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ORIGINAL PAPER

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ORGANIC ACIDS, SUGARS, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *Malus floribunda coccinella* FRUIT, PEEL AND FLESH

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ABSTRACT

Malus floribunda coccinella is a landscape tree that is generally planted for its pinky flowers and small reddish fruits. The red-fleshed fruits, called crab apples, are rich in anthocyanins and are assumed as an environmental pollution material during the fruit bearing season. The aims of this research were to determine the organic acids, sugars, sugar:acid ratio, color, phenolic compounds and antioxidant activity of the fruit and also to identify the phenolic compounds, monomeric anthocyanins content and antioxidant activities in the peel, flesh and whole fruit. Malic acid (25.394 g kg^{-1} FW) was the main organic acid of the fruit. In fruits, amounts of sucrose, glucose and fructose were found to be 0.497, 0.504 and 4.334 g 100 g^{-1} FW, respectively. Highest total phenolic concentration and antioxidant activity values were observed in the peel among the fractions, while protocatechuic and cinnamic acids, rutin, isorhamnetin-3-glucoside, quercetin, procyanidin B1, (+)-catechin and cyanidin-3-galactoside were predominant phenolics of the peel. Highest amounts of chlorogenic acid and (-)-epicatechin were determined in the flesh. Cyanidin-3-galactoside concentration in the flesh was approximately half the amount of that in the peel.

Key words: ABTS, anthocyanins, color parameters, DPPH, FRAP, wild apple

INTRODUCTION

Malus floribunda coccinella is a member of Malus genus and generally known as “wild apple” or “crab apple”. It is commonly cultivated as ornamental tree for its pink flowers, reddish leaves and dark red colored small fruits. Due to its highly sour taste and small size, there is no demand to be considered as a fresh table fruit for human nutrition by consumers and it becomes an environmental waste at the end of the season. In addition to its astringent and sour taste, the fruit of *Malus floribunda coccinella* also differs from many other commercial apples with its intense red colored flesh due to the anthocyanin accumulation. Phenolic compounds are the main phytochemicals of apples [Boyer and Liu 2004] and they contribute to astringent taste and/or color [Scalbert et al. 2005]. Amounts and profile of phenolics in apple may vary depending on many factors such as variety, growth conditions, agricultural practices, storage conditions and processing [Boyer and Liu 2004, Cebulj et al. 2017, Yıldırım et al. 2016]. At the same time, different phenolic compounds as well as their concentration could be seen among the peel and flesh of the fruits [Jakobek et al. 2013, Cebulj et al. 2017]. In commercial apple (*Malus domestica*) varieties, chlorogenic acid, catechin, epicatechin, phloretin glycosides, procyanidin B2 and quercetin glycosides

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are found to be the main antioxidant effective phenolics [Lee Ki Won 2003, Jakobek et al. 2013]. Cyanidin-3-galactoside is also the main anthocyanin in red skin apples and is accumulated generally in peels, except for the red flesh ones [Jakobek et al. 2013]. Among the fractions, polyphenolic concentration in apple is higher in peel as compared to the flesh and this case is attributed to UV light exposure [Bondonno et al. 2017]. The peel possesses higher antioxidant activity than the flesh due to high amounts of polyphenols [Kelly et al. 2003]. In addition to polyphenolics, acidity, sugar concentration and sugar : acid ratio have deterministic effects on the taste and sensorial quality of apple [Wu et al. 2007, Khan et al. 2013]. Malic acid is the main organic acid of apple and comprises 80% to 90% of total acids [Khan et al. 2013]. Sugars commonly detected in apples are fructose, glucose, sucrose and sorbitol, with changing their individual and total concentration depending on the variety [Filip et al. 2016, Ma et al. 2015, Wu et al. 2007].

Numerous studies have already been done on commercial apple varieties to identify their organic acids, sugars, phenolic compounds and antioxidant activities [Vieira et al. 2009a, Wu et al. 2007,, Ma et al. 2015]. To the best of our knowledge, no study has been carried out on the physical, physicochemical and nutritional characterization of *Malus floribunda coccinella* fruit up to now.

Aims of this research were: (1) to characterize the *Malus floribunda coccinella* fruit in terms of color, size, dry matter, organic acids, sugars and sugar:acid ratio, (2) to identify phenolic compounds in peel, flesh and the whole apple, and (3) to determine differences in total monomeric anthocyanin contents and antioxidant activities of the fractions.

MATERIAL AND METHODS

Material

Malus floribunda coccinella fruits used in this research were collected from 5 different landscaping trees in Selcuk University campus in September, 2016. Fruits were immediately transferred to laboratory and peeled. Thirty among the fruits were randomly selected to be subjected to analyze their color, weight, size, pH, total dry matter, organic acid and sugar. The remaining were divided into three homogenous groups. First group was used as a whole (peel + flesh) for extraction, in the second group, only the flesh was used for the extraction after removing the peel and seed, manually. While in the third group, the peels of fruits were used in the extraction.

Chemicals and reagents

Chemicals used in total phenolic and monomeric anthocyanin contents, acidity and antioxidant activity analyses were of analytical grade and were purchased from either Sigma Chemical Co. (St. Louis, MO) or Merck (Darmstadt, Germany). Protocatechuic, cinnamic, chlorogenic, *p*-coumaric, sinapic and ferulic acids, rutin, kaemferol-3-glucoside, isorhamnetin--3-glucoside, quercetin, procyanidin B1, (+)-catechin, (–)-epicatechin, apigenin, cyanidin-3-galactoside, glucose, fructose, sucrose, malic and citric acids were purchased from Extrasynthese (Genay, France). HPLC grade acetonitrile and methanol were from Merck (Darmstadt, Germany). Ultrapure water was generated by Milli-Q water purification system (Millipore, Bedford, MA, USA)

Color analysis

Reflectance color values (L^*, a^*, b^*, C^* and h) of skin, flesh and whole fruits were measured using a spectrophotometer (CM-5, Konica Minolta, Osaka, Japan) equipped with measuring aperture mask with 3 mm diameter.

Chemical analyses

Soluble solid content, total dry matter, pH and titratable acidity analyses were carried out according to the procedures of Cemeroglu [1992]. Soluble solid content was determined using a refractometer (HSR-500, Atago, Japan) at 20°C and expressed as brix. pH was measured with a pH meter (WTW, Weilheim, Germany) and titratable acidity was determined potantiometrically, in which sample was titrated with sodium hydroxide solution (0.1 N) to an end point of 8.1 using a pH meter and result was given as g of malic acid equivalent per 100 g^{-1} of fresh weight (FW). For dry matter analysis, sliced fruits (25 g) were put into an oven dryer (Nuve, Turkey) and dried at 70°C up to the constant weight.

Determination of individual organic acids and sugars

Four g of fruit was extracted in 50 mL ultrapure water using a homogenizer (WiseMixTM HG-150; Daihan Scientific, Korea) and then centrifuged (NF 800R, Nuve, Turkey) at 4500 rpm for 15 min. Supernatant was passed through the syringe filter (0.45 µm) before injection. The analyses of organic acids and sugars were carried out by an Agilent 1260 Infinity Series HPLC system equipped with diode array detector fororganic acids and refrective index detector for sugars. Separation was achieved by Aminex HPX-87H column (Bio-Rad, 300 × 7.8 mm). Mobile phase consisted of 0.005 N sulfuric acid and the flow rate was 0.6 mL min^{-1}. The DAD detector was set at 210 nm for organic acids. Temperature was kept at 50°C [Demir et al. 2014]. Identification of organic acids and sugar was made according to their retention times. The data were analyzed by ChemStation software.

Extraction procedure for total phenolic and monomeric anthocyanin contents, antioxidant activities and phenolic profile analyses

One g of each of the following: whole apple (including peel and flesh), peel and flesh were extracted with 50 mL acidified methanol:water (80 : 20) using a homogenizer (WiseMixTM HG–150; Daihan Scientific, Korea). Then, the extracts were centrifuged (NF 800R, Nuve, Turkey) at 4500 rpm for 10 min. Supernatant was removed and collected into a glass jar. The residual pellate was re-extracted with methanol:water mixture using a homogenizer. The supernatants from two extractions were cooled and stored at –18°C until further analysis.

Total phenolic content analysis

The total phenolic contents of the samples were determined using Folin–Ciocalteu procedure, described by Singleton and Rossi [1965]. The methanolic extract of samples (0.5 mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (0.2 N) and 2.0 mL sodium carbonate (75 g L^{-1}). The samples were read against the blank at 765 nm after 120 min using a spectrophotometer (U–1800, Hitachi, Japan). Results were expressed as milligram of gallic acid equivalents (GAE) g^{-1} of DW (dry weight).

Total monomeric anthocyanin content analysis

Total monomeric anthocyanin content in the extracts were determined by pH differential method as described by [Coklar and Akbulut 2017]. Briefly, two flasks each containining 1 mL of extract were diluted with 4 mL of buffers pH 1.0 and pH 4.5, respectively. After 30 min, absorbance at wavelengths 515 and 700 nm was recorded and the difference between both values were calculated according to Eq. 1. Total monomeric anthocyanin pigment content of samples was calculated by Eq. 2 and the results were reported as milligram of cyanidin-3-galactoside equivalent g^{-1} DW.

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5} \quad (1)$$

$$\text{Anthocyanin pigment (mg g}^{-1}) = (A \times MW \times DF)/\varepsilon \times l \quad (2)$$

where A is the absorbance differences, DF is dilution factor, MW is molecular weight of cyanidin--3-galactoside, ε is molar absorptivity of cyanidin--3-galactoside and l is the pathlength in centimeters.

Determination of phenolic profile

For phenolic profile analysis, 1 mL of each extract was purified using C18 SPE cartridges (Agilent, USA). Non-anthocyanin phenolics and anthocyanins were eluted with ethyl acetate and methanol, respectively. Ethyl acetate and methanol eluates were evaporated at 35°C, re-suspended in 1 mL methanol and then filtered through 0.45-µm pore size syringe filter (Sartorius AG, Goettingen, Germany) [Coklar 2017]. The analysis of phenolic compounds in extracts was carried out by an Agilent 1260 Infinity Series HPLC system equipped with diode array detector. Separation was achieved by a reverse phase C18 column (5 µm, 250 × 4.6 mm i.d.). Mobile phase consisted of acetic acid : water (A) and water:acetonitrile:acetic acid (B). Flow rate was 0.75 mL min^{-1} and gradient was as follow: 10–14% B (5 min), 14–23% B (11 min), 23–35% B (5 min), 35–40% B (14 min), 40–100% B (3 min), 100% B isocratic (3 min), 100–10% B (3 min), 10% B isocratic (4 min). The detector was set at 280, 306, 320 and 360 nm for non-anthocyanin phenolics and 520 nm for anthocyanins [Coklar and Akbulut 2017]. The identification of phenolics was confirmed by comparing their reten-

tion times and UV spectra. Quantification of each compound was carried out by external standard. Calibration curves of standard phenolic compounds were drawn applying at least 5 different concentrations and the R^2 values of curves were ranged from 0.9991 to 1.000. Mentioned data analyses were performed using ChemStation software.

Antioxidant activity analyses

FRAP assay. The ferric reducing antioxidant power (FRAP) of samples were determined according to the method described by Benzie and Strain [1999]. Briefly, 50 µL extract and 150 µL deionized water were mixed in 1.5 mL of freshly prepared FRAP reagent – acetate buffer with pH 3.6 (300 M) : TPTZ solution (10 M) : FeCl3.6H2O solution (20 M) at the ratio of 10 : 1 : 1, respectively. The reaction mixture was incubated at 37.8°C for 4 min. Absorbance was read at 593 nm, and the results were given as µmol Fe^{+2} g^{-1} DW.

ABTS assay. The protocol described by Re et al. [2005] was followed to determine the ABTS antioxidant activity of the extracts. To generate the ABTS• radical, 2.5 mL of potassium persulfate solution (2.45 mM) was added to 5 mL ABTS solution (7 mM). The mixture was incubated at room temperature nearly for 16 h. The stock solution was diluted with ethanol to an absorbance of 0.700 ±0.02 at 734 nm. The extract (10 µL) was added to 990 µL ABTS• solution. The absorbance at 734 nm was measured after 6 min and the reduction in the absorbance was recorded. Results were given as mmol trolox equivalent kg^{-1} DW.

DPPH assay. 2,2-Diphenyl–1-picrylhydrazyl (DPPH•) antioxidant activity was determined according to the Brand-Williams et al. [1995]. Briefly, an aliquot (0.1 mL) of the extract was added to 3.9 mL of a DPPH (6×10^{-5} M) methanolic solution. After 30 min of incubation at room temperature in the dark, the absorbance was measured at 515 nm. The results were expressed as mmol trolox equivalent kg^{-1} DW.

Statistical analysis. The results are presented as means ± standard deviations (SD). Total phenolic content, total monomeric antocyanin content, antioxidant activities, and phenolic compounds results were subjected to one-way analysis of variance (ANOVA) at a confidence level of 95% to determine whether there was any significant difference between the means of the flesh, the skin and the whole apple. Statistical analyses were performed using MINITAB (Released 14, Minitab Inc. USA).

RESULTS AND DISCUSSION

Some physical and physicochemical properties of the fruit

The values of some physical and physicochemical properties of the apple are shown in Table 1. In reflectance color analysis, higher *a** value indicates

Fig. 1. Fruits of *Malus floribunda coccinella*

Table 1. Some physical and physicochemical properties of *Malus floribunda coccinella* fruit

Properties		Values
pH		2.89 ±0.02
Titretable acidity•		2.21 ±0.05
Brix		8.32 ±0.11
Total dry matter (%)		14.41 ±0.24
Skin color	L^*	22.54 ±0.36
	a^*	17.46 ±1.84
	b^*	3.65 ±0.24
	C^*	16.64 ±0.65
	h	12.29 ±0.28
Flesh color	L^*	27.23 ±2.13
	a^*	29.42 ±0.74
	b^*	6.56 ±0.36
	C^*	30.21 ±0.68
	h	12.47 ±0.81
Size	height (mm)	29.38 ±1.25
	width (mm)	33.57 ±1.27
Weight (g)		11.93 ±0.43
Flesh/peel ratio		6.34 ±0.17

• g malic acid equivalent 100 g^{-1} FW

higher redness; a^* values were measured as 17.46 and 29.42 for skin and flesh, respectively. The size of the apple used in our study was smaller than the well-known apples often used in other researches (Fig. 1). Higher titratable acidity and lower pH values were found in *Malus floribunda coccinella* fruit when compared to commercial apple cultivars. Apple titratable acidity values in various researches range from 0.18 to –0.52 g malic acid equivalent 100 mg^{-1} in different commercial and red-fleshed apple cultivars [Vieira et al. 2009a, Piagentini and Pirovani 2017], while the value of the titratable acidity of apple used in our research was 2.21 g malic acid equivalent 100 g^{-1} FW. Similarly, the pH values of apples determined in most research works falls between 3.05 and 4.25 [Rupasinghe et al. 2010, Vieira et al. 2009a, Wu et al. 2007, Contessa and Botta 2016], but in *Malus floribunda coccinella* fruit, the pH value was found to be 2.89.

Organic acids and sugars of the fruit

Table 2 represents the organic acids and sugars found in *Malus floribunda coccinella* fruit. Citric and malic acids were the organic acids identified in the fruit. The content of malic acid was approximately 22 times more than citric acid. The value of malic acid determined in our research was found to be 25.394 g kg^{-1} FW. However, the value of malic acid identified in commercial apples in other researches varies between 0.5–18.9 g kg^{-1} [Ma et al. 2015, Contessa and Botta 2016]. Therefore, it can be concluded that the concentration of malic acid in *Malus floribunda coccinella* fruit is higher than the other apples used in various studies.

Table 2. Organic acids, sugars and sugar:acid ratio of *Malus floribunda coccinella* fruit

Properties		Values
Organic acids*		
	Citric	0.133 ±0.003
	Malic	25.394 ±0.187
Sugars**		
	Sucrose	0.497 ±0.004
	Glucose	0.504 ±0.012
	Fructose	4.334 ±0.095
Sugar: acid ratio		2.091 ±0.055

* g kg^{-1} FW; ** g 100 g^{-1} FW

The content of fructose was found to be 4.33 g 100 g^{-1} FW, which represented the highest valued sugar among the rest of sugars confirmed in crab apple fruit. Sucrose and glucose were other sugars found in the fruit, the contents of which were almost the same. The individual sugar contents and their total values when compared to previous literature works on commercial and red fleshed apples were predominantly low [Wu et al. 2007, Vieira et al. 2009a, Contessa and Botta 2016].

Sugar : acid ratio indicates the sweetness of the fruits and higher values point out the sweeter taste. As a consequence of lower sugar and higher acid concentrations, the sugar:acid ratio of the *Malus floribunda coccinella* fruit was fairly low as compared to values of commercial apples, which was reported by Wu et al. [2007].

Total phenolic and monomeric anthocyanin contents

In Fig. 2, the total phenolic and monomeric anthocyanins contents of different fractions of *Malus floribunda coccinella* fruit was presented. The results showed a decreasing pattern from the peel, whole fruit (including peel and flesh) and the flesh in both the total phenolic and monomeric anthocyanins contents as illustrated in Fig. 2. Values of the total phenolic contents in the peel, the whole fruit and the flesh were 56.85, 45.91 and 36.12 mg g^{-1} DW, respectively. Differences in the total contents of various fractions were statistically significant at the level of $p < 0.01$.

According to our results, total phenolic amount of *Malus floribunda coccinella* fruit was higher than that of common apples, which were reported in previous studies [Vieira 2009a, Piagentini and Pirovani 2017]. Raudone et al. [2017] reported that phenolic amount of different apple cultivars ranged from 20.60 to 31.03 mg g^{-1} DW. Similar to our results, previous studies reported that phenolic concentration in the peel of apple was higher than in the flesh [Balázs et al. 2012, Yuri et al. 2009]. Huber and Rupasinghe [2009] reported that total phenolic concentrations in the skin of commercial apples were between 19.3–25.3 mg 100 g^{-1}. Crab apples contain higher total phenolic contents than common apples [Sharma and Nath 2016]. On the other hand, Sharma and Nath [2016] have found lower phenolic contents in flesh and peel of crab apple (*Malus baccata*) as compared to our results.

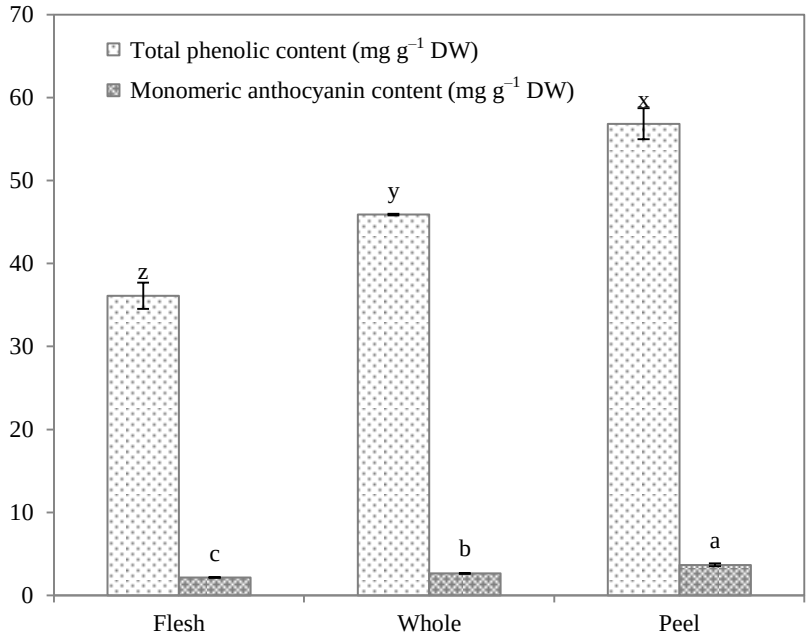

Fig. 2. Total phenolic and monomeric anthocyanin contents of different fractions of *Malus floribunda coccinella* fruit. Different letters within monomeric anthocyanin content and total phenolic content bars indicate statistically significant differences ($p < 0.05$)

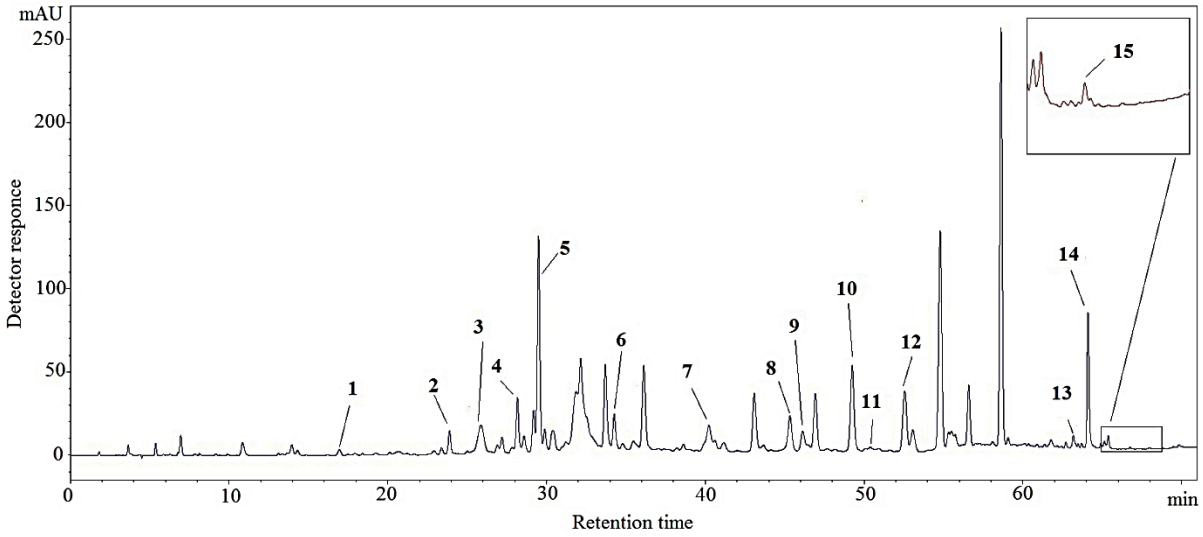

Fig. 3. HPLC chromatogram of phenolic compounds in *Malus floribunda coccinella* fruit at 280 nm (1. protocatechuic acid, 2. procyanidin B1, 3. cyanidin-3-galactoside, 4. (+)-Catechin, 5. chlorogenic acid, 6. (–)-epicatechin, 7. *p*-coumaric acid acid, 8. ferulic acid, 9. sinapic acid, 10. rutin, 11. kaemferol-3-glucoside, 12. isorhamnetin--3-glucoside, 13. cinnamic acid, 14. quercetin, 15. apigenin)

Contrary to lots of commercial apple cultivars, anthocyanins have been accumulated in the flesh of *Malus floribunda coccinella* fruit. Anthocyanin content in the peel was 2-fold higher than in the flesh. Values of the monomeric anthocyanin contents of the peel, the whole fruit and the flesh were respectively found to be 3.68, 2.66 and 2.15 mg $1g^{-1}$ DW. There was statistically significant difference among the monomeric anthocyanin content among fractions ($p < 0.01$)

We found much higher anthocyanin values for flesh and skin of *Malus floribunda coccinella* with respect to those reported by Wang et al. [2015] and Contessa and Botta [2016], who investigated the anthocyanin content of red-fleshed apple.

According to Wang et al. [2015], total monomeric anthocyanin values of different red-fleshed apple varieties were between 29.53–175.84 mg 100 g^{-1} FW for skin, and 1.21–55.97 mg $100g^{-1}$ FW for flesh.

Phenolic profile results

Fig. 3 presents the chromatogram of phenolic compounds of *Malus floribunda coccinella* fruit. Six phenolic acids (protocatechuic, cinnamic, chlorogenic, *p*-coumaric, sinapic and ferulic acids), five flavonoids (rutin, kaemferol-3-glucoside, isorhamnetin-3-glucoside, quercetin and apigenin), three flavan--3-ols (procyanidin B1, catechin and epicatechin) and one anthocyanin (cyanidin-3-galactoside) were detected in *Malus floribunda coccinella* fruit. Also in Table 3, the concentrations of phenolic compounds found in different fractions of the fruit are shown. We observed that the concentration of phenolic compounds detected in the whole fruit varies from 1.25 to 322.90 mg kg^{-1} DW. In whole fruit, the concentration of rutin was the highest and followed by cyanidin--3-galactoside, chlorogenic acid, catechin, quercetin, epicatechin, procyanidin B1 in a decreasing order. Apigenin was the lowest at concentration among the rest of phenolic compounds in the whole fruit.

Flavanols were the main phenolics of *Malus floribunda coccinella* fruit. As distinct from commercial apples, they were followed by anthocyanins in terms of the concentration. This result concurs well with the results of Wang et al. [2015], although the amounts of individual phenolics in their study were highly lower compared to our results. Similar results were reported by Li et al. [2014], who investigated the

phenolic compounds and antioxidant activity of ten different wild Malus species. They determined rutin as the main phenolic of crab apples. Furthermore, chlorogenic acid, (–)-epicatechin, quercetin and phloridzin were pointed out as other important phenolics.

When we considered the concentration of phenolic compounds in the flesh of the fruit, it ranged between 420.05 and 1.25 mg kg^{-1} DW. The main three phenolic compounds detected from the flesh were chlorogenic acid, cyanidin-3-galactoside and epicatechin with 420.05, 250.59 and 197.25 mg kg^{-1} DW, respectively, while apigenin revealed the lowest concentration. Despite the presence of kaempferol--3-glucoside in the whole and the peel of the fruit, it was not identified in the flesh.

In alike manner, while kaemferol-3-glucoside have been identified in the whole apple (peel and flesh) by Serra et al. [2010], Stefova et al. [2017] have determined kaempferol hexoside and kaempferol pentoside only in the peel of apples.

The concentration of phenolic compounds in the peel was also taken into consideration. The phenolic compounds found in the peel ranged between 3654.90 and 2.17 mg kg^{-1} DW. The most dominant phenolic compound identified in the peel was rutin with 3654.90 mg kg^{-1} DW and apigenin with 2.17 mg kg^{-1} DW was identified as the lowest in concentration.

As in many apple varieties, the amount of total phenolic acid in the flesh of *Malus floribunda coccinella* was higher than in the peel. As reported by Lee et al. [2017], phenolic acid concentrations in the skin and pulp of Fuji, Pink Lady, Golden Delicious, Granny Smith apples ranged from 63.7 to 449.0 mg kg^{-1} DW in skin and 157.0 to 781.8 mg kg^{-1} DW in pulp for chlorogenic acid, 1.4 to 125.0 mg kg^{-1} DW in skin and 44.3 to 287.5 mg kg^{-1} DW in pulp for caffeic acid, 30.1 to 60.5 mg kg^{-1} DW in skin and 11.3 to 26.1 mg kg^{-1} DW in pulp for *p*-coumaric acid, 3.3 to 31.6 mg kg^{-1} DW in skin and 0.8 to 3.1 mg kg^{-1} DW in pulp for ferulic acid, 0 to 3.9 mg kg^{-1} DW in skin and 0.9 to 1.3 mg kg^{-1} DW in pulp for sinapic acid, 19.1 to 76.4 mg kg^{-1} DW in skin and 3.6 to 9.6 mg kg^{-1} DW in pulp for protoctechuic acid.

Table 3. Phenolic compounds of different fractions of *Malus floribunda coccinella* fruit (mg kg^{-1} DW)

Specification	Flesh	Whole fruit	Peel
Protocatechuic acid	6.32 ±0.19b	6.82 ±0.18b	8.62 ±0.32a
Cinnamic acid	2.92 ±0.11b	4.55 ±0.35ab	6.39 ±0.71a
Chlorogenic acid	420.05 ±16.91a	242.58 ±17.42b	200.82 ±8.29b
p-coumaric acid	8.88 ±0.29b	17.96 ±1.24a	18.75 ±0.39a
Sinapic acid	12.73 ±0.32b	16.92 ±0.86a	16.96 ±0.39a
Ferulic acid	17.82 ±1.03b	29.02 ±1.41a	5.53 ±0.81c
Rutin	49.60 ±0.53c	322.90 ±11.46b	3654.90 ±64.68a
Kaemferol-3-glucoside	nd	15.23 ±1.09b	806.27 ±56.16a
Isorhamnetin-3-glucoside	5.05 ±0.13c	21.92 ±1.52b	270.83 ±2.61a
Quercetin	18.50 ±0.55c	118.18 ±12.36b	457.11 ±31.13a
Procyanidin B1	43.47 ±0.70c	93.68 ±6.34b	285.96 ±6.12a
(+)-Catechin	72.61 ±1.72c	176.66 ±3.49b	851.59 ±29.77a
(–)-Epicatechin	197.24 ±4.56a	118.03 ±6.22b	57.67 ±2.93c
Apigenin	1.25 ±0.04	1.25 ±0.05	2.17 ±0.52
Cyanidin-3-galactoside	250.59 ±2.11b	262.88 ±8.04b	540.86 ±48.16a

nd: not dedected
Different letters in the same row indicate statistically significant differences between flesh, peel and whole fruit ($p < 0.05$)

Chlorogenic acid, kaemferol-3-glucoside, isorhamnetin-3-glucoside, quercetin, procyanidin B1, catechin and cyanidin-3-galactoside were sufficiently higher in concentrations; therefore, these can be classified as the important phenolic compounds within the peel. Looking at the concentration of the following phenolic compounds: kaemferol-3-glucoside, rutin, isorhamnetin-3-glucoside, quercetin, procyanidin B1, cinnamic acid and catechin among the three fractions, they were higher in the peel and gradually decreased from the whole fruit to the flesh. Also, *p*-coumaric and sinapic acid concentrations determined in the peel and whole fruit were approximately the same, but higher in concentrations than in the flesh. On the other hand, protocatechuic acid and cyanidin-3-galactoside displayed high concentrations in the peel and almost similar in concentrations in both the whole fruit and the flesh. In epicatechin, the order of concentration was flesh > whole fruit > peel.

Catechin, (–)-epicatechin and procyanidin B1 were comprised of 6.56, 17.82 and 3.93% of total phenolic compounds in the flesh of apple, respectively. The percentage of catechin among all phenolics in the peel was approximately 2-fold higher than in the flesh. However, percentages of procyanidin B1 of peel and flesh were nearly equal to each other. Although phenolic compounds detected in apples and their concentrations both in peel and flesh show differences depending on many factors such as the variety [Jakobek et al. 2013, Górnaś et al. 2015], generally higher concentrations were reported in the peel as compared to whole fruit and flesh of apples.

Cyanidin-3-galactoside constituted 22.64 and 7.53% of the total phenolics in the flesh and in the peel of apple, respectively. Except the red-fleshed apples, there is no anthocyanin accumulation in the flesh of commercial apples [Kelly et al. 2003, Tsao Rong 2005]. Red fleshed apples contain lesser amounts of anthocyanin compare to crab apples. On the other hand, our results showed that *Malus floribunda coccinella* fruit contained more cyanidin-3-galactoside than other crab apples studied by Górnaś et al. [2015]. They reported cyanidin-3-*O*-galacto- side content of seven crab apple varieties (kerr, riku, quaker beauty, ritika, kuku, ruti and K-8/9-24) ranging from 3.1 to 235.4 mg kg^{-1} DW.

Antioxidant activity results

Three methods of antioxidant activity, namely ABTS, DPPH, and FRAP, were used in our study. Fig. 4 shows the results of antioxidant activities in various fractions of *Malus floribunda coccinella* fruit.

According to our results, ABTS, DPPH and FRAP antioxidant activity values of the peel were approximately 1.5 times higher than those found in the flesh. Whereas other researchers have declared 2.5–10 fold-higher antioxidant activities in peels of different apple varieties compared to their flesh [Drogoudi et al. 2008, Yuri et al. 2009, Vieira et al. 2011, Alberti et al. 2017]. According to Vieira et al. [2011], ABTS and DPPH values in flesh of 11 apple cultivars ranged from 380.82 to 960.70 μmol TE 100 g^{-1} FW, 346.44 to 891.21 μmol TE 100 g^{-1} FW, respectively.

Our FRAP value for the whole apple was higher than those found by Contessa and Botta [2016], who analyzed red-fleshed, commercial and ancient apple cultivars. Similarly, Vieira *et al.* [2011] evaluated the antioxidant activities of flesh and peels of 11 apple cultivars and found that FRAP values were between 140.21 and 261.74 μmol TE 100 g^{-1} FW for flesh and between 520.71 and 1160.58 μmol TE 100 g^{-1} FW for peel.

Our findings are in agreement with the studies of Tsao Rong [2005], Kelly et al. [2003], Lee Ki Won [2003] from the points of contribution of apple phenolics to antioxidant activity.

A large number of studies have been performed on antioxidant activity and phenolic compounds in different fractions of different *Malus domestica* apple cultivars [Drogoudi et al. 2008, Górnaś *et al.* 2015, Alberti et al. 2017, Lee *et al.* 2017]. A large number of studies subsist on antioxidant activity and phenolic compounds in various fractions of different *Malus domestica* apple cultivars [Vieira et al. 2009b, Vieira et al. 2011]. Higher antioxidant activity in flesh of *Malus floribunda coccinella* fruits compared to flesh of common apples could be explained by higher amount of phenolic compounds

and a lot of cyanidin-3-galactoside. According to the results of antioxidant activities and phenolic concentration in the fractions of the apple, there is a strong correlation between the antioxidant activity and phenolic compounds. As phenolic concentration increased among fractions, higher antioxidant activity results by three different methods were recorded. On the other hand, distribution of individual polyphenolics among all three fractions as well as total polyphenolic concentration had influence on antioxidant activities.

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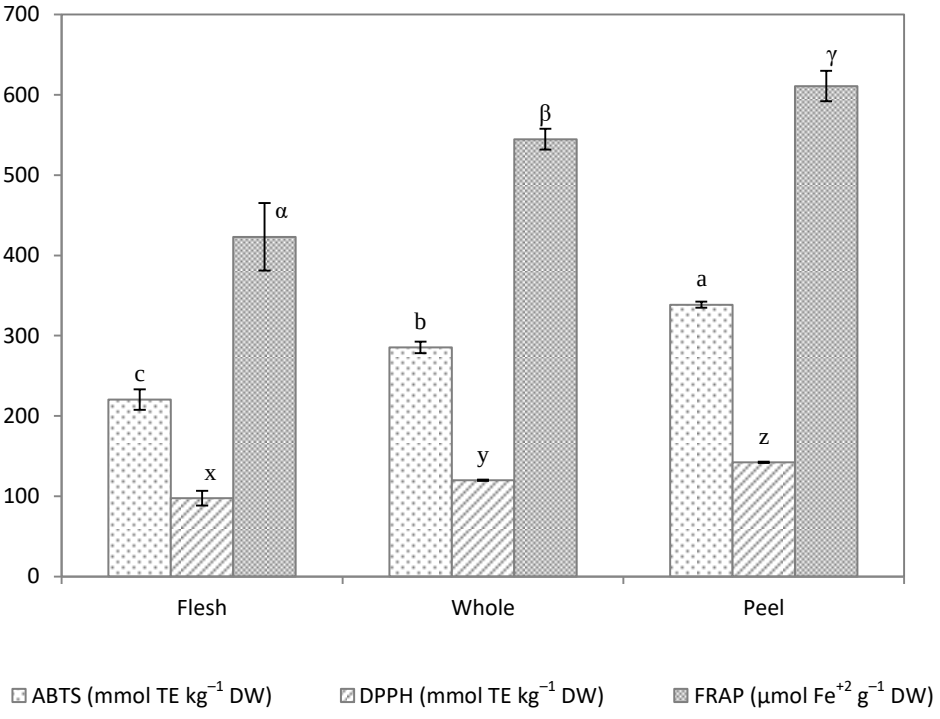

Fig. 4. Antioxidant activities of whole, peel and flesh of *Malus floribunda coccinella* fruit. Different letters and symbols within each antioxidant activity bar indicate statistically significant differences ($p < 0.05$)

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Polyphenols are one of the key antioxidants such as vitamin C and E, tocopherols, tocotrienols and carotenoid. Hydroxycinnamates exhibit equal or two times greater antioxidant activity than vitamin C or E, while flavan-3-ols, anthocyanidins and flavon-3-ols possess aproximately two to five-fold higher antioxidant activity than those of vitamin C and E [Rice-Evans et al. 1997]. Moreover, antioxidant activities of procyanidins (B1 and B2) are nearly 2 times higher compared to their monomers [Tsao Rong 2005]. On the other hand, lower antioxidant activity value was reported for (–)-epicatechin as compared to (+)-catechin Tsao Rong [2005]. Generally, anthocyanins posses higher antioxidant activities than those of non-anthocyanin phenolics [Coklar and Akbulut 2017]. In the light of this information, differences in antioxidant activities between fractions of the apple possibly could be attributed to differences in individual phenolic concentrations and their ratios in total phenolic account among peel, flesh and the whole apple.

CONCLUSION

To summarize, the peel and flesh of *Malus floribunda coccinella* fruit were abundant in phenolic compounds. The fruit also contained more polyphenols than commercial apples in previous studies and possessed higher antioxidant activities.

Except from *kaemferol*-3-glucoside, the same polyphenols were detected in both of the peel and flesh. Kaemferol-3-glucoside was not detected in the flesh. While higher levels of protocatechuic and cinnamic acids, rutin, isorhamnetin-3-glucoside, quercetin, procyanidin B1, catechin and cyanidin--3-galactoside were found in the peel, chlorogenic acid and epicatechin were predominant in the flesh. Cyanidin-3-galactoside concentration in the peel was approximately 2-fold higher than in the flesh. The fact that the fruit contained higher organic acid and lower sugar, the sugar:acid ratio was fairly low as compared to common apples. Due to fairly low sweetness and high antioxidant activity of the fruit, we recommend that future works should focus on processing the fruit into different products to enhance the consumer preference instead of raw consumption.

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