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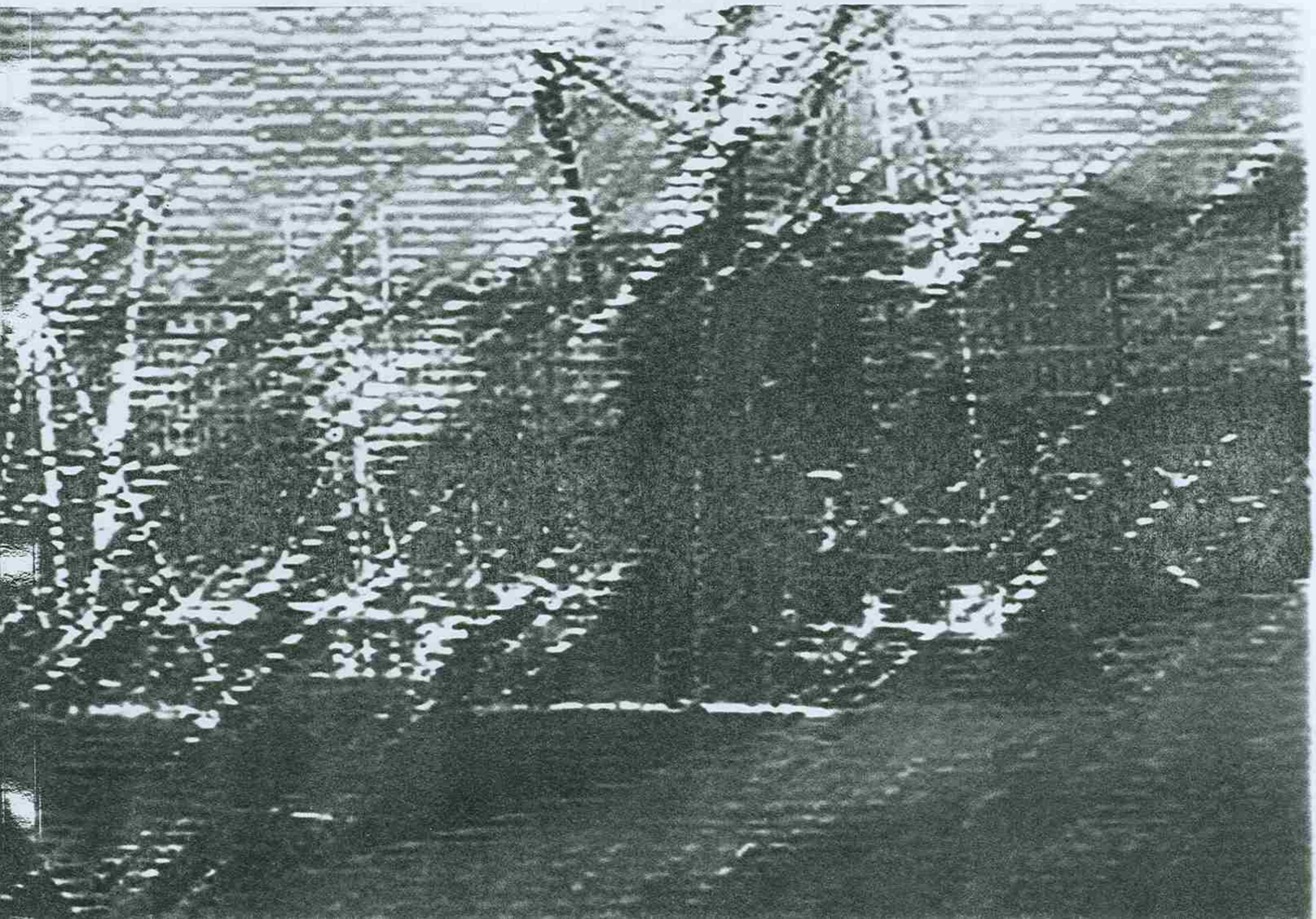

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A STUDY OF FRACTIONING AND HEAVY METAL COMPLEXATION IN WINES

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This work is concerned with the loss of colour observed in some of the red wines of Navarra. Flavonoid 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. In must, metals like Zn, Cu, Mn and Fe, present in low concentrations, act as cofactors of vitamins and enzymes, the latter being precursors of compounds responsible for the colour of wine. Some other metals like Pb and Cd are toxic[1,2] and Cr, Mn, Ca, Zn, Cu and Se have been described as inorganic nutrients and so their presence in wine is considered as positive in terms of consumers health[3].

Recent papers report the determination of the total metal content in wines produced in Galicia (Spain)[4], in several regions of Slovenia[5], with Monastrell variety grapes[6] and with young wines produced in the region of Murcia (Spain)[7]. In other cases, the antocyanin content of wine was evaluated depending on the time of contact of the skin of grapes with the wine during the fermentation process[8] or directly measured in the skin of grapes and in the corresponding wine[9].

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 was 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.

The analysis of the separated fractions obtained by chromatography indicate a preferential accumulation of certain metals (Zn, Cu, Mn) in the first fraction recovered, while iron was identified, at different concentrations, in most of the fractions. This behaviour points to antocyanines as the main ligand compounds for some of the metallic cations present in our samples.

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A STUDY OF FRACTIONING AND HEAVY METAL COMPLEXATION IN WINES. HISTORY OF A WINE.

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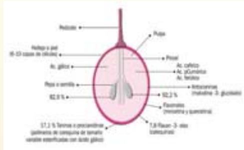
INTRODUCTION

Phenolic compounds are responsible for the colour of wine. These organic species are present in the skin, pulp and seeds of grapes but mainly in the skin (the skin is much more coloured than the other parts of the grape). The colour of these pigments and their stability depend mainly on the pH and the presence of several metallic cations. In must, metals such as Zn, Cu, Mn and Fe are present at low concentrations and function as cofactors of vitamins and enzymes precursors of those phenolic compounds.

The present work deals with the identification and quantitation of the metallic complexes responsible for the colour of wine and the study of their evolution with time throughout the fermentation of grapes.



METHODS

The studied samples were grape (*V. vinifera* cvs. Tempranillo grown in Navarre), must and wine, the last two fermented at different times ranging from 0 to 225 days, being 0 the time when grapes were introduced in the vessel where fermentation will take place.
Phenolic compounds were quantitated spectrofotometrically at 720 nm after samples were filtrated and dried.
Metals were identified and quantitated by ICP - AES. Previously, samples were fully digested with nitric acid, aided by a microwave radiation.
Samples were collected with the following frequency: days 1 to 15 daily; days 21 to 100 weekly and days 160 to 225, every two months. The last two samples were collected from two different barrels: one clarified (filtrated) and the other one without treatment (wine ferments in the presence of the skin, pulp and seeds of original grapes). All these samples were treated following the previously described method.

DISCUSSION

Results presented in this poster correspond to the first stage of the project. They show the evolution of the total content of phenolic compounds and metals during the fermentation process.
During the first days, polyphenol content experiments an important increase (figure B) while Fe and Cu concentrations decrease in the same way (column figure C). However, Mn and Zn concentrations remain almost constant along the time. Column figure A shows a close relationship present between Cu and Fe, and polyphenols.

Most of Mn and Zn in grape are concentrated in the seeds (insets figure column C) but their total content in wine is low. This result suggests that this metals are not extracted from the seeds to the must/wine during fermentation.

From the previous data and knowing that polyphenols are mainly present in the skin, one can think that there is no relationship between Zn, Mn and polyphenols.

All this work is a preliminary study which has allowed a selection of the most interesting cations (Fe and Cu) for the following studies of polyphenol complexation.

FUTURE WORK

Complexation studies in wine are difficult because this matrix is very complex. Fractionating of the different wine samples will facilitate the electrochemical complexation studies to be perform.

Figure D corresponds to a developed TLC obtained from a preliminary work directed to find the best solvent for the fractioning of samples. The solvent must mimic the original matrix (pH, ionic strength, alcoholic content) so that the metallic complexes remain stable.

Track 1 to 5 represent five different solvents containing phenolic compounds, after extraction from grape skin. Track 6 correspond to cyanidine. Figure D shows than solvent present in track 5 extracts a larger amount of polyphenols.

The following stage will concern the study of each fraction for: a) identification of the polyphenol compounds; b) quantitation of total metal content; c) electrochemical complexation studies.

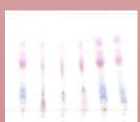
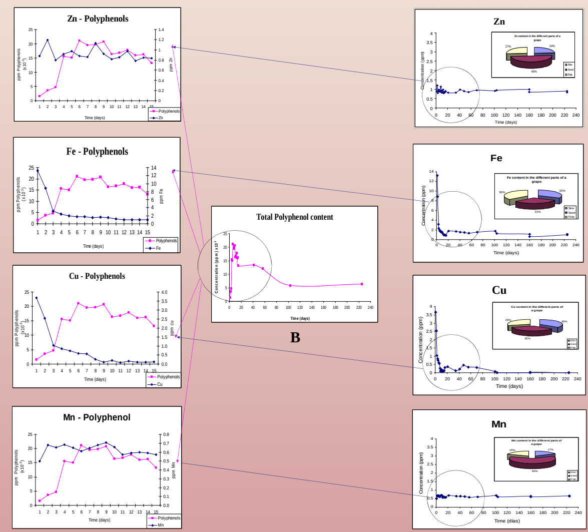


Figure D.- TLC for different extracting solvents containing phenolic compounds from grape skin.

RESULTS



A B C

Column figure **C** represents the evolution of metal content (Zn, Fe, Cu and Mn) during the fermentation process. Figure **B** corresponds to the evolution of the total polyphenol content in the samples during the same period of fermentation. Column figure **A** is a combination of **B** and **C** for the first 15 days.
Insets in column figure **C** indicate the distribution of each metal in a grape (skin, pulp and seeds).

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