

METHOD DEVELOPMENT AND APPLICATION FOR STUDYING PROANTHOCYANIDIN PROFILES OF RED WINES

<u>Marina Stefova,</u>^a Violeta Ivanova-Petropulos,^b Borimir Vojnoski,^c Jean-Mark Souquet,^d Emmanuelle Meudec,^d Veronique Cheynier^d

- ^a Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje, RN Macedonia
- ^b Goce Delcev University, Faculty of Agriculture, Stip, RN Macedonia,
- ^c Wine Department, Institute of Agriculture, Ss. Cyril and Methodius University, RN Macedonia
- ^d JRU Science for Oenology, INRA, Montpellier, France

marinaiv@pmf.ukim.mk

Polyphenols are widespread secondary metabolites found in plants and food sources such as fruits, herbs, chocolate, and red wine. They have antioxidant and nutraceutical properties and contribute to color and taste. In red wine, polyphenols, especially flavan-3-ols, play a key role in sensory perception. Condensed tannins, or proanthocyanidins (PAs), are oligomers and polymers of flavan-3-ols that significantly influence wine quality and can form red pigments under acidic, oxidative conditions. Procyanidins, which are condensed tannins composed of flavan-3-ol subunits, play a key role in wine astringency, bitterness, and color stabilization. Due to their polymeric nature, direct analysis is challenging. Therefore, a depolymerization approach such as acid-catalyzed cleavage in the presence of nucleophiles is usually employed to break down the procyanidin polymers into their constituent monomers and extension units. This approach enables detailed profiling of the procyanidin composition, including the average degree of polymerization (aDP) and the proportion of terminal versus extension units. Such data are essential for understanding the sensory characteristics of wine and how they evolve during vinification and aging.

In this study, a depolymerization strategy using phloroglucinolysis was applied to wines from the Vranec and Merlot grape varieties to characterize their proanthocyanidin profiles. Two sample preparation procedures using solid-phase extraction and precipitation with methanol were tested and compared. The latter was then used for assay of procyanidins in twenty-four experimental wine samples obtained under different vinification conditions. The depolymerization approach enabled the breakdown of proanthocyanidin polymers into monomeric and extension units, providing insight into their composition and allowing the estimation of the mean degree of polymerization (mDP). Flavan-3-ols were quantified using spectrophotometry and reversed-phase HPLC-DAD, while HPLC-ESI-MS/MS was employed for identification of flavan-3-ol monomers and phloroglucinol adducts. The results demonstrated that longer maceration time and higher SO₂ concentrations led to wines with significantly increased levels of proanthocyanidins, flavan-3-ols, and total polyphenols, highlighting the impact of vinification parameters on the polyphenolic composition and potential sensory attributes of red wine.