; Abad-García, Beatriz; Crespo-Ferrer, Inés; López-Márquez, Diana M.; Gallo, Blanca; Vicente, Francisca.
- PS1-282 Application of procrustes rotation and artificial neural networks methods to the characterization of meat products**  
Brito Miralles, Gonzalo; Peña Méndez, Eladia; Andrade, José Manuel; Diaz, Carlos; Havel, Josef; Garcia-Montelongo, Francisco.
- PS1-283 Determination of phenolic food antioxidants by HPLC-ED using bis(2-ethylhexyl) sodium sulfosuccinate/acetonitrile as mobile phase.**  
Bustamante-Rangel, Myriam; Delgado-Zamarreño, M. Milagros; Sánchez-Pérez, Ambrosio; Martín-Peña, Miguel E.; Carabias-Martínez, Rita.
- PS1-284 Optimization of a method to isolate, hydrolysis and subsequent GC-MS determination of volatile compounds from non-volatile precursors of grapes and other products.**  
Ibarz, M<sup>a</sup> Jesús; Hernández-Orte, Purificación; Cacho, Juan; Ferreira, Vicente.
- PS1-285 Determination of 1-octen-3-one at ng l<sup>-1</sup> through Gas Chromatography-Mass Spectrometry of their o-(2,3,4,5,6-pentafluorobenzyl)oximes formed directly in the solid phase extraction cartridge used for selective isolation**  
Culleré, Laura; Cacho, Juan; Ferreira, Vicente.
- PS1-286 Supercritical Fluid Extraction and HPLC determination of relevant polyphenolic compounds in grape skin**  
Chafer, Amparo; Pascual-Martí, Carmen; Salvador, Amparo; Berna, Angel.

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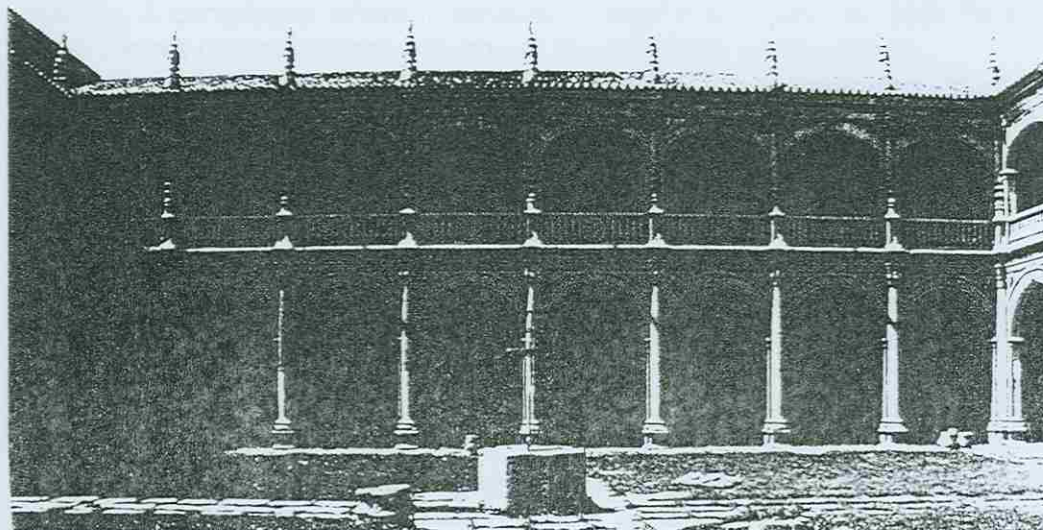
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**BOOK OF ABSTRACTS**



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## Electrochemical and theoretical complexation studies for Zn, Cu and individual anthocyanins in wine samples

I. Esparza, Í. Salinas<sup>a</sup>, C. Santamaria<sup>a</sup>, J.M. Garcia-Mina<sup>b</sup>, J.M. Fernández<sup>a</sup>

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The present work is a part of an overall project concerning: i) quantification of total and individual polyphenol content and trace metals (Zn, Cu, Mn and Fe) in wine samples; ii) identification of the possible complexes formed between some of the polyphenols and some metals; iii) evaluation of the role of metal complexes in the final colour of red wine.

Identification of the possible interactions between individual anthocyanins and metals was performed in selected samples of must and wine, collected at different vinification times. The chosen samples were fractionated through a Sephadex column following the procedure published elsewhere<sup>1</sup> and an average of 25 fractions were obtained for each sample. Fractionation of samples was done at the natural pH of must and wine to avoid modification of the metallic complexes present.

Each one of the obtained fractions was analysed and trace metals and anthocyanins were identified and quantified. Results showed that several individual anthocyanins (cyaniding-3-glucoside and malvidin-3-glucoside) and metals (Zn and Cu) were eluted together in the same fractions. It was concluded that those anthocyanins interact with Zn and/or Cu to some extent and the next step was the study of the possible metallic complexes formed between both species. A completely different behaviour pattern was seen for both Fe and Mn: these metals do not interact with any of the individual anthocyanins.

Differential pulse anodic stripping voltammetry and a theoretical study using Hyperchem® software were used in order to understand the interactions between anthocyanins and trace metals. Model organic compounds, resembling those previously identified were employed to perform both studies. The chosen compounds were (+) catechin and rutin.

Preliminary theoretical and electrochemical studies have shown a most probable 1:1 metal to anthocyanin complex. It should be remarked that these studies were performed at the natural pH of must and wine (pH = 4), rather than at the physiological pH as usually found in the literature.

<sup>1</sup>"Evolution of metal and polyphenol content over one year period of vinification: Sample fractionation and correlation metals -anthocyanins", I. Esparza, I. Salinas, I. Caballero, C. Santamaría, I. Calvo, J.M. García -Mina and J.M. Fernández, Anal. Chim. Acta, (submitted).

# ELECTROCHEMICAL AND THEORETICAL COMPLEXATION STUDIES FOR Zn, Cu AND INDIVIDUAL ANTHOCYANINS IN WINE SAMPLES

I. Esparza, Í. Salinas<sup>a</sup>, C. Santamaría<sup>a</sup>, J.M. García-Mina<sup>b</sup>, J.M. Fernández<sup>a</sup>

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## INTRODUCTION

The organoleptic characteristics of a red wine depend on a large number of factors -some of them still unknown- due to the high complexity of the wine matrix which contains many different organic and inorganic substances. It is well known that red wine -consumed in a moderate manner- has many beneficial properties for human health, most of them related to the presence of polyphenol compounds<sup>1</sup>. The chemical structure of these compounds is, for instance, responsible for the antioxidant activity of wine: polyphenols are involved in many chelation processes -mainly with copper and iron- which result in the inhibition of the catalytic activity of the metals and the corresponding formation of free radicals, becoming indirectly an antioxidant activity<sup>2</sup>.

On the other hand, the complexation reactions taking place between metals and polyphenols in wine modify the solubility and/or the stability of these compounds, inducing small changes in wine colour. These phenomena may arise as a compromise between the formation of new coloured compounds and the colour intensity loss due to precipitation of polyphenols, that may occur during the fermentative process.

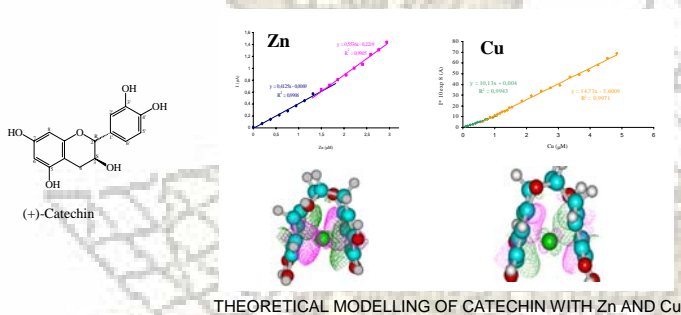
It is, therefore, important to identify and to quantify the concentration of metals and polyphenols present in red wine, what has already been done and published elsewhere<sup>3</sup>. Results showed a close relationship between an specific anthocyanin (cyanidine-3-glucoside) and several metals like Cu and Zn. The present work deals with the identification of the possible stoichiometries of the ligand-metal complexes, both theoretically and electrochemically, from model compounds resembling the anthocyanins present in wine.

## EXPERIMENTAL

Preliminary electrochemical studies have been performed on synthetic aqueous samples containing either catechin or rutin, buffered at the pH of the original sample (wine, pH 4) and also at pH 8. Differential pulse anodic stripping voltammetry (DPASV) was the electrochemical technique used throughout the study. Zn and Cu were the metals studied and (+)-catechin and rutin, were the model compounds chosen. The electrochemical experimental procedure involved the addition of known amounts of metal -either Zn or Cu- to the synthetic ligand solution. The evaluation of the slope of the current-metal concentration plot allowed the determination of the ligand-metal stoichiometry. Simultaneously, theoretical studies have been carried out using the same metal and model compounds, with the aid of Hyperchem® and Zindo 1 softwares.

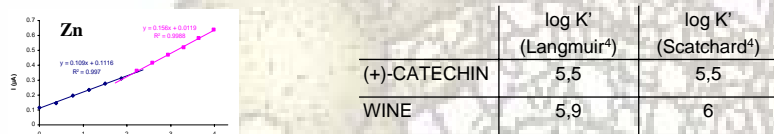
## ELECTROCHEMICAL TITRATION CURVES

### (+)-Catechin pH 4 → Stoichiometry 1:1 (Zn and Cu)

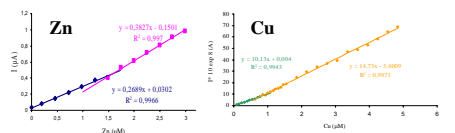


The electronic clouds belonging to aromatic rings seem to be involved in the complexes formation. Catechin bends and the space left can host a single metal molecule.

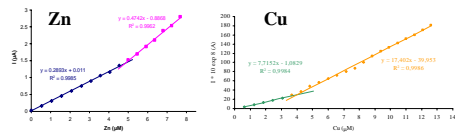
### APPLICATION: STUDY OF A MUST SAMPLE (21 days fermentation) AT pH 4. Determination and comparison of conditional stability constants for catechin and wine samples.



### (+)-Catechin pH 8 → Stoichiometry 3: 2 (Zn) → Stoichiometry 3: 1 (Cu)

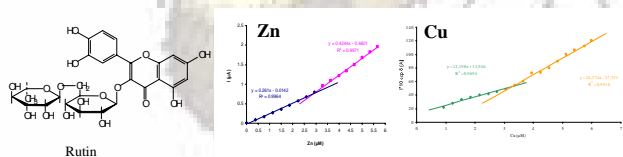


### Rutin pH 8 → Stoichiometry 5:1 (Zn) → Stoichiometry 4:1 (Cu)



Deprotonated functional groups (hydroxyl groups) now possibly participate in the chelating process at this pH. The corresponding theoretical study is on the way.

### Rutin pH 4 → Stoichiometry 3:1 (Zn and Cu)



Hydroxyl and carbonyl groups are possibly involved in the complexes formation, as this structure is too big to fold under the studied conditions.

## CONCLUSIONS

- pH affects strongly the stoichiometry of the studied complexes.
- The behaviour of Cu and Zn follow a similar pattern with either ligand.
- Experimental associations found between metals and anthocyanins<sup>3</sup> were confirmed in synthetic samples of ligands resembling anthocyanin chemical structures.
- Titration curves for wine and catechin (pH 4) are very close which points to cyanidine-3-glucoside as the main complexing agent present in wine.
- Theoretical modelling studies show an excellent agreement for both metals with catechin at pH 4.
- DPASV has proved to be a very useful technique since it is able to detect changes in the stoichiometry of complexes when experimental conditions are altered.

## FUTURE WORK

Further theoretical modelling will be calculated for the remaining experimental conditions not shown in this poster. Complexing studies will be performed on a two years old wine, ready to consume.

## REFERENCES

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