

ANTIOXIDANT CAPACITY AND BIOLOGICAL EVALUATION OF DIFFERENT LOCAL APPLE VARIETIES FROM AOSTA VALLEY

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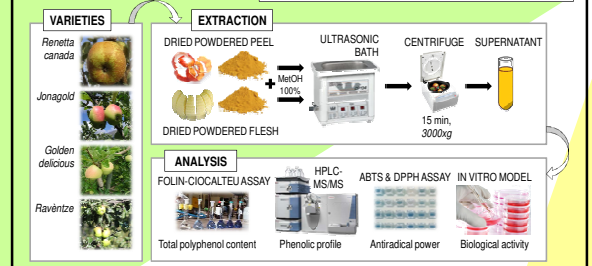
Introduction

Apple cultivars from mountain areas are an important source of antioxidant compounds due to the characteristics of the environment where they grow, and may have a great biological vaso-protective activity against degenerative disorders as cardiovascular disease.

In this study, four different apple cultivars (*Renetta canadensis*, *Jonagold*, *Golden delicious* and *Ravèntze*) from Aosta Valley (Italy) were harvested at commercial maturity from three distinct orchards.

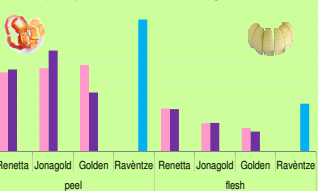
Powdered peels and fleshes were extracted in methanol and studied in terms of total polyphenol (TP) content, phenolic profile by LC-MS and antiradical power (ARP) by ABTS* and DPPH* assays. Moreover, biological activity of peel extracts was investigated by an in-vitro model of endothelial dysfunction induced by the pro-inflammatory cytokine TNF α .

Materials and methods



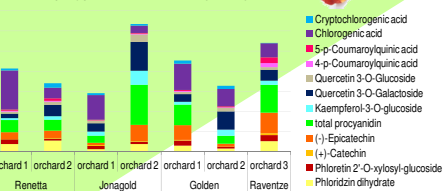
Results: phenolic profile

Total polyphenol content - mg_{CE}/100g_{DM}



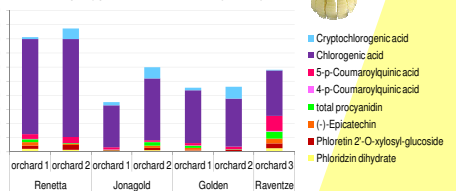
Total polyphenol content in peel and flesh varied depending of variety and orchard, reaching the highest values in *Ravèntze* cultivar (1498,20 and 546,11 mg_{CE}/100g_{DM} in peel and flesh, respectively).

Peel - polyphenol content mg/100g_{DM}



The phenolic profile of peels varied greatly among cultivars and orchards (especially in *Jonagold*). *Ravèntze* showed the highest levels of catechin (10,35 mg/100g_{DM}, p<0,05), 4-p-coumaroylquinic acid (19,77 mg/100g_{DM}, p<0,01) and 5-p-coumaroylquinic acid (29,40 mg/100g_{DM}, p<0,01).

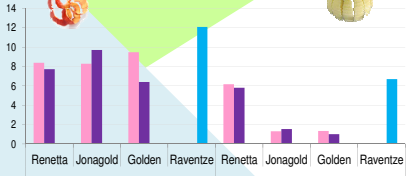
Flesh - polyphenol content mg/100g_{DM}



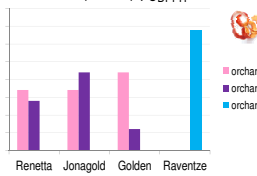
Fleshes showed a phenolic profile generally less rich than peels, but with important levels of chlorogenic acid in all the studied apples, with the highest concentration in *Renetta* cultivar (342,21 mg/100g_{DM}, p<0,01).

Results: antiradical power

ARP (ABTS) μ g_{TE}/L

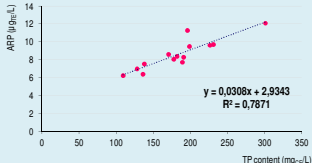


Peel ARP (DPPH) μ g_{DPPH}/mL

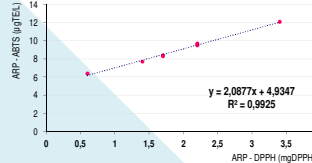


The highest ARP was observed in *Ravèntze* peel extract (12,1 μ g_{TE}/L), and in *Renetta* and *Ravèntze* flesh extracts (6,7 and 5,9 μ g_{TE}/L, respectively) with ABTS assay. The DPPH assay also showed, for peel extracts, the highest value in *Ravèntze* cultivar (3,4 mg_{DPPH}/mL).

Peel: TP-ABTS correlation



DPPH - ABTS correlation



IndGood correlations were observed between ARP (ABTS* assay) and TP content of peel extracts and between the results of ABTS* and DPPH* assays. The correlation of ARP (ABTS* assay) and TP content was low for flesh extracts (data not shown).

Conclusions

This preliminary study suggests that ancient and more recent apple cultivars cultivated in mountain areas are an important source of phenolic compounds. The extracts 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 apples coming from different orchards are probably due to a different composition of the phenolic profile, which is directly related to growth conditions of the fruit.

This study is continuing with additional sampling and analysis, in order to increase the informations about nutraceutical and healthy properties of typical apples of Aosta Valley region.

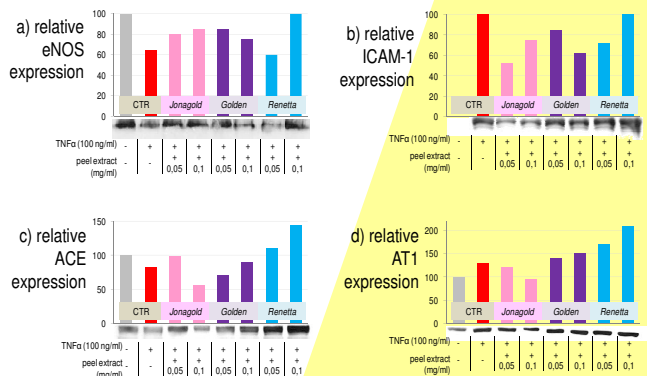
Acknowledgements

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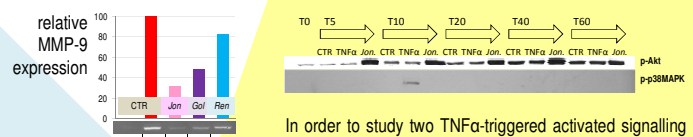
Results: biological activity

Among peel extracts tested, apples harvested from orchard 2 (*Renetta*, *Jonagold* and *Golden*) seem to show a biological activity, counteracting the effects of the pro-inflammatory cytokine TNF α . Apparently, extracts from apple harvested in orchards 1 and 3 did not show any substantial activity in counteract the effects of TNF α (data not shown).

Figures below display the expression levels of four key markers in endothelial dysfunction. Two non-cytotoxic concentrations for each extract were tested (0,05 and 0,1 mg/ml). Western blotting of cell lysates and densitometric analysis were performed after normalization for control levels of the marker.



The TNF α -induced decrease of eNOS (a) and increase of ICAM-1 (b) seem to be counterbalanced by all the extracts tested (except for *Renetta* 0,05 mg/ml and 0,1 mg/ml, respectively). The decrease of ACE (c) expression appears to be neutralized by *Renetta* extract at both concentrations tested, while only *Jonagold* extract display a potential counteraction for the TNF α -induced increase of AT1 (d).



Treatments with apple extracts, especially *Jonagold* and *Golden* (0,05 mg/mL), seem to counterbalance the TNF α -induced increase of MMP-9.

In order to study two TNF α -triggered activated signalling pathways (phosphorilation of Akt and p38MAPK), endothelial cells were subjected to TNF α and apple extracts for 5, 10, 20, 40 and 60 min. Seemingly, western blotting shows an activation of Akt and an inhibition of p38MAPK pathways due to treatment with apple extract. The figure above display, as an example, the effects of *Jonagold* apple extract.