

INHIBITORY ACTIVITY OF PHENOLIC COMPOUNDS AGAINST MICROORGANISMS INVOLVED IN OENOLOGICAL FERMENTATIONS

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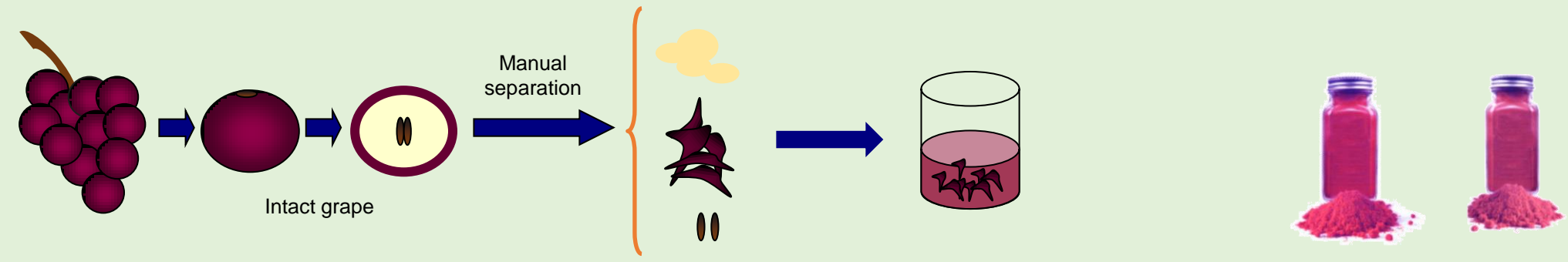
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Objectives: Stuck fermentations are a usual problem in high quality wines with high phenolic content. The aim of this work was to evaluate the antimicrobial activity of wine and grape phenolic extracts towards oenologic yeast and lactic bacteria strains. The use of a phenolic-subtractive oenological product which would minimize the stuck fermentations has also been studied. This product would reduce the contents of the phenolic compounds with the highest microbial development inhibitory activity, without causing a negative impact on the organoleptic quality of the wine

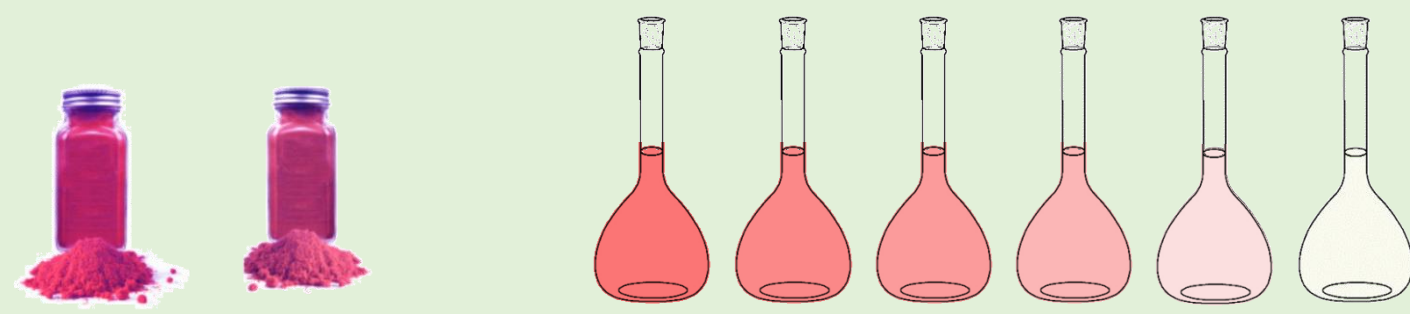
MATERIALS AND METHODS

Obtention of skin extracts: Tempranillo and Graciano grapes were harvested at technological maturity. Skins were manually separated from whole grape and extracted in methanol media until skin colorless. Extracts were pooled concentrated and finally lyophilized prior to evaluate their antimicrobial activity.



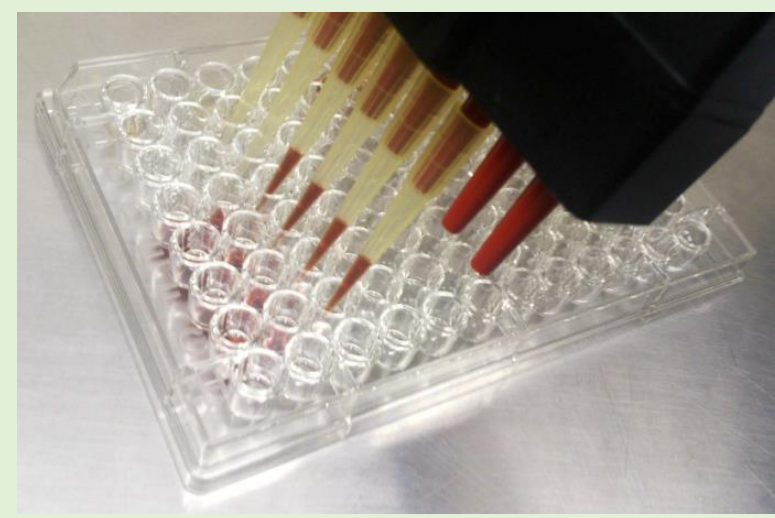
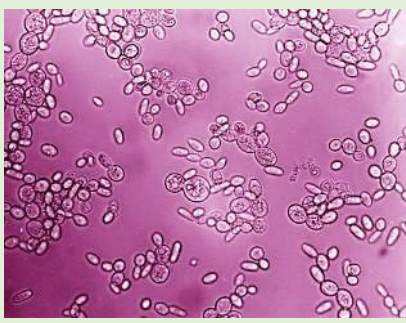
Skin extracts composition: Anthocyanin and Flavonol composition of the extracts was ascertain after HPLC-DAD-MS analysis as previously described^{1,2}

Effect on microbial development: microtiter plates were incubated with several different yeast or bacteria in the presence of grape skins at different concentrations. Effect was measured with a microtiter plates reader



20 enological yeast strains

3 *Brettanomyces*
8 comercial *Saccharomyces*
9 other non-*Saccharomyces* yeasts



17 enological bacteria strains:

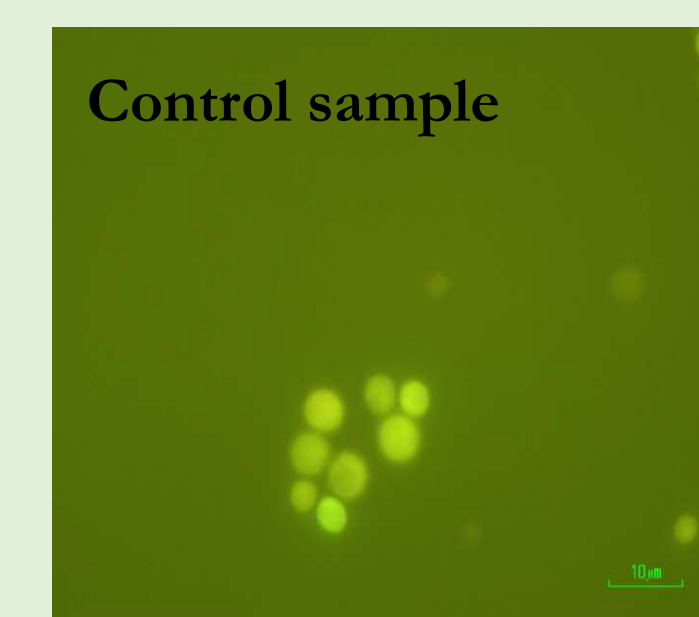
11 *Lactobacillus*
6 *Pediococcus*



Obtention of wine extracts: Tempranillo grapes were harvested at technological maturity and divided in 5 subsamples. Alcoholic fermentation was developed in five different tanks. One control wine and four wines obtained by subtracting treatment with technological adjuvants (A, B, C or D treatment) were made. The technological adjuvants were added to eliminate different types of phenolic compounds. Phenolic extracts from each wine were obtained by size-exclusion column chromatography.

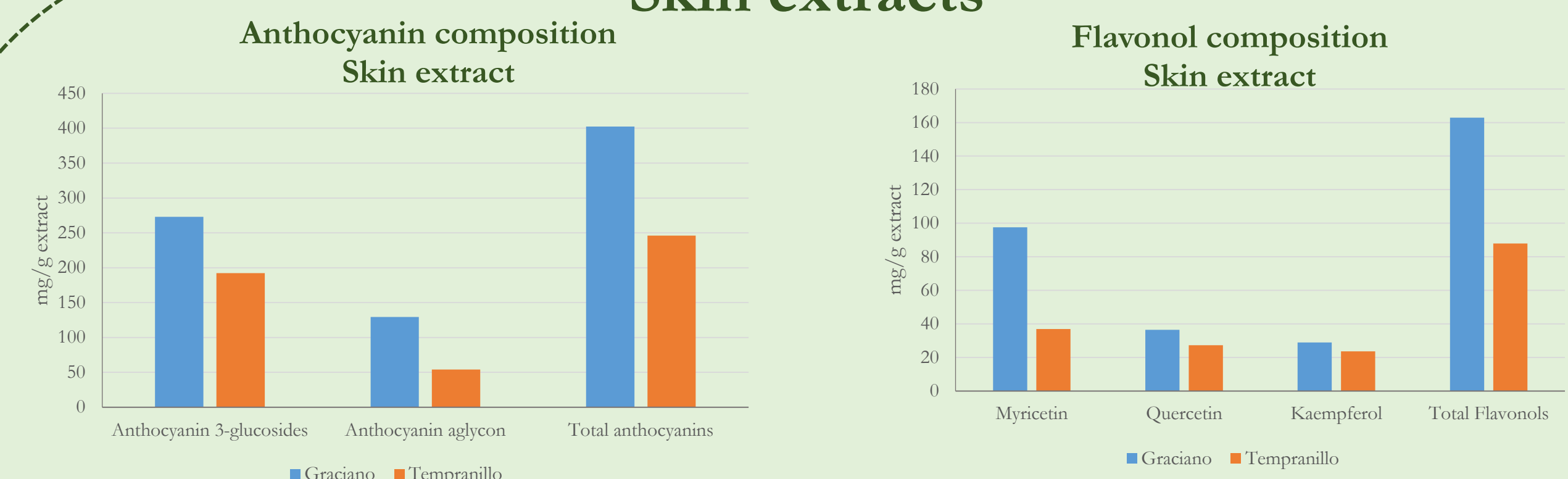


Effect on yeast viability: yeast cultures were treated with the five wine phenolic extracts (control + 4 subtractive products). The viability was measured by epifluorescence and microscopy counting of living cells.



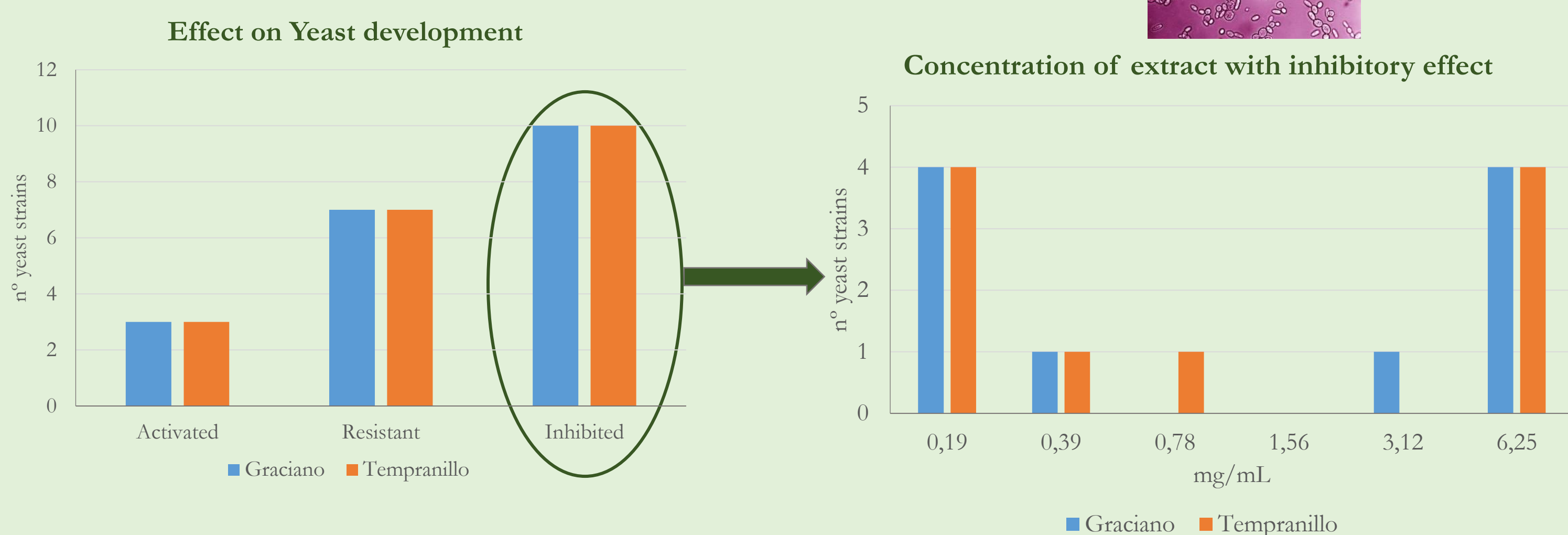
RESULTS

Skin extracts



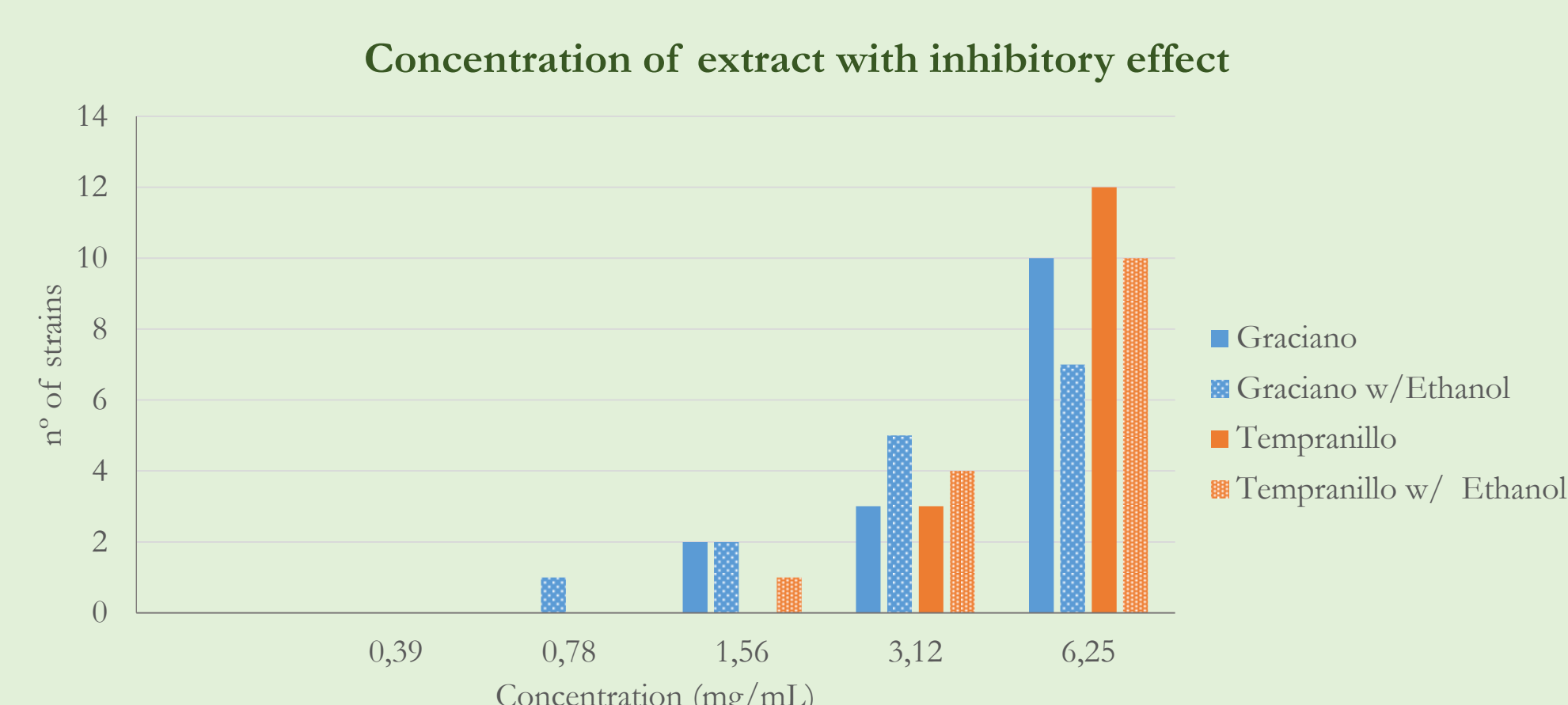
The composition of the skin extracts showed quantitative and qualitative differences between Graciano and Tempranillo grapes. Higher amounts of anthocyanins and flavonols were found in Graciano grapes. Higher percentage of quercetin and kaempferol derivatives were present in Tempranillo than in Graciano grapes

Effect on yeast development



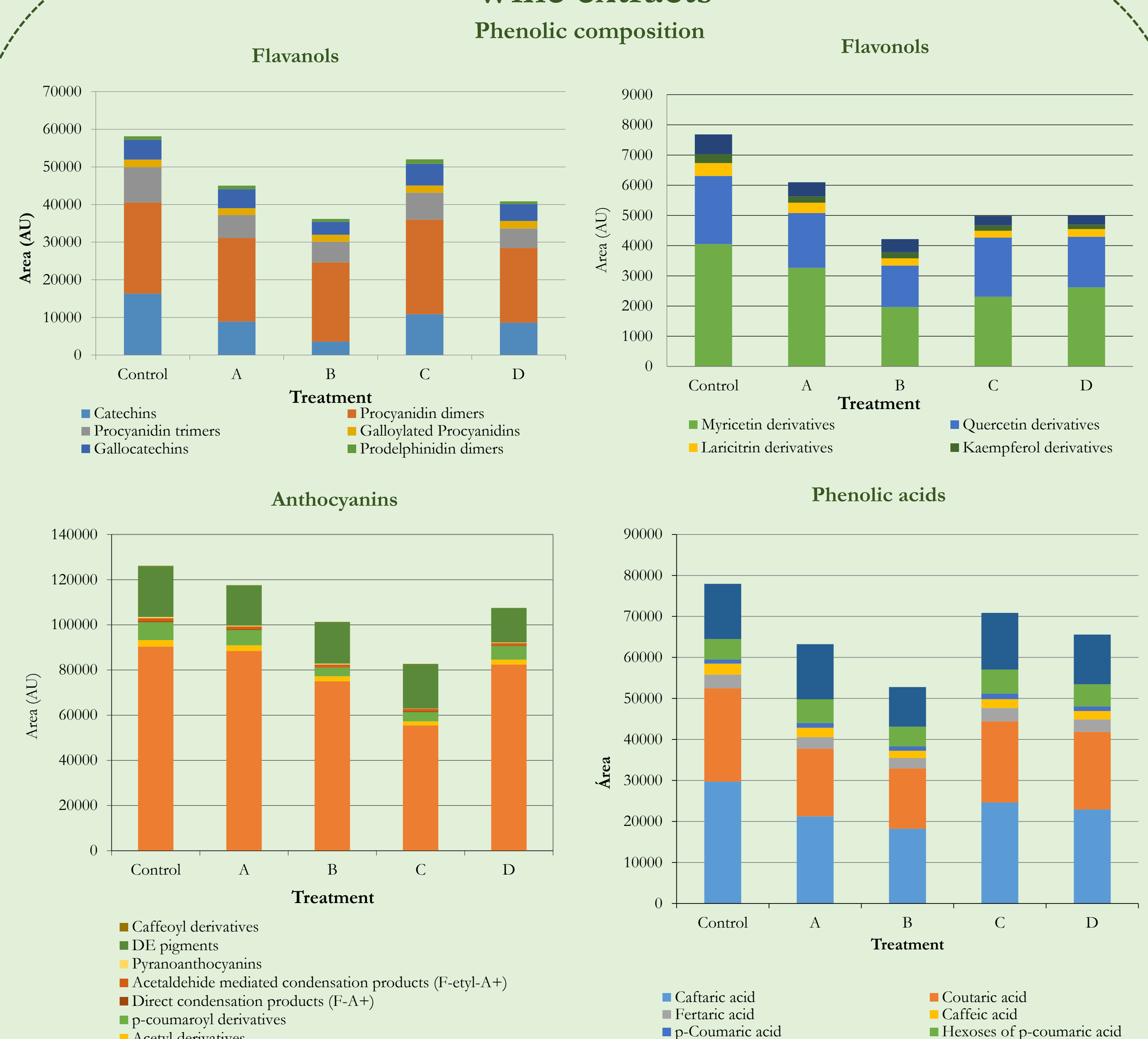
3 yeast strains were activated in the presence of grape skins extracts, 7 did not show any effect and 10 were inhibited. 4 of them were inhibited at all the studied concentrations. Despite the different concentrations of phenolic compounds in Tempranillo and Graciano extracts, few differences in the yeast development were found between the varieties. Among the inhibited strains, only one required higher concentration of Graciano extract than Tempranillo extract to be inhibited, pointing out the importance of qualitative differences. No fungicide effect was observed at the studied concentrations

Effect on lactic acid bacteria development



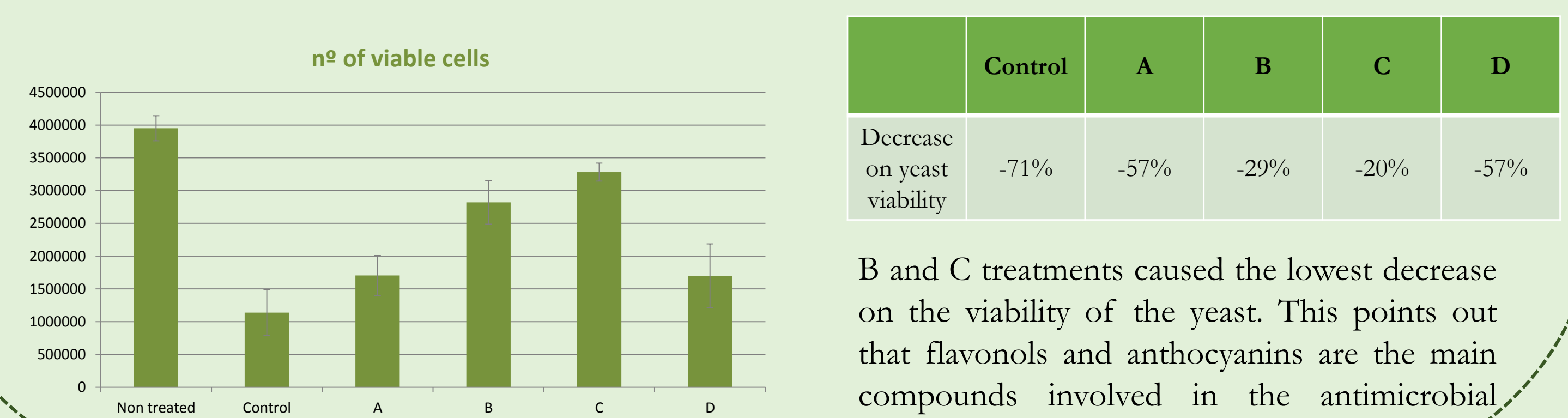
Bacteriostatic effect: The growth of fifteen of the seventeen lactic acid bacteria strains was inhibited by the presence of grape skin extracts, at concentration $\leq 6,25$ mg/mL, despite no bactericide effect was shown. Ethanol increased the inhibitory effect on the 17,6% of the cases. Graciano extract has inhibitory effect towards lactic acid bacteria at lower concentrations than Tempranillo extract.

Wine extracts



The use of phenolic subtractors modified the phenolic profile of the wines. Different subtractors showed different effects on the different families of compounds. A and D treatment caused a slight decrease of all families of compounds. B treatment caused an important decrease on phenolic acids, flavanols and flavonols and a medium decrease in anthocyanins. C treatment provoked an important decrease in anthocyanins and also in flavonols contents.

Effect on yeast viability



B and C treatments caused the lowest decrease on the viability of the yeast. This points out that flavonols and anthocyanins are the main compounds involved in the antimicrobial activity shown by the wine extracts

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References:

1-Alcalde-Eon, C.; García-Estévez, I.; Ferreras-Charro, R.; Rivas-Gonzalo, J.C.; Ferrer-Gallego, R.; Escribano-Bailón, M.T. *J. Food Compos. Anal.* **2014**, *34*, 99-113
2-Quijada-Morín, N.; Hernández-Hierro, J.M.; Rivas-Gonzalo, J.C.; Escribano-Bailón, M.T. *J. Agric. Food Chem.*, **2015** Article ASAP DOI: 10.1021/acs.jafc.5b00261

CONCLUSIONS

Most of the yeast strains used were inhibited in presence of Tempranillo and Graciano skin extracts. Qualitative differences in polyphenolic composition of these two grape varieties could be more important for this inhibitory effect than the quantitative differences. The grown of most of the LAB strains used was inhibited in presence of grape skin extracts, although no bactericide effect was shown. Graciano extract has inhibitory effect towards lactic acid bacteria at lower concentrations than Tempranillo extract. The subtracting treatment with technological adjuvants modified the phenolic profile of Tempranillo wines. Extracts from treatments that decrease the content in flavonols and in anthocyanins showed the lowest differences towards the control in the number of viable yeast.