



ISTITUT AGRICOLE REGIONAL



# LC-MS CHARACTERIZATION, ANTIRADICAL POWER AND BIOLOGICAL ACTIVITY OF POLYPHENOLIC EXTRACTS FROM ANCIENT GRAPE VARIETIES TYPICAL OF AOSTA VALLEY

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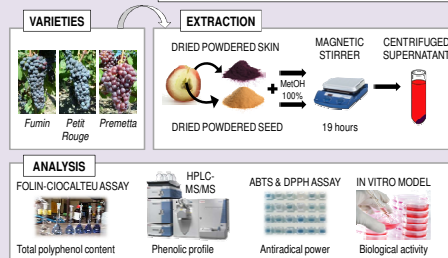
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## Introduction

Polyphenols are the most important phytochemicals in grape due to their biological activities and health-promoting benefits. The profile of phenolic compounds varies depending on varieties and it is influenced by vineyard management and by growing conditions.

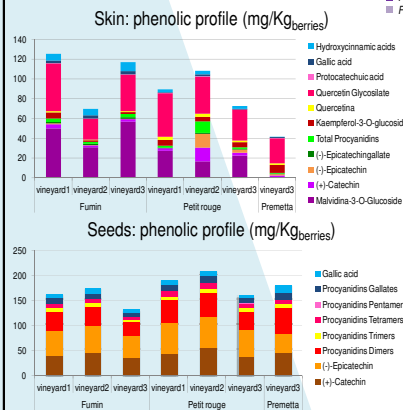
For this study three autochthonous grape varieties (*Fumin*, *Petit Rouge* and *Premetta*) grown in Aosta Valley (Italy) were harvested from three different vineyard and immediately dried. Powdered skins and seeds were extracted with methanol. The extracts were characterised in terms of total polyphenols (TP) content, antiradical power (ARP) using ABTS\* and DPPH\* assays and phenolic profile by LC-MS/MS (ESI-). Moreover, the biological vaso-protective activity of seed extracts was investigated by evaluating the expression of some endothelial activation markers in an in vitro model of endothelial dysfunction induced by the pro-inflammatory cytokine TNF $\alpha$ .

## Materials and methods



## Results: phenolic profile

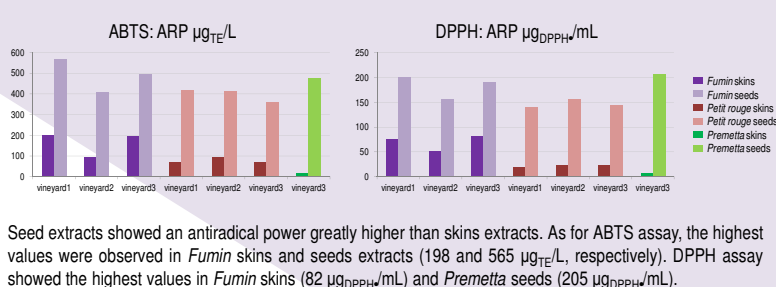
Total polyphenol content varied greatly among cultivars and vineyards, falling in the ranges from 215 to 1759 mg<sub>CE</sub>/Kg<sub>berries</sub> (skins) and from 1739 to 2137 mg<sub>CE</sub>/Kg<sub>berries</sub> (seeds). The highest values were reached in *Fumin* skin and *Premetta* seeds.



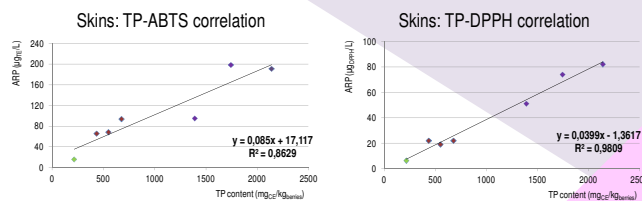
TP content (mg<sub>CE</sub>/Kg<sub>berries</sub>)

Phenolic profile showed some differences depending on cultivar and vineyard. Malvidine-3-glucoside and glycosylated flavonols were the most abundant polyphenols in skin extract ( $p < 0.001$ ), especially in *Fumin* and *Petit Rouge*, respectively. Kaempferol-3-O-glucoside too was present in larger amount in skin extracts ( $p < 0.001$ ), especially in *Premetta*. Flavonols and total procyanidins were the most abundant class of polyphenols present in seed extracts ( $p < 0.001$ ) without significant differences between grape varieties.

## Results: antiradical power

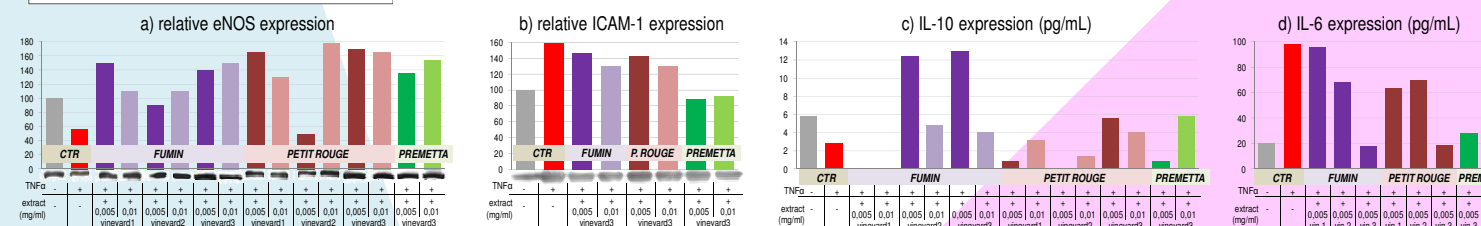


Seed extracts showed an antiradical power greatly higher than skins extracts. As for ABTS assay, the highest values were observed in *Fumin* skins and seeds extracts (198 and 565 µg<sub>Te</sub>/L, respectively). DPPH assay showed the highest values in *Fumin* skins (82 µg<sub>DPPH</sub>/mL) and *Premetta* seeds (205 µg<sub>DPPH</sub>/mL).

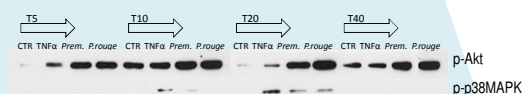


As for skins extracts, both ABTS\* and DPPH\* assays showed a good correlation with total polyphenol content of the extracts. Indeed, for seeds extracts this correlation was low (data not shown).

## Results: biological activity



Endothelial cells were subjected to TNF $\alpha$  (100 ng/mL) and seed extracts at two non-cytotoxic concentrations (0.005 and 0.01 mg/mL), for evaluate the expression of some dysfunction markers (eNOS, ICAM-1, AT-1, ACE, MMPs, ILs). The TNF $\alpha$ -induced decrease of eNOS (a) seems to be counterbalanced by all the extracts tested, except for *Petit rouge* from vineyard 2 at 0.005 mg/mL. TNF $\alpha$  produced an increase of ICAM-1 level, apparently counterbalanced by all the extracts tested from vineyard 3 (especially *Premetta* at both concentrations). A potential efficacy of the extracts was also observed in counteracting the TNF $\alpha$ -induced decrease of IL-10 (c), especially in *Fumin* from vineyards 2 and 3, which at 0.005 mg/mL led IL-10 levels even more high than control level. TNF $\alpha$ -induced increase of IL-6 expression (d) seems to be counterbalanced by all the extracts at 0.005 mg/mL, especially grapes from vineyards 3 which, apparently, restored the basal level of this inflammatory cytokine.



Two TNF $\alpha$ -triggered activated signalling pathways (Akt and p38MAPK) were studied, subjecting endothelial cells to TNF $\alpha$  and grape extracts for 5, 10, 20 and 40 min. Western blotting seems to show an increase of Akt phosphorylation (formerly activated by TNF $\alpha$ ) and an inhibition of p38MAPK pathway, due to treatments with grape seed extracts coming from vineyard 3 (*Fumin* data not shown). As for the other markers tested (AT-1, ACE, MMPs), it was not detected a substantial activity of the extracts in counteracting the inflammatory effect of TNF $\alpha$  (data not shown).

## Conclusions

This preliminary study suggests that ancient grape cultivars grown in mountain areas are an important source of phenolic compounds, show high levels of antiradical power and seem to be involved in preventing endothelial dysfunctions induced by cardiovascular diseases. Differences observed in antiradical power and biological activity of grapes coming from different vineyards are probably due to a different composition of the phenolic profile, which is directly related to growth conditions such as high solar radiation, altitude and harsh climate. This study is continuing with the assessment of the biological activity of skins extracts too, and with another year of sampling and analysis, in order to increase the informations about nutraceutical and healthy properties of typical grapes of Aosta Valley region.

## Acknowledgements

This study was funded by "FESR Competitività regionale 2007/2013" and "FSE Occupazione 2007/2013" programs. We would like to thank Les Crêtes ss and Otin ss for providing many of the samples analyzed in this study.