

BIOACTIVE POLYPHENOL PROFILES AND ANTIOXIDANT ACTIVITY IN ITALIAN APPLES VARIETIES

A.M. TAROLA^{*1}, A.M. GIRELLI² and F. D'ASCENZO³

¹Laboratory of Commodities Sciences, Department of Management, Sapienza University of Rome,
Via del Castro Laurenziano 9, 00161 Rome, Italy

²Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

³Department of Management, Sapienza University of Rome, Via del Castro Laurenziano 9, 00161 Rome, Italy

*Corresponding author: Tel.: +39 0649766312
E-mail address: annamaria.tarola@uniroma1.it

ABSTRACT

In this study ten organic apple varieties grown in Italy: Renetta Osiris, Gold Rush, Braeburn, Celato Cola, Limoncella, Cerina, Rosada, Topaz, Jonagored, Florina were analysed in order to evaluate some quality parameters. Individual phenolics compounds, total phenolics, glucose, fructose and antioxidant activity was determined in pulp and peel extracts. Results show that the chlorogenic acid was a predominant component in pulp and peel extracts, with highest value in Limoncella and Jonagored respectively. A significant correlation between phenolic composition and antioxidant activity was observed. Apple varieties Renetta Osiris and Gold Rush presented significant values on glucose and fructose in pulp.

Keywords: apple fruit, DPPH, HPLC, phytochemicals

1. INTRODUCTION

Apple (*Malus domestica*) is one of the most ancient fruits. It was born in Minor Asia, south of Black Sea and arrived in Greece from Egypt, where it was grown along Nile valleys (XIII century B.C.). Afterwards, it quickly spread all over Europe and during the XVI century it appeared in North America (FORSLINE *et al.*, 2003; COART *et al.*, 2006). It is currently the second fruit produced and consumed all over the world (70 million tons). Italy produces 1.9 millions tons of apples a year and is the fifth largest producer worldwide and the third largest exporter in the world after China and Poland. Italy, along with India, is one of the major consumers of apples per capita, each person eats an average of 15 pounds of apples per year (two a week). In Germany 10, in France 8, in the United States only 5 (FAOSTAT, 2013). Apples are grown all over the Italian territory, but production is traditionally concentrated in the mountain and foothill regions, particularly in Valle d'Aosta, Piemonte, Veneto and Trentino-Alto Adige regions. In the past, the old varieties of fruits locally grown were quickly abandoned accordingly to a higher rationality of production with the choice of more productive varieties. In 1920, about 50 varieties of apples were marketed but they were reduced to 10 today with a loss of 80%. During the twentieth century the development of the sector has grown in connection with the increase of production, using particular attention to the territory and natural cultivation techniques with a low environmental impact. Currently, a particular attention is dedicated to the improvement of biodiversity and to the organic production (BERTSHINGER *et al.*, 2004,; RAGANOLD *et al.*, 2001). The quality of apple fruits depends on the bioactive compounds: polyphenols, organic acids and carbohydrates. The concentration of the compounds differs with varieties, maturity stage and environmental conditions (EISELE and DRAKE, 2005). Phenolic substances are considered important in nutrition because of the positive effect on human health as a result of their high antioxidant capacity (SERRA *et al.* 2012, EBERHARD *et al.*, 2000). Epidemiologic studies associate the biological activity of these substances to the prevention of cancer and cardiovascular diseases, neuropathies and diabetes (RIBEIRO *et al.*, 2014; BOYER *et al.*, 2004; BALASURIYA *et al.*, 2012; SCALBERT *et al.*, 2000). The aim of this study is to evaluate the composition of different apples varieties, produced by organic methods representative of Italian biodiversity. The variability on the composition of bioactive compounds: individual phenolic compounds, total phenolics, fructose and glucose in pulp and peel tissue was determined. The influence of the total phenolic compounds on the antioxidant activity was evaluated.

2. MATERIAL AND METHODS

2.1. Standard and reagents

The standard compounds gallic acid, (+) catechin, (-) epicatechin, chlorogenic acid, phloretin, rutin, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), Formic Acid, Folin-Ciocalteu reagent, sodium carbonate, Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Sigma-Aldrich (MO USA). Methanol of HPLC grade and hydrochloric acid were purchased from Merck (Germany). The water used was purified in a Milli-Q system (Millipore, Bedford, MA, USA). The extracts samples and standard solution were filtered through membrane 0.45 µm (Millipore Bedford, MA, USA).

2.2. Apple fruit samples

Apples samples from the varieties: Renetta Osiris (Trentino Alto Adige), Gold Rush (Toscana), Braeburn (Toscana), Celato Cola (Sicilia), Limoncella (Abruzzo), Cerina (Lazio), Rosada (Lazio), Topaz (Trentino Alto Adige), Jonagored (Trentino Alto Adige), Florina (Trentino Alto Adige), were purchased from local organic producers in different Italian regions during the period September-October 2015. The analysis was conducted within two days.

2.3. Samples preparation and procedure

The apple samples of peel and pulp have been separated and the pulp (5 g) and the peel (5 g) fractions were immediately homogenized with methanol containing 1% hydrochloric acid. The samples have been extracted with 10 ml of solvent for 30 min and 10 ml for 30 min using a Ultrasonic sonicator at room temperature (25°C). The extract, pulp and peel separately, were combined and filtered through a membrane 0,45 μm pore size prior to injection in HPLC for determining the individual phenolic compounds, antioxidant activity and total phenolics. Extracts were stored at -4°C for 1 day. Later at -20°C for 1 week.

The recovery efficiency of phenolic compounds was determined by spiking amounts of standards compounds considered to the fraction pulp and peel of Braeburn apple prior to extraction. The recovery study was performed in triplicate.

2.4. Apparatus

HPLC analyses were performed on a Shimadzu HPLC system, a LC-10AT liquid chromatograph equipped with four pumps FCV-10AL, a degasser DGU-14A, a Rheodyne 7725i injector with a 20 μl sample loop (Rheodyne, Berkeley, CA, USA) and a photodiode array detector SPD-M20A. The eluted compounds were monitored at 280 nm and the adsorption spectra between 250 and 350 nm. Spectrophotometric determinations were performed with a Shimadzu UV-Vis 1800 spectrophotometer.

2.5. Identification and quantification of individual phenolic compounds

The column used was a C18 Supelcosil LC (150 mm x 4.6 mm, 3 μm particle size), and a guard column. The mobile phase was a mixture: formic acid in water (2%, pH 3, solvent B) and formic acid in methanol (2%, pH 3, solvent A). The gradient program was time 0.01 95% B, 15 min 50% B, 20 min 30% B, 25 min 20% B, 30 min 95% B. 5 min of equilibration was required before the next injection. The flow rate was 0.6 mL min and the analyses were conducted at room temperature (25°C). The injected volume was 20 μL . Peak identification was performed by comparing the retention times and diode array spectral characteristics with external standards. Quantification was performed using calibration curve of standards. Data were processed using Shimadzu LC solution software. The determination of sugar content, glucose and fructose were determined according method previously described (KARKACIER *et al.*, 2003).

2.6. Total phenolic content assay (TPC)

The total phenolics content in the apple extracts was determined using the Folin-Ciocalteu procedure according to procedure previously described (SINGLETON *et al.*, 1999) with some slight modifications: 50 μl extract solution was added to 2.5 ml of Folin-

Ciocalteu reagent, 2 ml of 20% % Na₂CO₃ solution. The mixture was incubated for 30 min at 40°C. Absorbance increase was measured at 765 nm. Chlorogenic acid was used as standard (0-100 mg l⁻¹), and a calibrate equation was obtained (R² = 0.9961). Total phenolic content is expressed as mg chlorogenic acid g⁻¹.

2.7. Antioxidant activity assay

The antioxidant activity was estimated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). Free Radical Scavenging Method according to method previously described with minor adaptation (SHARMA and BHAT, 2009). The DPPH reagent 25 mg were dissolved in 1000 ml of methanol(A), and the solution have been diluted 1:10 (B). Two ml of solution B were mixed with 100 µl of the extract methanolic solution and kept at 25°C for 30 min in a dark room. A decrease absorbance was determined at 515 nm using spectrophotometer. Radical Scavenging activity was calculated by the following formula:

$$\text{Scavenging \%} = [\text{Abs}_{t=0\text{min}} - \text{Abs}_{t=30\text{min}} / \text{Abs}_{t=0\text{min}}] \times 100$$

where A₀ was the absorbance of DPPH blank solution, and A was the final absorbance of the tested sample after 30 min of incubation. Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) 0.05 mM in methanol was used as a standard to convert the inhibition capacity of the apple extract solution to the Trolox equivalent antioxidant activity (r²= 0,9980).

2.8. Statistical data analysis and graphic program

All results were analyzed by Pearson correlation and R²coefficient was determined. These data were elaborated with Graph Pad Prism 5.0 software and statistical significance was established at p<0.05.

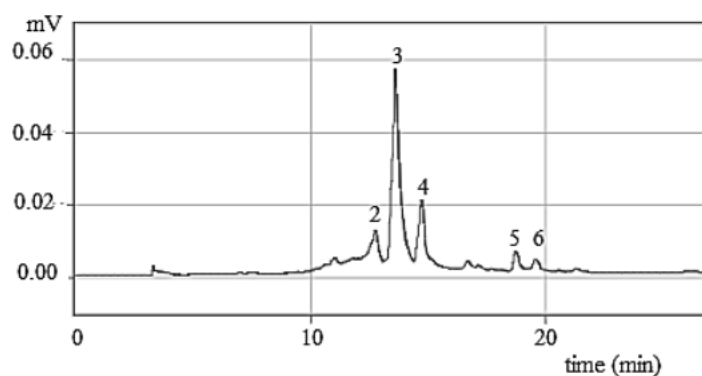
3. RESULTS AND DISCUSSION

In this study ten apple varieties, by organic production, were selected in order to represent the new orientation of the Italian quality organic production aiming to evaluate quality parameters. The apples types considered in the study are usually consumed as fresh fruits in Italy: Renetta Osiris, Gold Rush, Braeburn, Celato Cola, Limoncella, Cerina, Rosada, Topaz, Jonagored, Florina coming from the organic production of different Italian regions: Trentino Alto Adige, Toscana, Lazio, Abruzzo, Sicilia. Among them: Limoncella (Abruzzo), Cerina (Lazio), Gelato Cola (Sicilia) have been produced in small quantities and purchased from selected producers that aim to appraise enhances biodiversity of production territories.

3.1. Quantification of individual phenolic compounds

The determination of individual phenolic compounds was performed by RP-HPLC using the gradient elution method reported in the experimental section. Fig. 1 shows the chromatograms of the extracts from the pulp and peel apple of Braeburn varieties, obtained at λ 280 nm.

pulp



peel

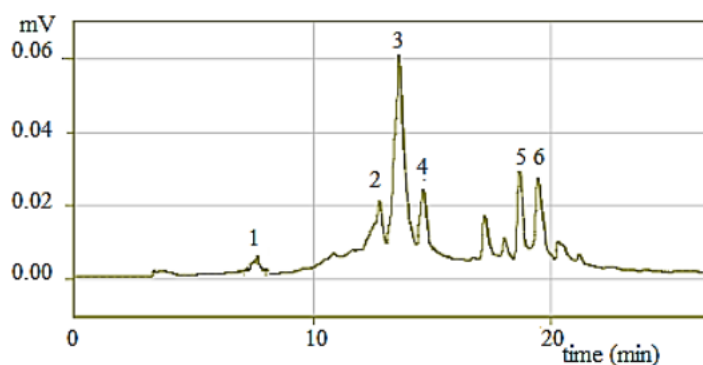


Figure 1. Chromatogram of apple Braeburn varieties, pulp and peel extracts using RP-HPLC, as 280 nm. Peaks: 1 gallic acid, 2 (+) catechin, 3 chlorogenic acid, 4 epicatechin, 5 rutin, 6 phloretin.

Table 1 shows the calibration parameters of the analytical method. Concentration of the linear range were determined, for all phenolic compounds, by triplicate analysis of four different standard concentrations. Recoveries were determined in both pulp and peel apple extract by spiking apple samples with a solution containing known amounts of all standard mixture compounds prior to the extraction procedure. The study was conducted in triplicate. The obtained values ranged from $92\% \pm 2.5$ SD - $98\% \pm 2.2$ SD for all analytes.

Compositions of the individual phenolic compounds: gallic acid, catechin, epicatechin, chlorogenic acid, rutin and phloretin in apples varieties, pulp and peel, are shown in Table 2 and Table 3. The results on apples pulp show that chlorogenic acid is the major compound present in all examined apples varieties and ranged, in the pulp, between $800.3 \mu\text{g g}^{-1}$ Limoncella to 52.7 in Braeburn, in the peel between $766.6 \mu\text{g g}^{-1}$ in Jonagored to 187.1 in Renetta Osiris.

Table 1. Retention time (T.) and calibration parameters of apple phenolics.

Compounds	T _{r min} ±SD	Linear range (µg ml ⁻¹)	R ²	Detection limit LOD (µg ml ⁻¹)
Gallic acid	7.5±0.04	2-50	0.9958	1.6
(+) Catechin	12.4±0.03	2.5-110	0.9902	1.5
Chlorogenic acid	13.6±0.06	1.5-200	0.9925	0.7
(-) Epicatechin	14.7±0.03	3.0-100	0.9928	0.6
Rutin	18.9±0.02	2.5-120	0.9958	0.5
Phloretin	19.8±0.03	08-80	0.9991	0.7

Mean value (n=3) µg ml⁻¹±SD, 1280 nm.

Table 2. Amounts of phenolics compounds in apples varieties, pulp methanolic extract.

Apples varieties	Gallic acid	Catechin	Chlorogenic acid	Epicatechin	Rutin	Phloretin
Renetta Osiris	4.7±0.4	34.1±2.2	224.1±2.2	30.3±0.8	35.5±1.3	1.0±1.2
Gold Rush	<1.6	14.9±0.8	243.6±2.0	70.6±1.3	20.1±0.8	3.4±0.8
Braeburn	<1.6	11.1±2.3	52.7±1.5	170.0±2.1	16.4±0.7	<0.7
Gelato Cola	4.4±1.7	60.7±2.5	400.1±3.1	89.4±1.7	20.1±0.6	1.7±0.8
Limoncella	6.3±1.8	21.2±1.8	800.3±3.5	72.6±1.5	47.2±0.8	<0.7
Cerina	5.0±1.0	69.3±1.6	328.5±2.8	79.3±2.1	46.8±1.1	<0.7
Rosada	8.3±1.1	110.5±2.8	247.7±1.6	30.0±1.6	13.6±1.1	2.5±1.1
Topaz	<1.6	44.6±1.6	230.1±2.7	60.5±1.4	18.0±1.2	3.1±1.2
Jonagored	7.7±2.1	36.3±1.5	400.2±1.6	18.3±0.8	13.4±0.8	3.2±1.0
Florina	<1.6	39.2±1.7	180.70±1.5	34.1±1.1	18.8±1.6	3.1±0.8

Data are expressed as the mean value (µg g⁻¹)±SD (n=3).

Table 3. Amounts of phenolics compounds in apples varieties, peel methanolic extract.

Apples varieties	Gallic acid	Catechin	Chlorogenic acid	Epicatechin	Rutin	Phloretin
Renetta Osiris	4.4±1.2	45.9±0.6	187.1±1.8	65.6±1.0	102.4±2.3	<0.7
Gold Rush	9.8±1.8	35.9±1.6	400.2±3.2	131.8±0.8	79.4±2.4	4.3±1.3
Braeburn	26.7±1.4	77.8±2.8	315.4±3.1	71.2±0.6	131.3±1.8	1.1±1.0
Gelato Cola	7.2±1.5	100.6±3.1	466.4±3.3	80.1±1.3	128.2±1.6	<0.7
Limoncella	12.3±1.6	110.4±3.1	700.2±3.6	100.3±1.7	42.7±2.2	1.2±0.7
Cerina	6.3±1.1	120.6±3.3	366.6±2.6	166.8±1.3	71.8±2.1	2.2±0.6
Rosada	13.9±1.2	84.7±2.2	500.1±2.7	92.6±0.8	27.7±2.5	<0.7
Topaz	2.3±1.6	141.5±2.8	400.3±2.5	140.1±2.1	233.5±1.8	<0.7
Jonagored	13.9±1.2	127.2±1.8	766.6±3.1	89.3±2.0	186.4±1.6	10.6±0.5
Florina	2.2±2.1	194.3±1.8	203.0±1.8	227.5±2.2	145.8±1.3	19.8±0.7

Data are expressed as the mean value (µg g⁻¹)±SD (n=3).

Furthermore (-)epicatechin, (+) catechin, rutin, phloretin and gallic acid were minor phenolic constituents. In particular (+) catechin ranged between 11.1 µg g⁻¹ in Braeburn

apple to 110.5 in Rosada in pulp extracts, while on the peel between 35.9 $\mu\text{g g}^{-1}$ Gold Rush to 141.5 in Topaz. Epicatechin ranged from 18.3 $\mu\text{g g}^{-1}$ Jonagored to 170.0 $\mu\text{g g}^{-1}$ Braeburn in the pulp extracts, while in peel in the range between 65.6-227.5 $\mu\text{g g}^{-1}$ corresponding to Renetta Osiris and Florina, respectively. Rutin content on pulp, ranged from 13.4 $\mu\text{g g}^{-1}$ Jonagored to 47.2 Limoncella, on the peel 27.7 $\mu\text{g g}^{-1}$ Rosada to 233.5 in Topaz. Phloretin content ranged from, 0.6 $\mu\text{g g}^{-1}$ on Cerina and 3.4 $\mu\text{g g}^{-1}$ in Gold Rush on pulp, while on peel 0.6 $\mu\text{g g}^{-1}$ Renetta Osiris and 19.8 Florina. A poorly significant difference was observed in gallic acid in pulp and peel of all the examined cultivars. However, an higher content of all the examined phenols in peel extracts compared to the one in the pulp one was found with the exception of phloretin in Renetta osiris, Gelato cola, Rosada and Topaz.

3.2. Total Phenolics and antioxidant activity

Total phenol compounds (TPC) of apple peel extracts are always higher than those in pulp. This is in accordance with the fact that phenolic compounds have the tendency to accumulate in the peel tissues of the plant because of their potential roles in protection against UV radiation and pathogens. In addition, it is clear that total phenol compounds (TPC) of apple peel extracts differed significantly among ten cultivars: TPC was the highest in Florina followed, in decreasing order, by Jonagored, Topaz, Rosada, Braeburn, Limoncella, Cerina, Gelato Cola, Rush, Renetta Osiris. Florina was the variety with the highest TPC ($9.60 \pm 0.8 \mu\text{g chlorogenic acid g}^{-1}$) in the peel among all cultivars under study, whereas the lowest value $1.70 \pm 0.1 \mu\text{g chlorogenic acid g}^{-1}$ was observed in Renetta Osiris. In pulp, the values ranged between 0.9 ± 0.1 to $4.9 \pm 0.5 \mu\text{g g}^{-1}$ chlorogenic acid; Rosada had the lowest values and Limoncella, Gelato Cola and Jonagored the highest value. It means that, also in the pulp, TPC is highly related to the specific cultivar. Evaluation of antioxidant activity was conducted by DPPH method which reveals the colorimetric decrease in absorbance of the radical DPPH due to the chemical trapping of the unpaired electron. Antioxidants interact with DPPH neutralizing its free radical character. Table 4 shows the results related to Total Phenolics and the DPPH activity of extracts obtained by using methanol acidified with 1% of HCl.

Table 4. Total phenolics and antioxidant activity in apple varieties, pulp and peel methanolic extract.

Apples varieties	Total phenolics*		Antioxidant activity** ($\mu\text{mol g}^{-1}$)	
	pulp	peel	pulp	peel
Renetta Osiris	1.0 ± 0.3	1.7 ± 0.1	243.9 ± 0.2	297.2 ± 0.2
Gold rush	1.0 ± 0.2	2.3 ± 0.3	236.2 ± 0.2	293.2 ± 0.2
Braeburn	1.4 ± 0.2	3.9 ± 0.4	294.8 ± 0.2	363.2 ± 0.3
Gelato cola	2.0 ± 0.4	3.0 ± 0.3	319.5 ± 0.6	360.8 ± 0.4
Limoncella	2.9 ± 0.5	3.4 ± 0.5	349.4 ± 0.5	307.0 ± 0.4
Cerina	1.9 ± 0.2	3.3 ± 0.7	290.7 ± 0.3	348.2 ± 0.4
Rosada	0.9 ± 0.1	3.9 ± 0.6	216.6 ± 0.5	351.8 ± 0.3
Topaz	4.9 ± 0.5	1.5 ± 0.6	311.1 ± 0.7	397.4 ± 0.4
Jonagored	2.0 ± 0.4	6.6 ± 0.6	325.4 ± 0.7	370.9 ± 0.5
Florina	1.5 ± 0.2	9.6 ± 0.8	319.8 ± 0.7	377.3 ± 0.6

Data expressed* (chlorogenic acid $\mu\text{g g}^{-1}$) \pm SD mean value (n=3).

** data expressed ($\mu\text{mol g}^{-1}$ Trolox) \pm SD mean value (n=3).

In the pulp, the value ranged between 216.6 ± 0.5 to 325.4 ± 0.7 ($\mu\text{mol g}^{-1}$ Trolox) respectively for Jonagored and Rosada varieties, in the peel 293.2 ± 0.2 to 377.3 ± 0.6 ($\mu\text{mol g}^{-1}$ Trolox) for Gold rush and Florina varieties. The highest values in peel are also evidenced for antioxidant activities. This is may be due to the different distribution of polyphenolics compounds: the peel, in addition to the compounds present in pulp, have additional phenolic compounds not found in the pulp such as anthocyanins and a high amount of quercetin glycosylate (VIERA *et al.* 2009). This appears more evident when we compared antioxidant activity in peel extracts between red and yellow apples. The relation between antioxidant activities and polyphenolic concentration is previously reported (VAN der SURS *et al.*, 2001; D'ANGELO *et al.*, 2007). In this study total phenolic compounds content was positively correlated with antioxidant activity in apple pulp ($r=0.870$) and peel ($r=0.699$) respectively. The results suggest that, in apple, phenolic compounds have a significant contribution to the antioxidant capacity according to literature data (LEE *et al.*, 2003).

3.3. Glucose and fructose content

In order to evaluate the sugar content, glucose and fructose have been quantified in apple pulp and peel extracts (Figure 2 and Figure 3). The fructose content ranged between 1.32 ($\text{g}100\text{g}^{-1}$ Florina) and 4.45 (Gold Rush); glucose from 1.25 ($\text{g}100\text{g}^{-1}$ Florina) to 3.0 (Rosada) in the pulp, while in the peel fructose ranged between 0.80 ($\text{g}100\text{g}^{-1}$ Florina) and 3.40 (Renetta Osiris), glucose 0.50 ($\text{g}100\text{g}^{-1}$ Florina) to 2.35 (Rosada). The results show the lowest amount in apple Topaz, Jonagored and Florina, whereas these cultivars present significant value in total phenolic content. On the contrary, apple varieties Renetta Osiris and Gold Rush, present significant values on glucose and fructose and low content of total phenolic compounds.

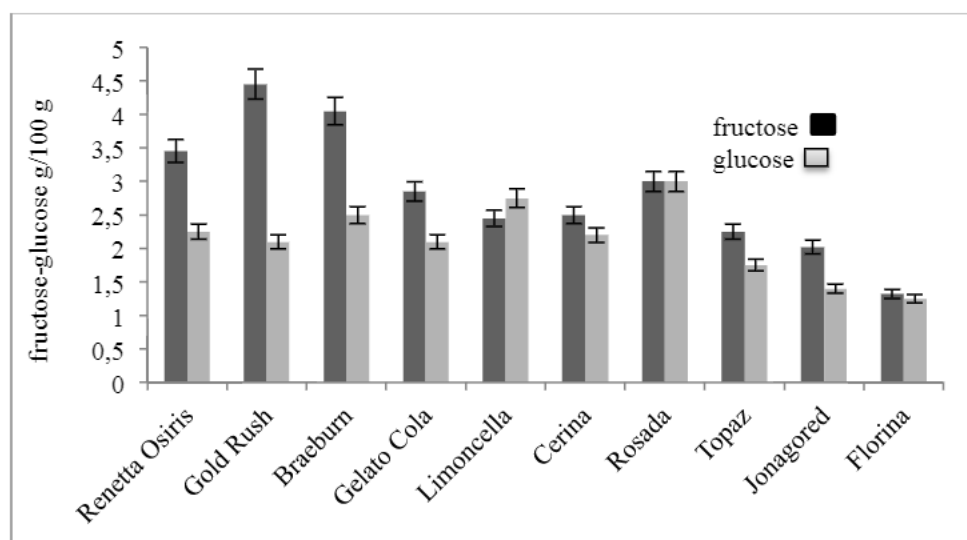


Figure 2. Fructose and Glucose content in pulp of apples varieties ($\text{g}100\text{g}^{-1} \pm \text{SD}$, $n=3$).

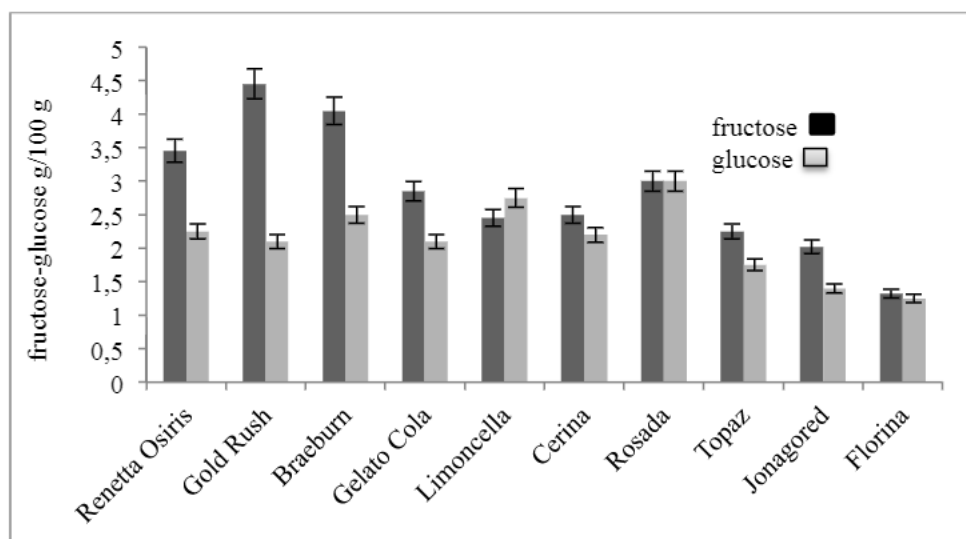


Figure 3. Fructose and Glucose content in peel of apples varieties ($\text{g } 100\text{g}^{-1} \pm \text{SD}$, $n=3$).

4. CONCLUSIONS

In conclusion, phenolic compounds, secondary metabolites naturally present in plant material, are important to determine quality characteristics of apple. The bioactive substances quantified show that the commodity is a good source of nutraceutical compounds and denote significant differences in the apples varieties considered. Moreover, the apple peel, generally considered as waste, of the examined commodities presents significant contents of individual phenolic compounds, glucose and fructose and, consequently a high antioxidant capacity.

ACKNOWLEDGEMENTS

The authors acknowledge the funding obtained from Sapienza University of Rome 2014.

REFERENCES

- Balasuriya N. and Rupasingh V.H. 2012. Antihypertensive properties of flavonoid-rich apple peel extract. *Food Chem.* 135(4):2320.
- Bertschinger, L., Mouron P., Dolega E. and Zürcher M. 2004. Ecological apple production: a comparison of organic and integrated apple-growing. *Acta Hort.* 638:321.
- Boyer J. and Liu R.H. 2004. Apple phytochemicals and their health benefits. *Nutritional J.* 3:1.
- Coart E., Van Glabeke S., De Loose M., Larsen A.S. and Ruizi R. 2006. Chloroplast diversity in the genus *Malus*: new insights into the relationship between the European wild apple (*Malus sylvestris* sMill.) and the domesticated apple (*Malus domestica* Borkh). *Mol. Ecol.* 15:2171.
- D'Angelo S., Cimmino A., Raimo M., Salvatore A., Zappia V. and Galletti P. 2007. Effect of Reddening-Ripening on the Antioxidant Activity of Polyphenol Extracts from Cv. 'Annurca' Apple Fruits. *J. Agric. Food Chem.* 55 (24):9977.
- Eberhardt M.V., Lee C.Y. and Liu R.H. 2000. Nutrition-antioxidant activity of fresh apple. *Nature* 405:903.
- Eisele T. and Drake S.R. 2005. The partial compositional characteristics of apple Juice from 175 apple varieties. *J. Food Comp. Anal.* 18:213.
- FaoStat 2013. World Apple Production. www.fao.org (accessed 20-02-2013).

- Forsline P.L., Aldwinckle H.S., Dickson E.E., Luby J.J. and Hokanson S.C. 2003. Collection, maintenance, characterization, and utilization of wild apples of Central Asia. *Hortic. Rev.* 29:1.
- Lee K, Kim Y, Kim D. and Lee C. 2003. Major phenolics in apple and their contribution in the total antioxidant capacity. *J Agric. Food Chem.* 51:6516.
- Karkacier M., Erbas M., Uslum. K and Aksu M. 2003. Comparison of different extraction and detection methods for sugar using amino-bonded phase HPLC. *J. Chrom. Sci.* 41(6):331.
- Raganold J.P., Glover J.D., Andrews P. K. and Hinman H.R. 2001. Sustainability of three apple production systems. *Nature.* 410:926.
- Ribeiro F., Gomes De Mura C., Aguiar O., De Oliveira F., Spadari R., Oliveira N., Oshima C. and Ribeiro D. 2014. The chemopreventive activity of apple Against carcinogenesis, antioxidant activity and cell cycle control. *Eur. J. Cancer Prev.* 23(5):477.
- Scalbert A. and Williamson G. 2000. Dietary intake and bioavailability of polyphenols. *Jo. Nutr.* 130:2073.
- Sharma OM P. and Bhat T. K. 2009. DPPH antioxidant assay revisited. *Food Chem.* 113: 1202.
- Serra A.T., Rocha J., Sepododes B., Matias A., Rodrigo P., Agostinho del Carvalho F., Bronze R., Duarte C. & Figureira M. 2012. Evaluation of cardiovascular protective effect of different varieties, Correlation of response with composition. *Food Chem.* 135 (4):2378.
- Singleton V.L., Orthofer R. and Lamuela-Reventos R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by mean of Folin-Ciocolteu reagent. *Method Enzymol.* 299:152.
- Van der Sluis A., Dekker M.; De Jager A. and Jongen W.M.F. 2001. Activity and Concentration of Polyphenolic Antioxidants in Apple: Effect of Cultivar, Harvest Year, and Storage Conditions. *J Agric. Food Chem.* 49 (8):3606-3613.
- Vieira FG., Borges GS.; Copetti C., Gonzaga LV., Nunes EC. and Fett N. 2009. Activity and contents of polyphenolic antioxidants in the whole fruit, flesh and peel of three apple cultivars. *Arch. Latinoam. Nutri.* 59 (1):101.

Paper Received July 24, 2018 Accepted September 21, 2018