Fruit Growing Research, Vol. XXXV, 2019 DOI 10.33045/fgr.v35.2019.17
http://publications.icdp.ro/index.php

EVALUAREA COMPUȘILOR BIOACTIVI ȘI A PARAMETRILOR DE CALITATE DIN DIFERITE SOIURI DE MERE ECOLOGICE ȘI DIN PULBERI NATURALE CU VALOARE ADĂUGATĂ OBȚINUTE DIN ACESTEA

BIOACTIVE COMPOUNDS AND QUALITY PARAMETERS IN DIFFERENT ORGANIC APPLE VARIETIES AND THEIR NATURAL VALUE ADDED POWDERS

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Abstract

The interest on organic plant-based foods is constantly growing due to their health benefits and ecological importance along with increasing demands of the consumers for quality foods produced sustainably. Organic apples were known to present high content in polyphenols, compounds which are recognized to have multiple biological activities and various health benefits as potential agents for preventing and treating many oxidative stress-related diseases, such as cardiovascular diseases, cancer, ageing, diabetes mellitus and neurodegenerative diseases. The purpose of this article is to evaluate the variations in quality parameters (firmness, total soluble solids and titratable acidity) and bioactive compounds (anthocyanins and vitamin C) of three organic apple varieties (Gala, Golden and Red Prince), used to obtain natural value added powders, rich in polyphenols and antioxidant activity. The nutritional analyses revealed that the studied organic apples are high quality and could be used to obtain natural value added powders by lyophilization, in order to preserve most of these valuable compounds inside. The powders obtained from apple pulp contain high quantities of ascorbic acid, more than powders from peel are mixture (peel and pulp). The powders obtained from peel revealed high quantity of polyphenols and high antioxidant potential, which is related more with the polyphenol content than with ascorbic acid content.

Cuvinte cheie: ecologic, mere, pudre, compuși bioactivi, calitate
Key words: organic, apples, powders, bioactive compounds, quality

1. Introduction

The interest on organic plant-based foods is constantly growing due to their health benefits and ecological importance along with increasing demands of the consumers for quality foods produced sustainably. Organic apples were known to present high content in polyphenols, compounds which are recognized to have multiple biological activities (Stan et al., 2017) and various health benefits as potential agents for preventing and treating many oxidative stress-related diseases, such as cardiovascular diseases, cancer, ageing, diabetes mellitus and neurodegenerative diseases (Li et al., 2014; Pandey et al., 2009).

The quality of organic fresh apples depends of several pre-harvest factors as: environmental factors, horticultural practices applied in the orchard (Musachi & Sera, 2018; Lin-Wang, et al., 2017), varieties (Budan et al., 2012; Ubi et al., 2006) harvesting moment (de Jager & Awad, 2002), etc. that could affect the content of biochemical compounds (Manach et al., 2004), as well as the taste and physico-chemical properties of processing (Bezdadea-Cătuneanu et al., 2019; Kumar et al., 2018).

Fresh or processed, apple are valuable source of minerals (K, Fe, Mg, Ca, Zn), dietary fibres and bioactive compounds (Catană et al., 2018). Apple pomace powders are important sources of polyphenols (17.83-38.83 mgGAE/g) and high dietary fibre content (60.62-64.75%) being important sources to increase the fibre content of foods (bakery products, pastry products etc.). Many studies have been carried out estimating dried apple quality characteristics. However, very few studies have been carried out in the organic sector (Crichton et. A., 2018; Moscetti et. A., 2018).

Polyphenol concentrations in foods vary according to numerous genetic, environmental, and technologic factors, some of which may be controlled to optimize the polyphenol content of foods (Shrestha et al., 2018). The main tasks ahead are identifying the plant varieties that are the richest in the polyphenols of interest, improving growing methods, and limiting losses during the course of industrial processing and domestic cooking (Manach et al., 2004). The health effects of polyphenols depend on both their respective intakes and their bioavailability, which can vary greatly.

The purpose of this article is to evaluate the variations in quality parameters (firmness, total soluble solids and titratable acidity) and bioactive compounds (anthocyanins and vitamin C) of three organic apple varieties (Gala, Golden Delicious and Red Prince), used to obtain natural value added powders, rich in polyphenols and antioxidant activity.

2. Materials and methods

The apples varieties (Gala, Golden Delicious and Red Prince) were harvested at the optimal ripening stage in September – October 2018, from the organic orchard of county Arad and transported immediately to analysis. All samples were analyzed for different physical (firmness) and nutritional attributes (ascorbic acid, total soluble solids, total anthocyanins and minerals). The samples were stored two months in cold rooms at 3°C and 85% relative humidity in Laboratory of Postharvest Technologies form Research Center for Studies of Food Quality and Agricultural Products from UASVM Bucharest.

Prior to freeze-drying, apples from each variety were washed and sliced into small pieces (*apple peel and pulp samples*), or the samples were peeled and cut, then separate into *peel samples* and *pulp samples*. All these samples were immediately frozen at -86˚C then lyophilized at – 40˚C until became powders and analyzed for different nutritional attributes (ascorbic acid, antioxidant activity, total phenolic compounds).

All samples were analyzed as fresh samples, immediately after harvesting, and as powders, immediately after lyophilization. Results as mean ± SD of triplicate measurements.

Reagents. Analytical standard such as ascorbic acid, was purchased from Merck (KgaA, Darmstadt, Germany). All other reactive used for analysis were purchased from Merck (KgaA, Darmstadt, Germany), except for formic acid which was purchased from Sigma-Aldrich (GmbH, Germany). Water used in the study was produced with the Milli-Q Direct Water Purification System (Millipore SAS, France).

Firmness was determined using a digital penetrometer (53205 TR Italy) equipped with an 8 mm piston, and the results reported as Kg/cm^2, represent the average of 6 to 10 different measurements. Dry matter content was performed using Partner MAC 50 thermal balance at 105°C using 1 g of homogenized sample. The results were expressed in percentage (w/w).

Total soluble solids were measured with using digital refractometer (Kruss DR301-95) as previously described in our work (Stan et al., 2019, Bezdadea-Cătuneanu et al., 2018) and the results were expressed in percentage (w/w). pH values and total titrable acidity were measured using a TitroLine automatic titrator. Before analysis the samples were grinded and homogenized; 5 g of fresh sample was weighed and 25 mL of distillated water was added. The initial pH was measured and the samples were titrated with 0.1N NaOH up to a 8.1 pH (Saad et al., 2014; AOAC Official Method 942.15). The total titrable acidity was expressed in g malic acid /100g of fresh fruit (Gherghi et al. 2001).

Ascorbic acid content was determined after extraction of 1 g of raw material, grinded with 2 mL of orthophosphoric acid (2%, v/v) for 1 minute at room temperature. The mixture was quantitatively passed into a 15 mL centrifuge tubes, and brought to a final volume of 10 mL with orthophosphoric acid (2%, v/v). After extraction, all samples were centrifuged, filtered and stored for HPLC analysis (Stan et al. 2019). Ascorbic acid quantification was realized using HPLC-DAD equipment (Agilent Technologies 1200 Chromatograph). Chromatographic separation of compounds was performed using an ZORBAX XDB-C18 (4.6 x 50 mm, 1.8 µm i.d.) column. The following conditions were used for analysis: column temperature was 30°C, injection volume was 2 µL, isocratic flow rate of 0.5 mL/min using 0.05% formic acid in water as mobile phase (Chanforan et al., 2012).

For **the total anthocyanin content (TAC),** 0.3 g of fresh sample was extracted with 5 mL of methanol acidified with 1% hydrochloric acid (v/v), stirred for 30 min and centrifuged. The obtained supernatant was passed into 15 ml centrifuge tubes, and then remaining residue was subjected at successive extractions until the residue becomes colorless. The total anthocyanin content was determined according to the pH differential method (AOAC Official Method 2005.02; Giusti & Wrolstad, 2001). The absorbance of the extracts was measured at 530 and 700 nm for extracts diluted in pH 1.0 and pH 4.5 buffers (Specord 210 Plus spectrophotometer, Analytik Jena, Jena, Germany). The dilution factor was 5. The results were expressed as cyanidin-3-glucoside equivalents per 100 gram of fresh weight (mg Cyan-3-Glc equiv./100g FW) using an extinction coefficient of 34300 $Lcm^{-1}mol^{-1}$ (Giusti and Wrolstad 2001).

For the determination of **total polyphenolic content (TPC)** and the antioxidant activity were used 70% aqueous methanol extracts prepared as follows: to 0.2 g of dry sample (powders) were added 10 mL of 70% aqueous methanol and incubated in the dark overnight at 4°C. After that, the extracts were shaken at 500 rpm for 1h and then centrifuged at 5000 rpm and 4°C for 10 min. The supernatant was recovered in a centrifuge tube and the residue was re-extracted two more times with 10 ml of 70% aqueous methanol. All three supernatants were combined and then the volume of each extract was adjusted to 30 mL with the extraction solvent.

The total phenolic content of the extract solutions was determined by the Folin-Ciocalteu spectrophotometric method described by (George et al., 2005). An aliquot of 0.5 mL of extract was mixed with 2.5 mL of water-diluted Folin-Ciolcateu reagent (1/10). The mixture was incubated for 2 min at room temperature, and 2 mL of sodium carbonate (75 g*L^{-1}) was added. The mixture was incubated for 15 min at 50 °C and finally cooled in a water-ice bath. The specific absorbance at 760 nm was immediately measured (UV–VIS Specord 210 Plus spectrometer, Analytik Jena, Jena, Germany) The results were expressed as mg of gallic acid equivalents per 100 gram of dry sample (mg GAE/100 g DS).Triplicates of independent extract solutions were analyzed.

The antioxidant activity of methanolic extracts was performed by **the DPPH assay** after the method described by (Bujor et al., 2016, Alberti et al., 2017) with minor modifications. Briefly, 0.2 mL of the sample extract was added to 2 mL of 0.2 mM solution of DPPH in methanol. Then the absorbance was measured at 515 nm after 30 minutes. Methanol was used as a blank reference. The results were expressed as micromoles of Trolox equivalents (TE) per gram of dry sample using Trolox calibration curve. Triplicates of independent extract solutions were analyzed.

3. Results and discussions:

Differences in the relative concentrations of individual components in different cultivars may occur depending on the fruit maturity, environmental factors, horticultural practices applied in the orchard and storage conditions (Musacchi & Serra, 2018). Fresh organic apples were used to determine the firmness and total soluble solids at harvest moment (Fig. 1. and Fig. 2). Apples from Golden delicious variety show significant high firmness (8,04 Kg/cm^2) comparatively with Gala (6,404 Kg/cm^2) and Red Prince (6,863 Kg/cm^2), due to their variety characteristics. These values are correlated with titratable acidity (Fig. 3) with a quantity if malic acid in Golden delicious fruits are twice (0.485 %) than those in Gala fruits (0.244%) and 1.5 times than Red Prince fruits (0.302%).

Nutritional quality of organic apples was characterized by the content of total anthocyanins (Fig. 4.) and ascorbic acid (Fig. 5). It could be well observed that Red Prince fruits contain high quantities of anthocyanins (1.568 mg/100 g F.W.) and Golden delicious fruits contains high quantities of ascorbic acid (4.17 mg/100 g F.W.).

Minerals (nutrients and heavy metals) content of fresh apples was determined and results are reported in Table 1. Data show high content of K^+ (385.75 mg/100 g F.W.) in Golden delicious fruits, of Ca (38,44 mg/100 g F.W.) in Red Prince fruits, Mg (23,83 mg/100 g F.W.) in Gala fruits. The quantity of Pb is under the limit of detection and the values of Zn and Cd are below the values to be reported as heavy metals.

According with all these results the organic apples are high quality and could be use to obtain natural value added powders by lyophilization, in order to preserve most of these valuable compounds inside.

The powder was obtain from peel, pulp, and mixture of both and their characterization is comprised most the antioxidant ability and free radical scavenging capacity, with correlation with content of polyphenolics and ascorbic acid, according to (Li et al., 2014). All these results are reported in Figure 6 and Figure 7.

It could be observed that both of varieties (Gala and Golden delicious) contains the main quantities of polyphenols in peel (about 9 times more than in pulp or mixture), means 1.106,37 mg GAE/100 g powder, respectively 1109,57 mg GAE/100 g powder in Gala and Golden delicious. The ascorbic acid content show a variation between samples type and varieties, with the highest values in pulp (23,95 mg/100 g powder) obtained from Golden delicious fruits and 19.621 mg /100 g powder obtained from Gala fruits.

The DPPH scavenging activity was assessed for dried powder extracts (Figure 8). For both concentrations tested, the DPPH activity is rather similar for apple pulp and apple with peel and pulp of Golden and Golden cultivars in agreement with the similar variation of TPC. The DPPH activity of Gala and Golden peel is higher, as also observed for TPC.

All these new products obtained from organic apples show a high potential to be used as functional ingredients and can be used to fortify organic food products (bakery, diary, pastry products) in order to increase their nutritional and their antioxidant potential.

4. Conclusions

The firmness and titratable acidity were both significantly higher in Golden variety.

The Red Prince variety showed higher total soluble solids and total anthocyanin content than Gala and Golden varieties, what recommends their use for processing immediately after harvesting or in the first few weeks, while the Golden variety can be stored under controlled conditions for further processing.

The data also pointed that the Golden variety have high vitamin C content as well as higher firmness and titratable acidity values compared to the red apple varieties.

The powders obtained from apple pulp contain high quantities of ascorbic acid, more than powders from peel ar mixture (peel and pulp).

The powders obtained from peel revealed high quantity of polyphenols and high antioxidant potential, which is related more with the polyphenol content than with ascorbic acid content.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CCCDI – UEFISCDI, project number ERANET-COREORGANIC-SusOrgPlus, within PNCDI III.

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Tables and Figures

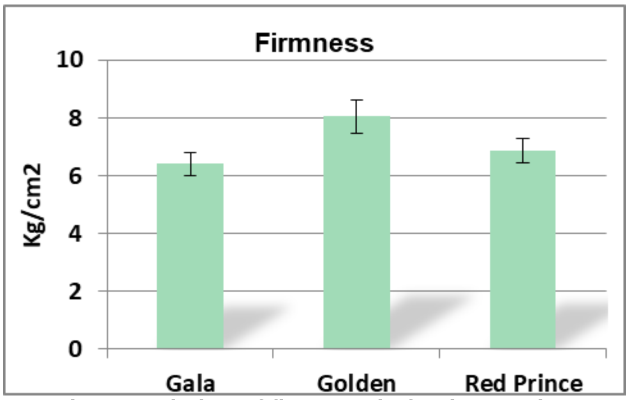

Fig. 1. Variation of firmness in fresh organic varieties at harvesting moment

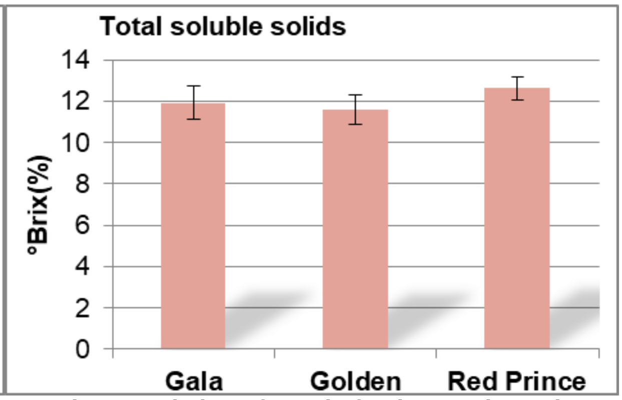

Fig. 2. Variation of TSS in fresh organic apple varieties at harvesting moment

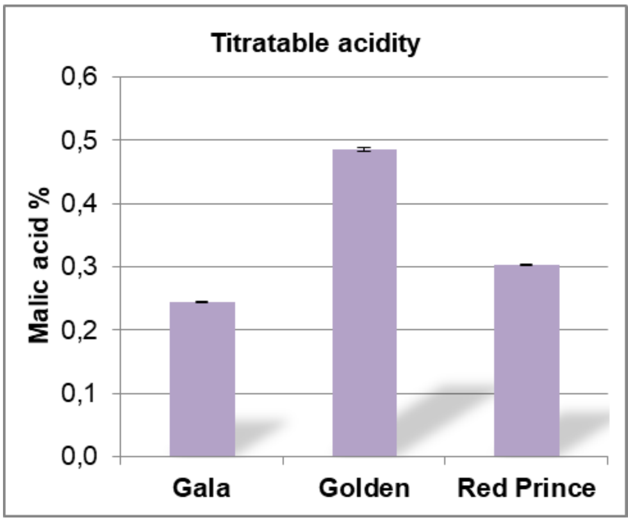

Fig. 3. Variation of titratable acidity in fresh organic apple varieties at harvesting moment

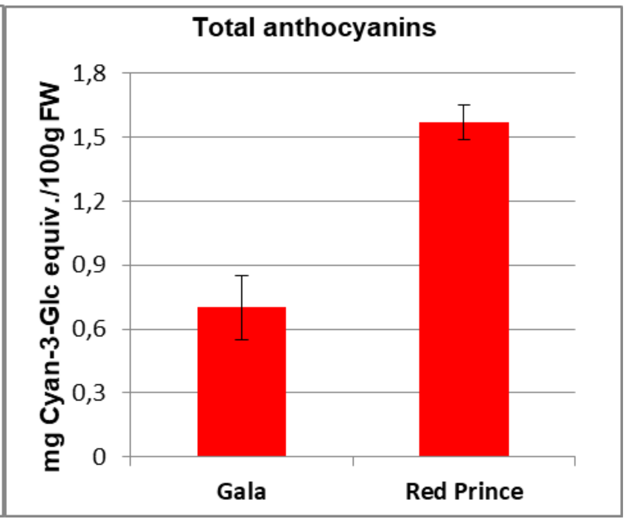

Fig. 4. Variation of total anthocyanins in fresh organic apple varieties at harvesting moment

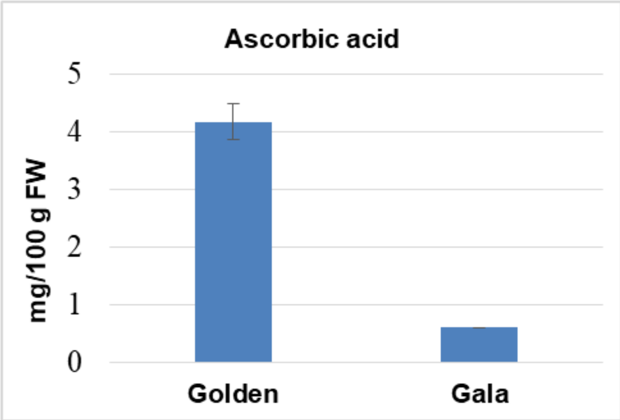

Fig. 5. Variation of ascorbic acid in fresh organic apple Golden Delicious and Gala

Table 1. The elemental content of fresh organic apple at harvesting moment (nutrients and heavy metals)

Apples variety	Golden Delicious		Gala		Red Prince	
Minerals	(mg/ 100 g f .w.)	Std dev	(mg/ 100 g f .w.)	Std dev	(mg/ 100 g f .w.)	Std dev
Nutrients						
K	385.75	3.05	255.22	3.22	365.67	6.72
Ca	14.91	0.57	30.28	0.38	38.44	0.52
Mg	18.49	0.31	23.83	0.16	19.90	0.18
Na	1.89	0.08	4.72	0.17	2.20	0.14
Fe	0.61	0.01	1.03	0.08	0.77	0.08
Mn	0.12	0.00	0.18	0.01	0.28	0.01
Co	0.00011	0.000001	0.00040	0.00002	0.0005	0.00004
Heavy metals						
Zn	0.15	0.01	0.20	0.02	0.23	0.02
Cd	0.0052	0.00025	0.00063	0.00003	0.00082	0.00001
Pb	< LOD	-	< LOD	-	< LOD	-

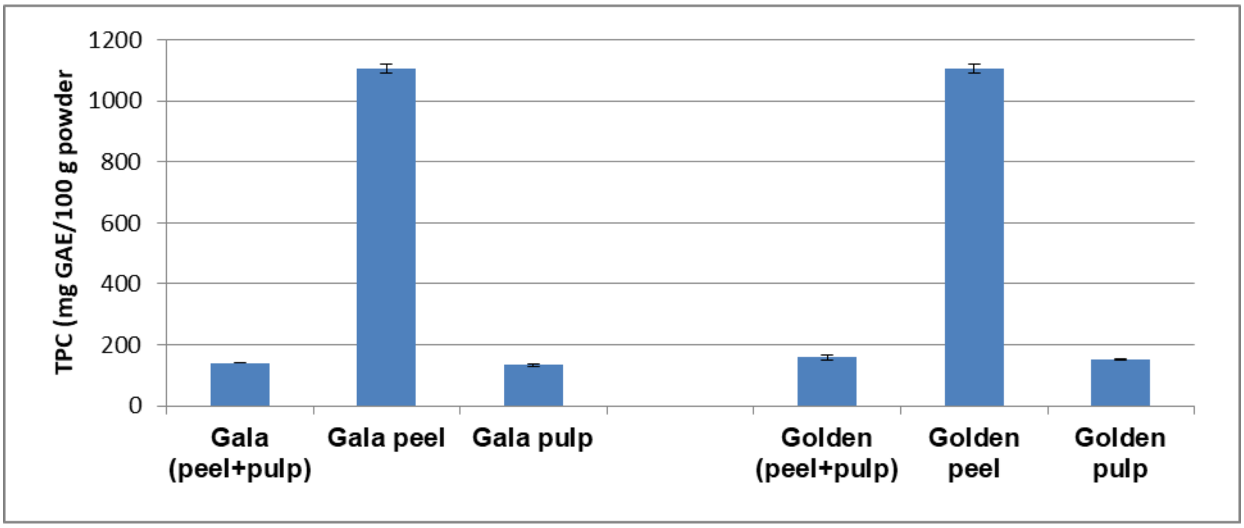

Fig. 6. Variation of total polyphenolic content in organic apple powders

DOI 10.33045/fgr.v35.2019.17

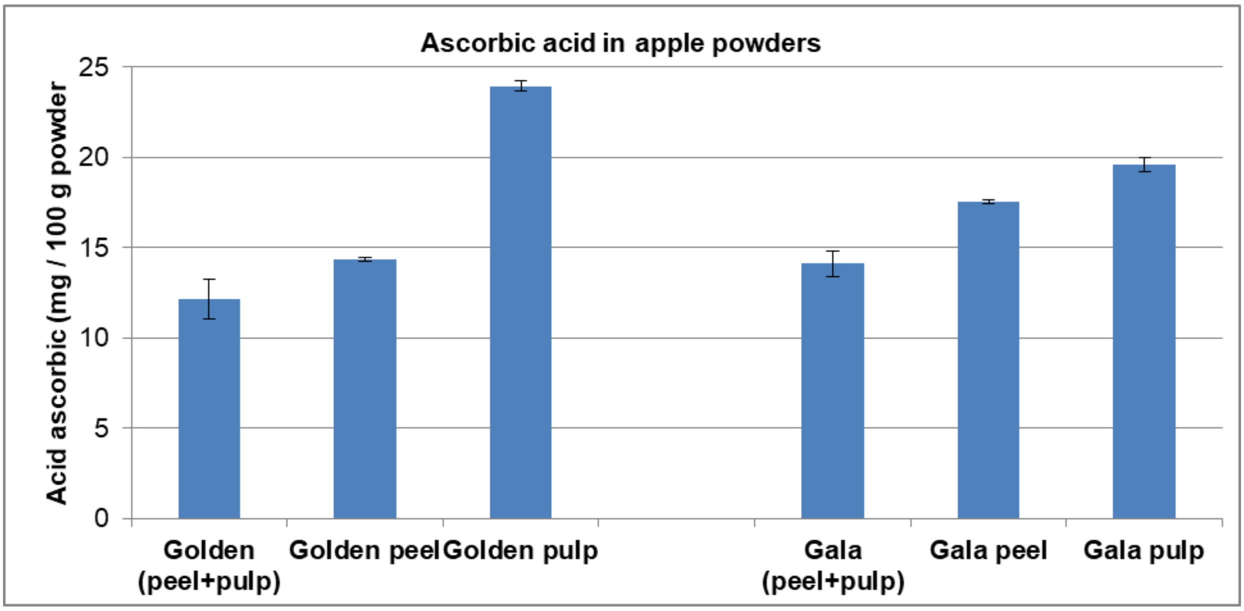

Fig. 7. Variation of ascorbic acid content in organic apple powders

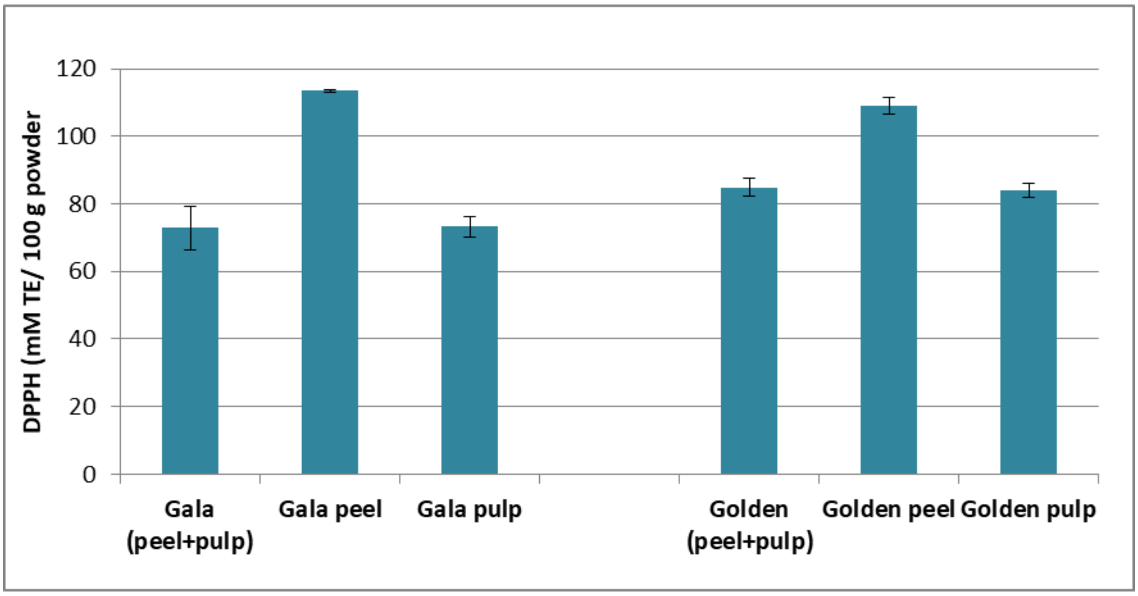

Fig. 8. Variation of antioxidant activity in organic apple powders