BIOCHEMICAL CHARACTERIZATION AND BIOLOGICAL EVALUATION OF POLYPHENOLIC EXTRACTS FROM LOCAL APPLE VARIETIES GROWN IN AOSTA VALLEY

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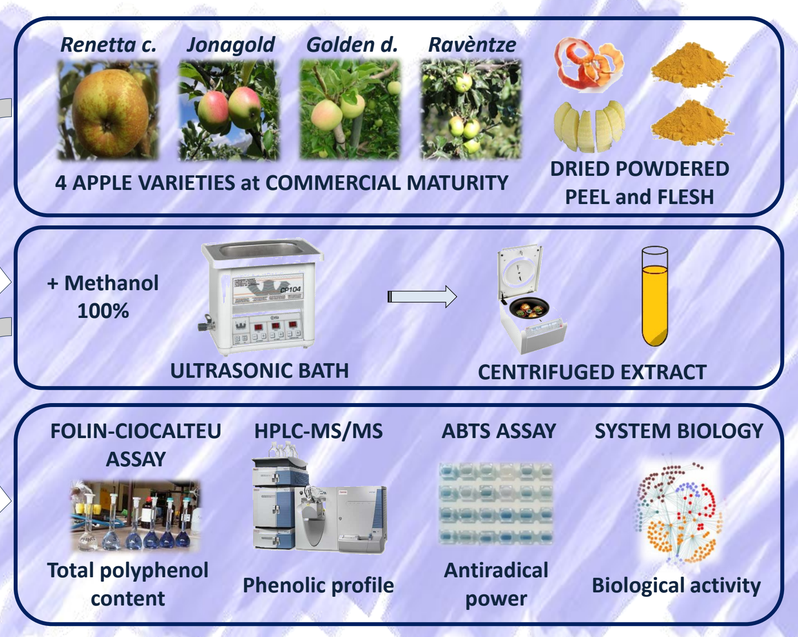
Introduction

Ancient and more recent apple varieties cultivated in local areas might represent an important source of phenolic compounds due to the characteristics of the environment where they grown. The aim of this work was to study phenolic profile and biological activity in the peel and flesh of four apple cultivars grown in Aosta Valley: *Ravèntze* (an ancient and autochthonous cultivar), *Renetta canada*, *Jonagold* and *Golden delicious*. Sampling was performed during three years (2013-2015).
Apples were harvested at commercial maturity and immediately dried. Powdered peels and fleshes were extracted in methanol using an ultrasound assisted technique, and characterized in terms of total polyphenols content (TP), phenolic profile, antiradical power (ABTS$^{\bullet+}$ assay) and biological activity (bioinformatic study with a system biology approach).

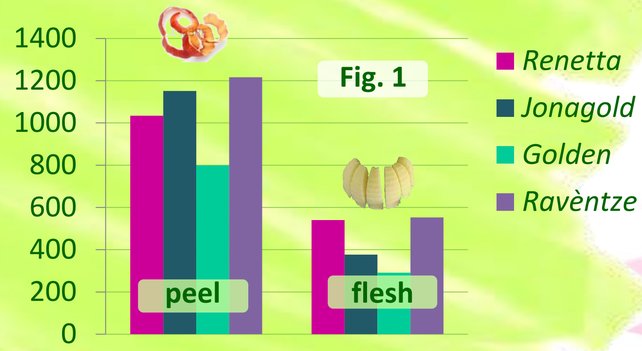
Materials & Methods



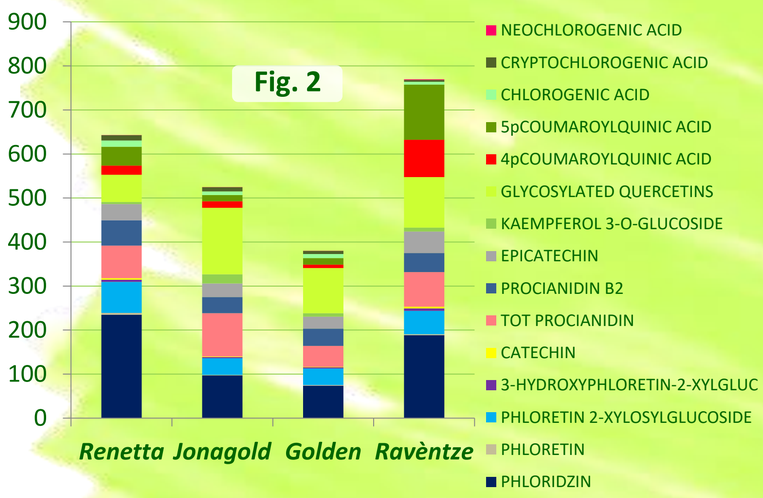
Results - phenolic profile

Total polyphenol content in peel and flesh varied depending of variety, reaching the highest values in *Ravèntze* cultivar (1498,20 and 660,7 $mg_{CE}/100g_{DM}$ in peel and flesh, during the first and second year of sampling, respectively). Figure 1 shows mean TP values.

mean TP content - mg/100g $_{DM}$

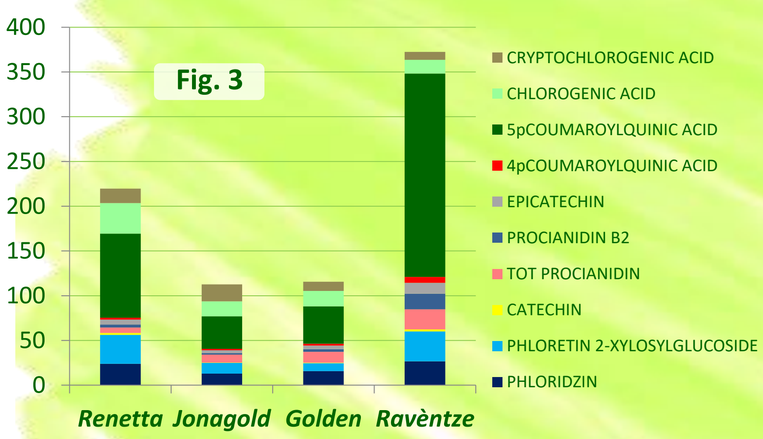


mean phenolic profile of peel - mg/100g DM



The predominant phenolics found in peel extracts (mean values shown in Figure 2) were dihydrochalcones, procyanidins, glycosylated quercetins and coumaroylquinic acids.
Renetta cultivar had the highest mean content of phloridzin and phloretin-2-xylosylglucoside (234,96 and 70,67 $mg/100g_{DM}$ respectively, **p<0,01).
Jonagold and *Ravèntze* showed the largest mean amount of glycosylated qurcetins (150,86 $mg/100g_{DM}$, **p<0,01).
Lastly, the phenolic profile of *Ravèntze* apple was higher in regards of 4p- and 5p-coumaroylquinic acids (85,21 and 124,89 $mg/100g_{DM}$ respectively, **p<0,01).

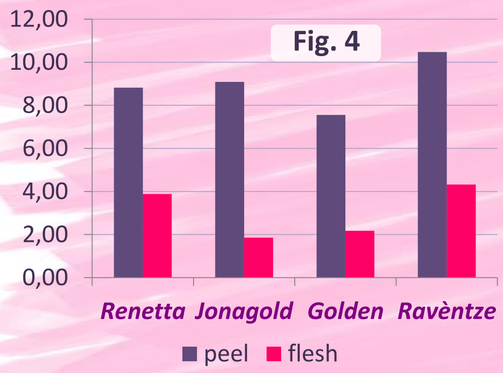
mean phenolic profile of flesh - mg/100g DM



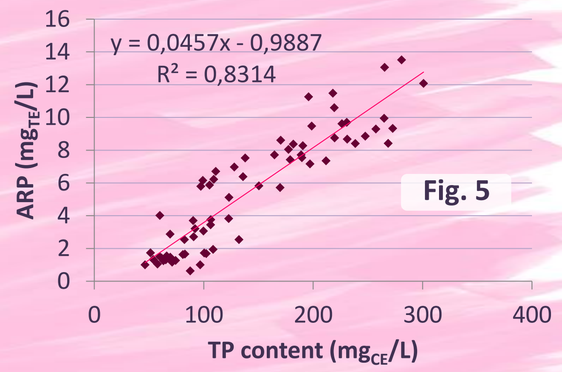
5-p-coumaroylquinic acid was the predominant phenolic compound in the flesh of all the apples studied (mean values shown in Figure 3), and showed the highest concentration in *Ravèntze* cultivar (267,23 $mg/100g_{DM}$, **p<0,01).
Renetta cultivar had the highest amount of chlorogenic acid (34,15 $mg/100g_{DM}$, **p<0,01).
As for dihydrochalcones, *Renetta* and *Ravèntze* are the cultivars more abundant in phloretin and phloretin 2-xylosylglucoside (**p<0,01).

Antiradical power - Results

ARP - mean ABTS - $\mu g_{TE}/L$



TP-ARP correlation



Antiradical activity of peels and fleshes showed great differences between the three years of sampling (mean values are represented in Figure 4). The highest ARP values were observed in *Ravèntze* peel and flesh extracts (13,52 µgTE/L and 6,71 µgTE/L, respectively). A good correlation (R^2 of 0,8) was observed between antiradical activity and TP content of peel and flesh extracts (Figure 5).

Biological activity - Results

26 nutraceutical bioactive compounds were found in the extracts analyzed; 6 among them showed enough concentration to potentially regulate up to 13 target molecules involved in cardiovascular and antitumoral pathways (more detailed results are shown in Figures 6 to 11). Coloured dotted lines in each graph represent the concentration level at which the concerned nutraceutical could possibly have an activity in up- or down- regulation of the target molecule in the related square box.

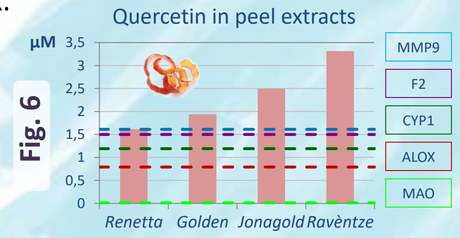


Fig. 6: all the peel extracts showed enough quercetin content to regulate 7 targets (ALOX5, ALOX15, CYP1, CYP19A1, F2, MAO, MMP9).

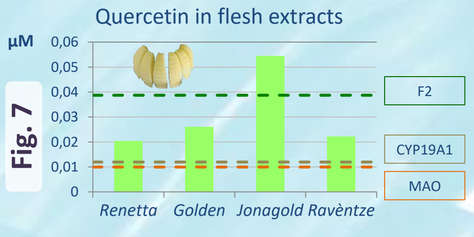


Fig. 7: all the flesh extracts showed enough quercetin content to regulate the MAO and CYP19A1 targets; *Jonagold* flesh quercetin content was enough to have an effect on F2 too.

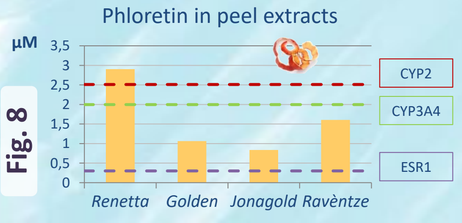


Fig. 8: all the peel extracts showed enough phloretin content to regulate 3 target molecules (CYP2, CYP3A4, ESR1).

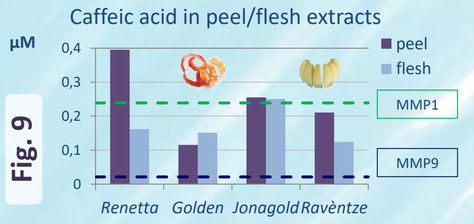


Fig. 9: all the peel and flesh extracts showed enough caffeic acid content to regulate MMP9; the content in *Renetta* (peel) and *Jonagold* (peel and flesh) extracts was enough to have an effect on MMP1 too.

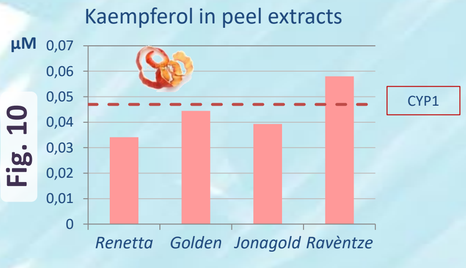


Fig. 10: *Ravèntze* peel extract showed enough kaempferol content to regulate the target CYP1; *Jonagold*, *Golden* and *Ravèntze* peel extracts had also sufficient isorhamnetin content to regulate the same target (data not shown).

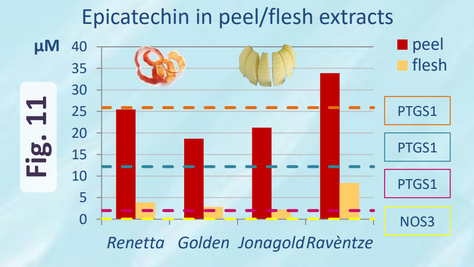


Fig. 11: All the peel and flesh extracts studied, especially *Ravèntze* peel, showed enough epicatechin content to regulate the targets NOS3 and PTGS1 (which is involved in more then one metabolic path).

Conclusions

This study suggests that ancient and recent apple varieties cultivated in mountain areas show high levels of phenolic compounds and antiradical activity, due to environmental conditions of growth. The autochthonous apple variety *Ravèntze*, in particular, has a high content of nutraceuticals like phloridzin, glycosylated quercetins and coumaroylquinic acids.
The level of antiradical activity is high mostly in apple peel, and seems to be positively related to TP content of the extracts.
The extracts of peel and flesh of the four varieties studied show enough content of quercetin, phloretin, caffeic acid, kaempferol, isorhamnetin and epicatechin to, potentially, regulate 13 different target molecules involved in cardiovascular, inflammatory and antitumoral pathways. This could suggest a possible nutraceutical effect of the apples studied.

Acknowledgements

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