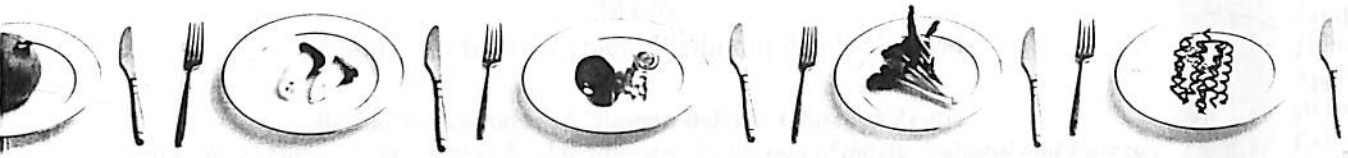

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Phenolic Profile of Typical Piedmont Apple Cultivars

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KEYWORDS
apple; phenolics; HPLC/DAD; HPLC/MS.

SUMMARY
Forty-one phenolic compounds (22 phenolic acids, 10 flavonoids, 3 glycosilated flavonols and 6 mixed phenolics) in 13 typical Piedmont apple cultivars were looking for. Phenolic profiles were collected for apples harvested in 2008 and were compared with that of an international cultivar (*Golden delicious*). Apple were peeled, cutted in small pieces, cryogenic frozen with liquid N_2 to avoid oxidative processes and then freeze-dried. Lyophilized apple powder was extracted two times with 90/10 methanol/water under sonication, vacuum dried and then 2 ml were filtered and injected in HPLC/DAD and HPLC/MS. Analytes were separated on a C_{18} column (250 x 4,6 mm, 5 µm) and the DAD detector was setted in full scan modality from 210 to 360 nm. For quantitation purpose, four point calibration curves (0.1, 0.5, 1 e 5 mg/L) were calculated. HPLC/MS was used to confirm DAD data. Because of the high number of analytes three different chromatographic methods were created and used. Analytes presence was confirmed by S.I.M. and MS^2 spectra analysis. Phenolic compounds were found and quantified in apple samples. Their total concentrations vary with the cultivar and are consistent with the collected literature data.

INTRODUCTION

Phenolic compounds or simply phenolics are compounds that possess one or more hydroxyl groups attached to one or more aromatic rings and represent a large and very important group of secondary metabolites of plant with a variety of functions in plant growth, development and defence (Vermerris and Nicholson 2006). Phenolic compounds include signalling molecules, pigments and flavours that can attract or repel, as well as compounds that can protect the plant against insects, fungi, bacteria and viruses. Most phenolics are found in nature in the form of esters or glycosides as free compounds rather than polymers like tannins and lignin and their content in plant vary with species, physiological stage and climatic conditions (Naczk and Shahidi 2004).
Recent studies demonstrate that plant but in particular fruits are important in the human diet for their effort in antioxidants compounds that are correlated to the health disease prevention. In particular apples consumption has been associated with lowered risk of cancer and cardiovascular diseases caused by oxidative processes. Polyphenolics and vitamins seem to be the major responsible of their great antioxidant power (Tsao *et al.* 2003). Aim of this work is to analyze the phenolic composition of ancient variety of apples coming from Piedmont (Italy) and compare it with an international cultivar took as reference.

MATERIALS AND METHODS

Samples

Thirteen cultivars were analyzed, 12 ancient cultivar and 1 international cultivar (*Golden delicious*). Apples had all grown in the same place in province of Turin (Piedmont, Northwest Italy) and were all harvested in 2008.

Extraction method

Samples were peeled, cutted into small pieces and immediately frozen with liquid nitrogen (Tsao *et al.* 2003).

Two grams of frozen sample were lyophilized for 48 h (D'Abrosca *et al.* 2007) and 0,1 g of lyophilized powder was used for the successive extraction with 5 mL of a solution of methanol/water 90/10 (v/v) (Bandoniene and Murkovic 2002). Sample was sonicated for 30 min and then centrifuged (10000 rpm, 10°C); these operations were repeated for two time (Hagen *et al.* 2007). Extract was then dried with a rotovapor (40°C, 50 mbar; Fattouch *et al.*, 2007) and finally 2 mL were filtered (0,45 μm) and injected in a HPLC system equipped with a Diode Array Detector (DAD) and a Mass Spectrometer.

HPLC/DAD analysis

An HPLC binary gradient pump system with an automatic injector was used. Separation was achieved on a C_{18} RP Lichrosphere (250 × 4.6 mm, 5 μm) column. Elution solvents were acetic acid/water (2/98 v/v) and acetonitryl. The mobile phase flow was 0,8 mL/min and the volume injected was 20 μL.

Analyses were performed with an UV Diode Array Detector equipped with a 5 cm length cell, operating in full scan modality.

Forty-one phenolic compounds were analyzed: 22 phenolic acids, 10 flavonoids, 3 glucosilated flavonoids and 6 mixed compounds. Separation was valley-valley for 17 analytes, not valley-valley for 13 and there was a coelution for 6 of them.

For quantification purpose four points calibration curves (0.1, 0.5, 1 e 5 mg/L) were calculated with an R^2>0,99, standard deviation <0,1. Limit of Detection (L.O.D.) was ≤ 0,1 mg/L for all analytes.

Analytes in the samples were identified by comparison with phenolic acids absorption spectra recorded at the concentration of 1 mg/L (Similarity value has to be ≥ 0,9 for positive identification).

HPLC/MS analysis

HPLC/MS was used for DAD data confirmation.

Separation was achieved on a Luna C_{18} (150 × 2.0 mm, 5 μm). Three different classes of compound were analyzed in three different chromatographic runs: one for phenolic acid, the second for flavones and isoflavones and the third for mixed compounds like chlorogenic acid, epicatechingallate and flavonoid glucosilated. Solvents used were formic acid/water (0.1/99.9) and methanol for phenolic acids and flavonoids. Formic acid/water (2/98) and methanol were used for mixed compounds and glycosilated flavonoids analysis. Flow was selected at 0,2 mL/min and the volume injected was 20 μL.

Mass tuning was achieved for each phenolic compounds that were analyzed by negative ionization recording molecular ions by S.I.M. (Single Ion Monitoring) and fragmentation patterns by MS^2.

Mass analyzer best performances were obtained working on simultaneously scans of a maximum of 4 analytes' ions, so it was needed a very good chromatographic separation.

RESULTS AND DISCUSSIONS

Analysis of 13 apple samples shown the presence of 10 phenolic compounds: chlorogenic acid and the two procyanidins b1 and b2 are the preeminent compounds, while phenolic acids (ferulic, gallic and caffeic acids) and phloridzin are present in lower concentration.

Ten cultivar present a T.P.C. (Total Phenolic Content = sum of phenolic compounds) higher than the *Golden delicious* cultivar, that was taken as comparison.
Data collected are consistent with the collected literature data.

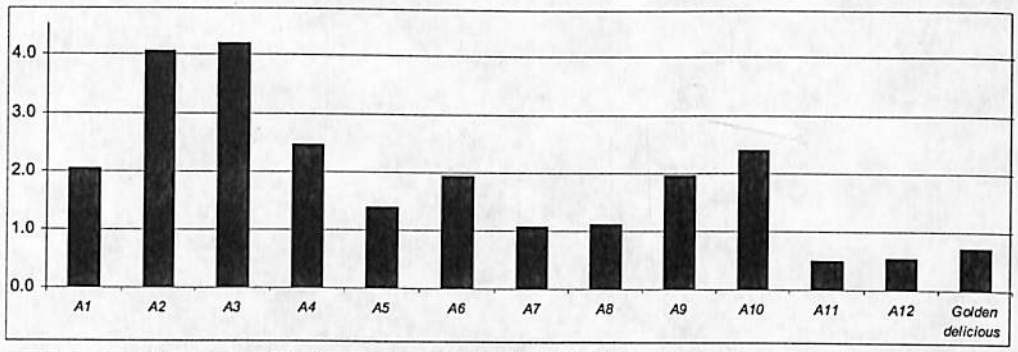

Fig.1 T.P.C. (Total Phenolic Content) concentration (mg/g) of ancient Piedmont cultivars compared to an international cultivar (*Golden delicious*).

CONCLUSIONS

These data are interesting in a revaluation context of typical products because ancient Piedmont cultivars have higher concentration of antioxidant compounds respect to the international cultivar *Golden delicious*. Anywhere more studies have to be done to understand phenolic profile variation in apples harvested in different years and the contribution of each phenolic compound found in apples to the human health.

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