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# Determination of Some Physical, Chemical and Nutritional Properties in the Peel and Flesh of Three Crab Apple Species at Five Edible Maturity Stages

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**HIGHLIGHTS**

- Protein concentrations were higher in the peels than the fleshes.
- Anthocyanin accumulation in the fleshes was the highest in *M. floribunda*.
- *M. evereste* is the richest species in terms of total phenolic and tannin.

**Abstract:** The main aim of this study was to determine the changes in quality properties and bioactive compounds of 3 crab apple species (*Malus evereste, Malus floribunda and Malus floribunda coccinella*) harvested at 5 different edible maturity stages. Acidity, soluble solids, organic acid and sugar contents were determined in the whole apple. The fleshes and peels of the crab apples were analyzed for antioxidant activities, color, protein, total monomeric anthocyanin, and total phenolic contents. Maturity stage had no significant effect on size, pH, titratable acidity, and citric acid content of crab apples. Peels of all three species had higher total phenolic, monomeric anthocyanin, tannin, and protein concentrations than their fleshes. *M. evereste* was the richest source of total phenolic and tannin among the species and it exhibited the highest antioxidant activity. Unlike the total phenolic compounds, anthocyanin accumulation was the highest in the flesh of *M. floribunda*. Concentrations of sugars in all species increased throughout the maturity. While the highest levels of glucose and fructose determined in *M. evereste* at the last stage, sucrose concentration was higher in *M. floribunda* that those in other two species at all stages. The trend in malic acid accumulation showed differences between the species. As a result, it can be concluded that the fruits of all species can be harvested in the first two weeks of September.

**Keywords:** color; red fleshed apple; protein; tannin; organic acids.

## INTRODUCTION

Crab apples are mostly grown for their charming flowers or fruits [1]. They are that type of apples generally considered as inedible and/or wild apples. Their trees are planted purposely for the beautification of the environment and ornamental landscaping. Due to the canopy shape-like of their leaves, they also serve as shelter during hot seasons. The colorful nature of its flowers attracts insects to it which helps pollination to occur and henceforth enabling good yield of the fruits. Crab apples, classified under the family Rosaceae are naturally small in size unlike the commercial apple cultivars. Crab apples have an intense sour taste which takes its source from a high level of malic acid.

In maturity, softening of flesh texture, turning from green to red, increments in sugar contents, developing of flavor, and nutritional qualities are the biochemical and physiological changes that fruits go through [2,3].The fruits of crab apples are naturally red in color and could possess nutraceutical and antioxidant properties. Considering the red color of the peel and flesh, the fruit is likely to contain significant amount of anthocyanins which possess antioxidants effects. Major organic acid in a matured apple fruit is malic acid, however, other organic acids like citric and quinic acid can be detected in low concentrations [4].

Fruits that are colorful in nature have features that supplies significant health benefits for mankind such as anti-cancer activities, anti-atherosclerotic effects and strong antioxidant activities [5]. It is therefore highly recommended to be consumed more fruits and vegetables, especially red colored ones which are more likely to contain high antioxidant activities. Additionally, pigments such as anthocyanins, chlorophylls and carotenoids are the basic determinants of fruit color [6]. Changes in *L**, *a** and *b** parameters of fruits especially red colored fruits during maturation is correlated to the turning of the fruit color from green to red, where accumulation of anthocyanins and loss of chlorophyll takes place [7]. During fruit maturity, oxidative enzymes polyphenol-oxidase, peroxidase, diminishing of chlorophyll, formation of new pigments like carotenoids and or anthocyanins and activation of pigments synthetized in the earlier stage of the development of fruit are among the factors responsible for changes in color in fruits [8]. Imeh and Khokhar [9] reported that changes such as biochemical, physiological and structural modifications in fruits during maturity could be the effects of climatic, agronomic, and environmental conditions.

In literature, there are only a few studies about crab apple species. However, no comprehensive study has been done to compare the physicochemical properties of the peel and flesh of *M. evereste*, *M. floribunda coccinella* and *M. floribunda* fruits during maturity. To fill the potholes which have been left behind in literature regarding some selected cultivars of crab apples prompted us to embark on this study. This study is thereby aimed to find out the physicochemical and nutritional properties of *M. evereste*, *M. floribunda coccinella*, and *M. floribunda*. In addition, the study was also aimed at deciding the optimum harvesting time of the fruits.

## MATERIAL AND METHODS

### Plant material

Three crab apple species, namely *Malus evereste*, *Malus floribunda coccinella*, and *Malus floribunda*, were used in this study. Fruits were harvested from 3 trees of each species cultivated for landscaping in Selcuk University campus. Samples were picked on August 18, August 24, September 6, September 14, and September 21, 2017. The dates of picking were indicated as 1., 2., 3., 4., and 5. stages, respectively. Approximately, 2 kg of fruits were collected at each harvesting stage and transferred to laboratory. Half of the each species was analyzed for soluble solids content, color, size, pH, protein, organic acid and sugar. The remaining was used to prepare the peel and flesh extracts. Manually removed peels and flesh were extracted.

### Color analysis

Color values (*L**, *a**, *b**, *C** and h) of surfaces and fleshes of the fruits were measured by using a spectrophotometer (CM-5, Konica Minolta, Osaka, Japan) equipped with measuring aperture mask with 3 mm diameter [10].

### Determination of some chemical and physical analyses

Soluble solids content, pH and titratable acidity analyses were carried out according to the procedures of Cemeroglu [11]. Soluble solids content was determined by using a refractometer (HSR-500, Atago, Japan) at 20 $^{o}$C. 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 by using a pH meter and result was given as g malic acid equivalent/100 g fresh weight. Diameter

and width of the apples were measured by using a vernier caliper and given as mm. Amount of nitrogen was detected by Dumas method. Freeze-dried and ground flesh and peel of apples (0.2 g) was weighed into tin foil capsule and placed in the nitrogen analyzer (Leco, TruSpecCN, USA). To calculate the total protein content in the seed, 6.25 conversion factor was used and results were given as g/100 g dry weight (DW).

**Determination of organic acids and sugars**

Four g of fresh fruit was extracted in 50 mL ultrapure water (Millipore, Bedford, MA, USA) by 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 from syringe filter (Sartorius AG, Goettingen, Germany) with a pore size of 0.45 µm before the injection. The analyses of organic acids and sugars were carried out by an HPLC system (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with diode array detector for organic acids and refractive index detector for sugars. Separation was achieved by Aminex HPX-87H column (300 × 7.8 mm). Mobile phase was sulfuric acid (0.0025 M) and the flow rate was 0.6 mL/min. The DAD detector was set at 210 nm for organic acids. Temperature was kept at 50 $^{o}$C [10]. Results were given as g/100 g FW.

**Extraction procedure for total phenolic, tannin, and monomeric anthocyanin contents, antioxidant activity analyses**

One g of freeze dried and ground flesh or peel were extracted with 50 mL acidified methanol:water (80:20) by using an ultrasonic bath (TI-H-20, Elma, Singen, Germany) with 20 ml of methanol at 35 kHz for 25 min at 35 $^{o}$C. Then, the extracts were centrifuged (NF 800R, Nuve, Turkey) at 4500×g for 10 min. Supernatant was removed and collected into a glass jar. The residue was re-extracted with methanol:water mixture. The supernatants from two extractions were pooled and stored at -18 $^{o}$C until further analysis.

**Total phenolic content analysis**

The methanolic extract (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). The samples were read against the blank at 765 nm after 120 min using a spectrophotometer (U–1800, Hitachi, Japan). Results were expressed as mg of gallic acid equivalents GAE /g DW [12].

**Total monomeric anthocyanin content analysis**

Total monomeric anthocyanin content in the extracts was determined by pH differential method as described by Coklar and Akbulut [13]. Briefly, two flasks each containing 1 mL of extract was diluted with 4 mL of buffers pH 1.0 and pH 4.5, respectively. After 30 min, absorbances at wavelengths 515 and 700 nm were recorded and the difference between the values was calculated according to Equation 1. Total monomeric anthocyanin pigment content of the samples was calculated by Equation 2 and the results were reported as mg of cyanidin-3-galactoside equivalent/g DW

$$A=(A_{520nm}-A_{700nm})_{pH=1.0}-(A_{520nm}-A_{700nm})_{pH=4.5} \quad (1)$$

$$\text{Total monomeric anthocyanin content (mg/g)}=((A \times MW \times DF))/(\varepsilon \times l) \quad (2)$$

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 path length in centimeter.

**Tannin content analysis**

One mL of methanolic extract was added to 2 ml of bovine serum albumine solution at a concentration of 1.0 mg/ml (in 0.20 M acetate buffer, pH 5.0, containing 0.17 M sodium chloride), kept at 4 $^{o}$C overnight to precipitate protein and tannin complex, and then centrifuged at 9000 x g for 15 min. Pellet was dissolved in 4 ml of SDS-triethanolamine. One ml 0.01 M ferric chloride in 0.01 N hydrochloric acid was added and immediately vortexed. The absorbance was recorded at 510 nm after 15 min. The results were calculated according to the standard curve that was prepared using 0.1-1 mg/ml tannic acid solutions and given as mg tannic acid equivalent/g of DW [14].

## Antioxidant activity analyses

*ABTS assay*

The extract (10 µL) was added to 990 µL ABTS• solution generated with potassium persulfate solution (2.45 mM) and ABTS solution (7 mM). The absorbance at 734 nm was measured after 6 min and the reduction in the absorbance was noted. Results were given as mmol Trolox Equivalent (TE)/100 g DW. [15].

*DPPH assay*

Briefly, an aliquot (0.1 mL) of the extract was added to 3.9 mL of a DPPH ($6 \times 10^{-5}$ M in methanol) 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 TE/100 g DW [16].

## Statistical analysis

The results are being presented as means ± standard deviations (SD). Results of analyses 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 maturity stages, crab apple species or fractions. Statistical analyses were performed by using MINITAB (Release 14, Minitab Inc. USA).

## RESULTS AND DISCUSSION

## Physicochemical and physical characteristics of crab apples

Table 1 summarizes the titratable acidity, pH, soluble solids content, and sizes of three crab apple species in accordance with the maturity. The pH values at the first stage of maturity were measured as 3.41±0.10, 3.20±0.04, and 3.03±0.08 for *M. evereste*, *M. floribunda coccinella*, and *M. floribunda*, respectively. At the last stages, while the *M. evereste* had the same pH value (3.41±0.13), an increase in *M. floribunda coccinella* (3.31±0.10) and a decrease in *M. floribunda* (2.98±0.02) were observed. However, the changes in pH of three species were approximately constant between the maturity stages and statistically insignificant ($p>0.05$). On the other hand, there were statistically significant differences between the pH values of the species ($p<0.01$). The pH values of common apples studied in previous studies are between 3.05-4.25 [17-19]. While pH values of *M. evereste* and *M. floribunda coccinella* fall in the range of pH values reported in common apples, *M. floribunda* had lower pH values.

**Table 1.** Some physical and physicochemical characteristics of crab apples

| Species | Maturity stage* | pH | Soluble Solids Content (%) | Titratable acidity** | Diameter (mm) | Width (mm) |
|---|---|---|---|---|---|---|
| *Malus evereste* | 1 | 3.41±0.10 | 10.07±1.86 | 2.21±0.20 | 18.53±8.53 | 11.14±0.22 |
| | 2 | 3.39±0.06 | 9.79±0.59 | 2.24±0.13 | 17.83±7.47 | 11.07±0.12 |
| | 3 | 3.36±0.03 | 10.25±0.97 | 2.38±0.06 | 16.97±5.73 | 11.34±0.13 |
| | 4 | 3.39±0.05 | 12.31±1.18 | 2.21±0.17 | 17.82±7.22 | 11.16±0.43 |
| | 5 | 3.41±0.13 | 12.44±1.46 | 1.89±0.23 | 17.80±6.91 | 11.85±0.24 |
| *Malus floribunda coccinella* | 1 | 3.20±0.04 | 8.23± 0.26 | 1.73±0.09 | 24.15±1.51 | 22.31±2.17 |
| | 2 | 3.20±0.04 | 8.75±0.56 | 1.73±0.03 | 24.80±1.85 | 23.97±4.25 |
| | 3 | 3.22±0.10 | 9.97±0.06 | 1.81±0.10 | 25.75±1.96 | 25.00±4.11 |
| | 4 | 3.20±0.09 | 10.93±0.59 | 1.66±0.14 | 25.77±0.98 | 25.31±5.94 |
| | 5 | 3.31±0.10 | 11.09±0.64 | 1.59±0.16 | 26.79±0.62 | 28.12±6.05 |
| *Malus floribunda* | 1 | 3.03±0.08 | 9.57±0.33 | 2.02±0.24 | 38.17±1.66 | 32.85±1.37 |
| | 2 | 3.00±0.02 | 9.79±1.60 | 2.17±0.25 | 39.08±2.96 | 31.91±1.30 |
| | 3 | 2.98±0.03 | 10.25±0.40 | 2.32±0.29 | 40.47±1.72 | 33.90±2.21 |
| | 4 | 2.95±0.02 | 11.04±0.23 | 2.43±0.27 | 42.03±1.74 | 35.36±2.48 |
| | 5 | 2.98±0.02 | 11.96±1.32 | 2.42±0.04 | 42.66±3.25 | 35.78±2.49 |

1*1: August 18, 2: August 24, 3: September 6, 4: September 14, 5: September 21
** g malic acid equivalent/100 g FW

Having taken into account titratable acidity, fluctuations in values occurred during maturity and their differences were statistically insignificant ($p>0.05$). The order of titratable acidity was at the first stage as *M. evereste>M. floribunda>M. floribunda coccinella*. Titratable acidities of *M. evereste*, *M. floribunda coccinella*, and *M. floribunda* were found to be 1.89±0.23, 1.59±0.16and 2.42±0.04 g malic acid equivalent/100 g FW,

respectively, at the last stage of maturity with being decreases in *M. floribunda*, and *M. floribunda coccinella* and an increase in *M. floribunda*.

Titratable acidity values of apples determined in all species throughout the maturity were higher than those in different commercial and red-fleshed apple species (0.18-1.28 g malic acid equivalent/100 g) which are studied previously [17,19]. Decrements in the titratable acidities of *M. floribunda coccinella* and *M. evereste* during the maturity agree well with the result of Kvikliene and coauthors [20].

In a general perspective, an increase in soluble solids contents was determined in the three species along the stages of maturity. The values changed from 10.07±1.86 to 12.44±1.46 % for *M. evereste*, 8.23±0.26 to 11.09±0.64 % for *M. floribunda coccinella*, and 9.57±0.33 to 11.96±1.32 % for *M. floribunda*. Differences between the species and the maturity stages were statistically significant ($p<0.01$).

Similar results were reported during maturity of common apples [20]. This increment could be as a result of hydrolysis of starch components into the soluble sugars during fruit maturity [21,22]. In *M. evereste* and *M. floribunda coccinella* varieties, similar attitude was observed where the acidity gradually increased to the third period.

The diameter and width values of the apples at the last stage of maturity were 17.80±6.91 and 11.85±0.24 mm for *M. evereste*, 26.79±0.62 and 28.12±6.05 mm for *M. floribunda coccinella*, and 42.66±3.25 and 35.78±2.49 mm for *M. floribunda*, respectively (Table 1). While differences in diameter and height between species were statistically significant ($p<0.01$), changes in the size of fruits throughout the maturity were found to be statistically insignificant ($p>0.05$).

## Color values of crab apple species

*L**, *a**, *b**, chroma (*C**), and hue angle (h) values for the peel and the flesh of crab apples are shown in Table 2. When taking into consider the colors of fleshy portion of the apples, maturity didn't have a statistically significant effect on *L**, *a** and *C** of *M. evereste*, *M. floribunda coccinella* and *M. floribunda* fruits. However, both maturity stages and species had a significant effect on the *b**, and h values ($p<0.01$). As a general increases in *b**, and h values were observed. Though the differences in the *L** and *a** values of the fleshes were statistically significant ($p<0.01$) between the species, they were found insignificant between the maturity stages.

The tendency of red color (*a**) among the flesh of the species was highest in *M. floribunda*, followed by *M. floribunda coccinella* and least in *M. evereste*. At the last stage of maturity *a** values for *M. floribunda*, *M. floribunda coccinella*, *M. evereste* were measured as 36.12±1.75, 23.34±9.87, and 19.90±6.92, respectively.

In respect to the peel color of crab apples used in this study, all color parameters of the peel of three apples changed by maturity. Moreover, differences in color parameters, except the *b** value, among the maturity stages were found to be statistically significant ($p<0.01$). While the *a** value increased from 18.09±0.45 to 22.32±1.68 in *M. floribunda* throughout the maturity, decreases were observed in the value both in the *M. evereste* (from 25.28±0.76 to 23.92±1.87) and *M. floribunda coccinella* (from 22.55±1.91 to 19.88±3.49). It was observed that there was a tendency as an increment in *L**, *a**, and h values of the peels during the maturity in all crab apples. Similar the results in maturity, there were significant differences in the *L**, *a**, *C**, and h values of the crab apples among the species. The highest values of *L** (42.70±8.09), *b** (16.51±6.85), *C** (27.51±1.91), and h (39.55±17.60) were occurred at the last stage of maturity in *M. floribunda coccinella*. Conversely, lowest *b** (10.82±1.28), *C** (25.01±1.46), and h (26.00±3.89) values were determined for *M. floribunda* at the last stage. The order of apples in terms of the peel redness was as *M. evereste>M. floribunda>M. floribunda coccinella*.

Anthocyanins are glycosides of anthocyanidins and natural pigments ranging in color from red to purple. Cyanidin and peonidin are anthocyanidins responsible for the orange-red colors, while malvidin, delphinidin and petunidin are responsible for the blue-red colors. Structural variations such as the number of hydroxyl groups, position of the sugars attached, acylation of sugars with acids cause differences in color of anthocyanins [23]. Cyanidin-3-galactoside is main anthocyanin that responsible for apples with red colored peel [10]. Since cyanidin-3-galactoside and other anthocyanins are also accumulated in the fleshes of *M. evereste*, *M. floribunda*, and *M. floribunda coccinella*, their fleshes are seemed as reddish.

## Protein amounts of peel and flesh of crab apples

Figure 1 shows the protein results observed in the peel and flesh of three crab apples. At the first stage of maturity, protein contents were determined as 2.30±0.28, 1.08±0.35, and 1.78±0.08 g/100 g DW for the peels of *M. evereste*, *M. floribunda coccinella*, and *M. floribunda*, respectively. While there were fluctuations in the values of all three species throughout the maturity, a decrement tendency occurred to last stages.

On the other hand, according to the results of statistical analysis, maturity had no significant effect on protein accumulation in the peel. Highest protein concentrations were found out at the peel of *M. evereste* among the species at all maturity stages. Differences in the protein amounts between the species were statistically significant ($p<0.01$). Peels of all apple species were contained higher protein than those of their fleshes. Similar the results in peels, *M. evereste* contained higher amount of protein than other species which were close to each other in terms of their protein concentrations. While the species had a significant effect on protein content in fleshes ($p<0.01$), effect of maturity was statistically insignificant. According to the protein contents of fleshes, 1.07±0.47 mg/100 g DW was the highest value recorded at the 5$^{th}$ stage of maturity in *M. evereste*, meanwhile, fluctuations were seen in 3 species during maturity.

In a previous study, protein concentration in Golden delicious apple was reported as 0.14 g/100 g FW at the first stage of maturity and decreased to 0.10 g/100 g FW at the last stage [24].

**Table 2**. Color values of the surface and flesh of the crab apples

| | Species | Stage* | *L** | *a** | *b** | *C** | H |
|---|---|---|---|---|---|---|---|
| Peel | *Malus evereste* | 1 | 29.61±3.40 | 25.28±0.76 | 11.10±1.47 | 27.69±0.77 | 23.75±3.35 |
| | | 2 | 28.81±2.34 | 24.21±2.73 | 10.17±2.00 | 26.30±3.24 | 22.37±2.18 |
| | | 3 | 31.10±2.06 | 21.87±1.80 | 10.62±2.77 | 24.53±2.84 | 25.43±4.67 |
| | | 4 | 37.37±4.06 | 28.06±3.02 | 14.86±1.45 | 32.04±2.29 | 28.26±4.61 |
| | | 5 | 36.22±3.68 | 23.92±1.87 | 12.56±3.31 | 27.28±3.19 | 26.76±4.98 |
| | *Malus floribunda coccinella* | 1 | 31.38±4.27 | 22.55±1.91 | 10.38±2.39 | 25.08±1.16 | 24.94±6.66 |
| | | 2 | 30.73±2.85 | 21.38±1.24 | 9.73±2.55 | 23.79±0.54 | 24.52±6.80 |
| | | 3 | 29.67±2.56 | 20.21±0.90 | 8.48±2.69 | 22.17±1.84 | 22.55±6.32 |
| | | 4 | 40.44±7.35 | 23.73±4.74 | 14.34±3.41 | 28.85±1.89 | 31.64±10.66 |
| | | 5 | 42.70±8.09 | 19.88±3.49 | 16.51±6.85 | 27.51±1.91 | 39.55±17.60 |
| | *Malus floribunda* | 1 | 27.44±0.43 | 18.09±0.45 | 6.84±0.52 | 19.36±0.38 | 20.51±1.70 |
| | | 2 | 28.51±1.48 | 19.36±1.81 | 7.43±0.68 | 20.77±1.81 | 20.85±1.84 |
| | | 3 | 28.10±2.72 | 17.15±0.44 | 5.43±0.24 | 18.39±1.08 | 19.87±4.25 |
| | | 4 | 34.94±2.64 | 24.99±2.81 | 11.08±1.73 | 27.45±3.12 | 23.90±2.38 |
| | | 5 | 37.16±1.90 | 22.32±1.68 | 10.82±1.28 | 25.01±1.46 | 26.00±3.89 |
| Flesh | *Malus evereste* | 1 | 46.79±2.94 | 20.81±4.51 | 10.95±2.30 | 24.06±2.65 | 29.64±10.17 |
| | | 2 | 48.07±3.95 | 18.91±5.10 | 10.91±1.93 | 22.63±3.70 | 32.39±10.82 |
| | | 3 | 45.62±4.62 | 22.58±4.55 | 12.48±2.66 | 26.27±2.71 | 30.19±9.93 |
| | | 4 | 53.48±3.04 | 20.56±3.92 | 19.08±2.48 | 28.65±1.11 | 43.37±9.04 |
| | | 5 | 54.04±4.70 | 19.90±6.92 | 18.77±2.29 | 28.11±3.57 | 45.14±12.78 |
| | *Malus floribunda coccinella* | 1 | 47.79±9.82 | 23.44±9.78 | 6.39±0.82 | 24.57±9.51 | 17.58±5.25 |
| | | 2 | 44.31±8.88 | 25.52±7.23 | 6.34±1.83 | 26.42±7.31 | 14.72±2.45 |
| | | 3 | 45.10±7.31 | 26.09±5.94 | 6.00±1.06 | 26.88±5.85 | 14.08±3.49 |
| | | 4 | 46.07±7.99 | 26.76±6.44 | 11.31±1.40 | 29.18±6.30 | 24.11±4.14 |
| | | 5 | 49.66±8.98 | 23.34±9.87 | 12.38±1.49 | 26.80±9.12 | 31.75±8.67 |
| | *Malus floribunda* | 1 | 38.82±1.59 | 31.69±2.02 | 5.31±1.245 | 32.17±2.21 | 9.32±1.66 |
| | | 2 | 37.87±4.52 | 31.20±1.38 | 6.29±2.79 | 31.95±1.87 | 11.13±4.67 |
| | | 3 | 35.41±4.22 | 30.05±0.47 | 8.53±1.76 | 31.31±0.40 | 15.66±3.34 |
| | | 4 | 35.99±2.09 | 32.82±0.63 | 12.57±0.83 | 35.18±0.89 | 20.81±0.99 |
| | | 5 | 37.60±1.78 | 36.12±1.75 | 14.97±1.30 | 34.18±3.65 | 22.37±0.82 |

*1: August 18, 2: August 24, 3: September 6, 4: September 14, 5: September 21

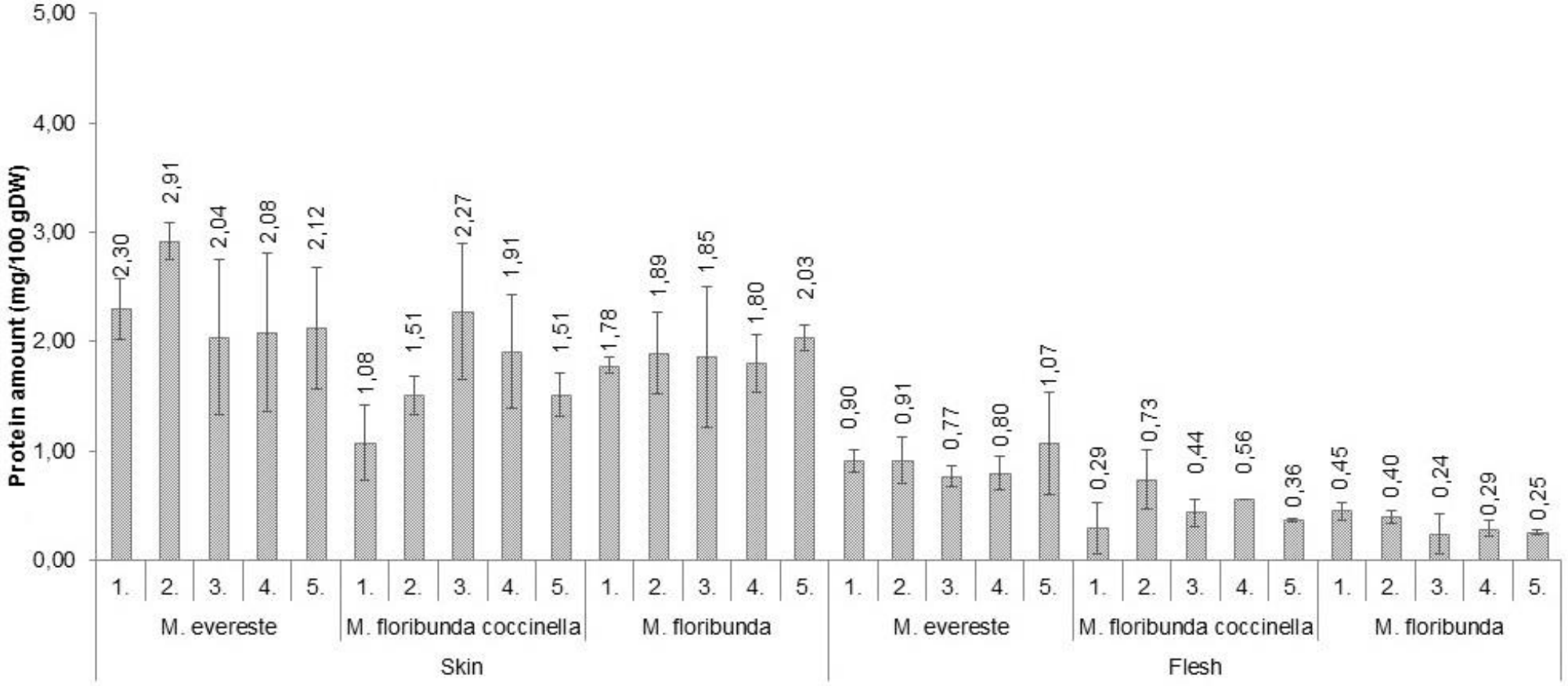


**Figure 1.** Protein amounts of peel and flesh of crab apple species

Fifty-three different proteins which have roles in energy and metabolism, stress response and defense, maturity and senescence, signal transduction, cell structure, protein synthesis were been identified in the apple by Shi and coauthors [25]. They take part in the metabolic pathway including glycolysis, pentose-phosphate pathway, anti-oxidative systems, photosynthesis, and cell wall synthesis, especially during the respiration and during the senescent stages of fruit development. Ten of these proteins synthesize during the late stages of maturity. Generally, an increase followed by a decrease in protein concentration during maturity is seen in apples.

**Sugars and organic acids of crab apples**

Organic acids cannot be overlooked in fruits as it's the major component that determines fruit taste. Additionally, sugar content and profile of fruits play vital roles in determining the taste. The taste of a particular fruit can either increase or decrease the demand of consumers as it's what they generally considered most. During harvesting, it is therefore necessary to consider the right period to harvest a specific fruit to meet the high demand of consumers.

Sugar and organic acid results performed by HPLC are shown in Table 3. The predominant sugar component detected among the species throughout maturity was fructose and at the last stage of maturity its concentration in *M. evereste*, *M. floribunda coccinella*, *M. floribunda* was found to be 6.35±0.78, 4.12±0.28, and 4.29±0.13 g/100 g FW, respectively. Similar to results of this study, in a previous research on common apples fructose has been found to be predominant sugar [26].

While highest glucose amount (1.12±0.08 g/100 g FW) was determined in *M. evereste* at the last stage of maturity, sucrose concentration was highest at the 4$^{th}$ stage of maturity in *M. floribunda*. Constant increases in fructose and glucose during maturity were found in other studies [27-29]. While fructose > glucose > sucrose was the order of increasing in sugar composition in *M. evereste* and *M. floribunda coccinella* species, the order changed in *M. floribunda* as glucose < sucrose < fructose during maturity. Statistically, the effect of the species and maturity stages on all sugars detected in crab apples used in this study were significant ($p<0.01$).

In like manner to our results, constant degradation in sucrose level was reported in damson plum fruit [29]. Starch is accumulated in apple fruits during the first 2-3 months of fruit development and its hydrolysis begins in the later stages of maturity [31,32]. Starch hydrolysis contributes the increases in free sugars and soluble solids content [32]. At the same time during maturity some of the sucrose found in apple is converted to fructose and glucose. Glucose levels increase in accordance with breaking down of starch and decrease due to using in respiration and converting into fructose and sucrose. Generally, fluctuation in the amount of the glucose and increases of fructose and sucrose levels is attributed to these factors [33].

Malic and citric acids were the major organic acids present in the apples. Malic acid has always been in a comfortable dominance so long as organic acids are concerned for Malus spp. fruits. For the organic acid, malic acid was dominant followed by citric throughout maturity in the 3 species. A recent study made by Zhang and coauthors [4] on honeycrisp' apple confirms that the major organic acid in apple fruits is malic acid which increases during maturity.

Although malic acid content increased with maturity in *M. evereste* from 2.02±0.50 to 2.32±0.20 g/100 g FW and *M. floribunda* from 1.48±0.22 to 1.73±0.11 g/100 g FW, it remained approximately constant in the first three stages of maturity and afterwards decreased towards the last stage in *M. floribunda coccinella* species. According to the results of the last maturity stage, the highest malic acid amount was determined in *M. evereste* (2.32±0.20 g/100 g FW) among the species, followed by *M. floribunda* (1.73±0.11 g/100 g FW) and *M. floribunda coccinella* (1.14±0.11 g/100 g FW). Both maturity and species had a statistically significant effect on the accumulation of malic acid in crab apple at levels of $p<0.05$ and $p<0.01$, respectively.

Among the all crab apples, *M. floribunda* had the lowest citric acid amounts in all maturity stages. The order of apples in accordance to their citric acid concentrations was as *M. floribunda* < M*. floribunda coccinella* < *M. evereste*. The differences between the citric acid concentration among the species were statistically significant ($p<0.01$). Although citric acid amounts change throughout the maturity, especially in *M. floribunda* and *M. evereste*, the changes were found to be statistically insignificant ($p>0.05$).

Malate accumulation in fruit is derived from mainly photosynthetic assimilation, or through catabolism of sugars translocated from the leaves. Malate synthesis and degradation are simultaneously occurred in fruits maturity.

**Table 3**. Sugars and organic acids of crab apples according to the species and maturity stage

| Species | Maturity stage* | Sucrose** | Glucose** | Fructose** | Citric acid** | Malic acid** |
|---|---|---|---|---|---|---|
| *Malus evereste* | 1 | 0.34±0.01 | 0.47±0.12 | 3.53±0.85 | 0.51±0.03 | 2.02±0.50 |
| | 2 | 0.41±0.06 | 0.62±0.11 | 4.41±0.43 | 0.53±0.10 | 2.45±0.17 |
| | 3 | 0.50±0.03 | 0.81±0.01 | 5.03±0.14 | 0.48±0.04 | 2.58±0.07 |
| | 4 | 0.58±0.11 | 1.09±0.11 | 5.98±0.47 | 0.59±0.15 | 2.54±0.26 |
| | 5 | 0.56±0.08 | 1.12±0.08 | 6.35±0.78 | 0.60±0.06 | 2.32±0.20 |
| *Malus floribunda coccinella* | 1 | 0.59±0.16 | 0.76±0.16 | 4.71±0.10 | 0.44±0.02 | 2.00±0.15 |
| | 2 | 0.76±0.14 | 0.95±0.11 | 5.20±0.11 | 0.44±0.32 | 2.05±0.07 |
| | 3 | 0.81±0.12 | 1.02±0.03 | 5.55±0.20 | 0.41±0.32 | 2.03±0.02 |
| | 4 | 0.83±0.16 | 0.99±0.17 | 4.92±0.01 | 0.35±0.01 | 1.53±0.38 |
| | 5 | 0.84±0.05 | 0.83±0.06 | 4.12±0.28 | 0.33±0.07 | 1.14±0.11 |
| *Malus floribunda* | 1 | 0.90±0.02 | 0.46±0.04 | 3.50±0.16 | 0.02±0.00 | 1.48±0.22 |
| | 2 | 0.93±0.10 | 0.42±0.04 | 3.55±0.02 | 0.01±0.00 | 1.51±0.15 |
| | 3 | 1.17±0.06 | 0.48±0.02 | 3.85±0.16 | 0.01±0.00 | 1.67±0.27 |
| | 4 | 1.41±0.05 | 0.66±0.11 | 4.29±0.12 | 0.01±0.00 | 1.71±0.10 |
| | 5 | 1.21±0.06 | 0.65±0.01 | 4.29±0.13 | 0.01±0.00 | 1.73±0.11 |

*1: August 18, 2: August 24, 3: September 6, 4: September 14, 5: September 21
** g/100 g FW

On the other hand, the compound is actively metabolized and decreases in malate levels throughout the maturity are arisen from using in catabolisms including the tricarboxylic acid cycle and respiration, gluconeogenesis, amino acid interconversions, ethanol fermentation, and the production of secondary compounds such as phenolic compounds [34]. Malate concentrations in fruits show differences factors such as spices, cultivar and environmental conditions [34].

**Total phenolic, total monomeric anthocyanin and tannin contents**

Total phenolic (TP), total monomeric anthocyanin (TMA) and condensed tannin (CT) contents in the peel and flesh of 3 crab apple species were investigated for 5 different edible maturity stages of picking during maturity. The results are presented in Table 4. Although there were fluctuations in TP, TMA, and CT concentrations between the maturity stages, as a general, decreases in the concentrations of aforementioned compounds in both fractions of all crab apples towards the end of maturity occurred. Except the total phenolic and monomeric anthocyanin contents in the peel, the decreases were statistically significant ($p<0.01$). The highest TP, TMA, and CT values were determined either in the peel and flesh of the *M. evereste* among all varieties. In the peel fraction of the species, TP, CT and TMA contents were highest in *M. evereste* during the 2nd stage of maturity with the values of 152.00±6.28, 57.85±4.60, and 2.93±0.92 mg/g DW and the values fell down to 118.80±9.77, 49.77±4.52, and 1.56±0.31 at the end of the maturity, respectively. The lowest TP and CT contents for the peel were respectively recorded as 31.22±5.18 and 16.49±3.36 mg/g DW at the 5th stage of maturity in *M. floribunda* species. TP value *of M. evereste* was approximately two times higher than *M. floribunda coccinella* and 5 times higher than *M. floribunda* at the all stages of maturity. While the CT values in the flesh fractions of *M. floribunda coccinella* and *M. floribunda* were close to each other, the flesh of *M. evereste* contained two-fold higher CT content than both these species. Concentrations of polyphenolics in the peel fractions of all crab apples were approximately 2,5-3 times higher than those of tannin contents. Species had statistically significant effect ($p< 0.01$) on TP and CT values of the peels and fleshes of the crab apples. However, there were no significant differences between all crab apples in terms of anthocyanins concentration in the peel ($p>0.05$).

Total phenolic and tannin concentrations in the flesh of *M. evereste* and *M. floribunda coccinella* were nearly equal to each other. However, *M. floribunda* flesh contained nearly two-fold higher CT than TP values. Anthocyanin contents of the *M. floribunda*, *M. evereste*, and *M. floribunda coccinella* were at the first stage were 0.95±0.03, 0.54±0.06, and 0.48±0.05 mg/g DW, respectively, and decreased to 0.75±0.09, 0.37±0.05, and 0.30±0.02 mg/g DW at the last stage of maturity. Differences in the TP, CT, and TMA values in the fleshes of three species were statistically significant at the level of $p<0.01$.

Apple contains condensed tannins which are important due to the imparting to flavor as bitterness and astringency and also contribute to the enzymatic browning of damaged tissue and their concentration varies

factors such as cultivar, fraction, maturation, and storage [35]. Lees and coauthors [35] reported that tannin contents of the peels, pulps and seeds of 11 different common apple varieties or types were at the ranges of 14.2-42.9, 1.8-5.9 and 0.7-1.7 mg/g DW. According to their results, the highest tannin value was determined for the peel among the three fractions. Bahukhandi and coauthors [36] notified lower tannin concentrations in the peels (1.37-21.74 mg/g FW) and fleshes (0.75-15.16 mg/g FW) of 20 apple species. Similar to the results of this study, previous studies reported higher tannin values in the peel of apples than their fleshes. When compared to the results of previous studies, peels and especially fleshes of crab apple species used in this study contain higher tannin contents than most of common apple species.

Several other studies on different varieties of apples reported higher TP contents in the peel to the flesh. According to results of Wang and coauthors [37], it could be seen that TP and TMA contents of crab apple species used in this study were considerable higher in both fractions (peel and flesh) than the species of apples used in their study.

**Table 4.** Total phenolic (TP), total monomeric anthocyanin (TMA) and condensed tannin (CT) contents and antioxidant activities of flesh and peel of crab apples

| Fraction | Species | Stage* | TP** | Antioxidant activity | | CT** | TMA ** |
|---|---|---|---|---|---|---|---|
| | | | | DPPH | ABTS | | |
| Peel | *Malus evereste* | 1 | 148.41±12.96 | 36.05±2.96 | 104.42±12.39 | 56.98±5.22 | 2.48±0.49 |
| | | 2 | 152.00± 6.28 | 40.64±2.63 | 110.93±8.45 | 57.85±4.60 | 2.93±0.92 |
| | | 3 | 125.59±7.50 | 33.44±2.85 | 81.36±11.09 | 48.43±3.24 | 1.93±0.42 |
| | | 4 | 131.82±2.95 | 32.33±2.00 | 97.05±7.14 | 51.64±2.96 | 2.34±0.87 |
| | | 5 | 118.80±9.77 | 32.04±0.99 | 92.39±8.10 | 49.77±4.52 | 1.56±0.31 |
| | *Malus floribunda coccinella* | 1 | 76.80±22.01 | 21.65±4.06 | 59.45±20.11 | 24.45±7.96 | 1.45±0.58 |
| | | 2 | 69.03±17.96 | 21.16±3.31 | 50.37±12.49 | 21.90±5.71 | 1.94±0.93 |
| | | 3 | 88.88±22.69 | 21.67±2.13 | 59.07±7.38 | 22.26±5.57 | 2.38±0.87 |
| | | 4 | 100.07±45.31 | 20.72±2.27 | 55.80±10.86 | 22.13±6.14 | 1.88±0.67 |
| | | 5 | 61.57±12.74 | 19.93±3.83 | 47.32±10.99 | 19.06±5.13 | 1.95±0.40 |
| | *Malus floribunda* | 1 | 47.93±4.40 | 17.04±0.69 | 31.58±2.10 | 24.92±1.46 | 2.03±0.15 |
| | | 2 | 39.28±4.00 | 15.45±0.89 | 27.71±1.94 | 23.69±2.84 | 1.90±0.24 |
| | | 3 | 37.63±5.18 | 14.86±1.02 | 25.97±4.39 | 19.47±3.06 | 1.84±0.39 |
| | | 4 | 37.43±6.83 | 13.80±0.82 | 27.38±3.05 | 18.90±2.40 | 1.95±0.71 |
| | | 5 | 31.22±5.18 | 13.14±1.97 | 22.24±1.90 | 16.49±3.36 | 1.51±0.23 |
| Flesh | *Malus evereste* | 1 | 22.50±0.66 | 20.18±0.72 | 64.11±4.81 | 29.19±1.25 | 0.54±0.06 |
| | | 2 | 20.83±1.77 | 18.89±0.63 | 61.72±1.86 | 26.00±0.02 | 0.57±0.05 |
| | | 3 | 17.89±1.57 | 17.20±0.09 | 55.10±1.28 | 24.03±0.34 | 0.50±0.13 |
| | | 4 | 17.54±1.20 | 15.98±0.07 | 49.14±1.95 | 22.65±1.22 | 0.42±0.08 |
| | | 5 | 17.37±1.88 | 16.03±1.49 | 51.99±6.67 | 22.94±2.05 | 0.37±0.05 |
| | *Malus floribunda coccinella* | 1 | 12.00±1.59 | 12.70±1.22 | 35.33±6.25 | 12.59±2.43 | 0.48±0.05 |
| | | 2 | 12.08±1.45 | 11.11±1.47 | 33.14±6.19 | 10.56±2.28 | 0.50±0.10 |
| | | 3 | 9.62±2.05 | 10.04±1.23 | 27.72±6.64 | 8.90±2.03 | 0.35±0.01 |
| | | 4 | 9.21±2.39 | 9.80±1.42 | 27.71±3.45 | 9.12±2.83 | 0.37±0.03 |
| | | 5 | 8.02±1.08 | 9.32±1.17 | 25.01±4.13 | 8.26±1.74 | 0.30±0.02 |
| | *Malus floribunda* | 1 | 4.57±0.35 | 7.12±0.35 | 12.55±1.57 | 11.27±1.34 | 0.95±0.03 |
| | | 2 | 4.36±0.77 | 6.87±0.97 | 10.74±1.78 | 10.18±1.23 | 0.99±0.02 |
| | | 3 | 3.74±0.61 | 5.94±0.44 | 9.44±0.44 | 8.04±0.61 | 1.01±0.05 |
| | | 4 | 4.41±1.50 | 5.37±0.35 | 9.74±1.48 | 7.44±0.64 | 0.99±0.10 |
| | | 5 | 3.05±0.21 | 5.34±0.19 | 7.61±1.68 | 6.61±0.28 | 0.75±0.09 |

*1: August 18, 2: August 24, 3: September 6, 4: September 14, 5: September 21
** mg/g DW
*** mmol TE/100 g DW

Furthermore, similar to the tannin results of the fleshes of *M. evereste* and *M. floribunda*, Bahukhandi and coauthors [36] reported higher CT content in the peel compare to the flesh of 20 apple species grown in India. In a previous study by Takos and coauthors [38], it was reported that condensed tannin concentration in the peel of Cripps Red apple was approximately 80% less in the last stages than that in early stages of fruit maturity. Kondo and coauthors [39] reported in their study that polyphenolic concentration in the peel and fleshes of Fuji, oorin, and redfield apple species decreased towards the maturity. We found much higher anthocyanin values for the fleshes and peels of three crab apple species with respect to those reported by Wang and coauthors [37] who investigated the anthocyanin content of red-fleshed apple. According to Wang and coauthors [37], total monomeric anthocyanin values of different red-fleshed apple varieties were between 29.53- 175.84 mg/100 g FW for peel, and 1.21-55.97 mg/100 g FW for flesh.

## Antioxidant Activities

The antioxidant activities of the peel and flesh of crab apple species were analyzed by DPPH and ABTS assays. The results are as shown in Table 4. Considering the peel of the crab apple species, the highest antioxidant capacity by both of two methods (DPPH and ABTS) was seen in *M. evereste* followed by *M. floribunda coccinella* and the least was *M. floribunda*. According to the results of the peel, the values of DPPH were 36.05±2.96, 21.65±4.06 and 17.04±0.69 mmol TE/100g DW for *M. evereste*, *M. floribunda coccinella* and *M. floribunda* respectively at the first maturity stage. Slight decreases were noticed in the values for all three crab apples at the last stage. Similar to results of DPPH, higher ABTS values were determined at the first stages of maturity than that of last period. ABTS values decreased from 104.42±12.39 to 92.39±8.10 mmol TE/100 g DW in M. evereste, from 59.45±20.11 to 47.32±10.99 mmol TE/100 g DW in M. floribunda coccinella, and from 31.58±2.10 to 22.24±1.90 mmol TE/100 g DW in M. floribunda.

For the flesh, the results of DPPH and ABTS were found to be highest for *M. evereste* and lowest in *M. floribunda*. The highest values for DPPH and ABTS in the flesh were 20.18±0.72 and 64.11±4.81 mmol TE/100 g DW recorded at the first maturity stage in *M. evereste* species while the least values were seen at the 5$^{th}$ maturity stage in *M. floribunda* species as 5.34±0.19 and 7.61±1.68 mmol TE/100 g DW, respectively.

Statistically, the DPPH and ABTS values of both fraction were affected by the variety and maturity stage at levels of $p<0.01$, except the ABTS values of the peel in accordance with the maturity stage. Differences between the ABTS results obtained for peel throughout the maturity were statistically insignificant ($p>0.05$). When taking into consider the results of DPPH and ABTS, antioxidant capacity was higher in the peel than the flesh for the 3 crab apple species.

A study on different apple species which is in support of our results reported that antioxidant activity in the peel exceeds than that in the flesh [36]. Vieira and coauthors [40] found in their study of three apple species similar to our results that total antioxidant capacity (ABTS) was higher in the peel than the flesh. A lot more studies that confirm higher antioxidant activities in the skin to the flesh in different apple cultivars are reported in literature [41,10]. Phenolic compounds are the main antioxidant effective compounds of apples [42,41]. While non-anthocyanin phenolic compounds are accumulated at the early steps of fruit development and anthocyanins are intensely synthesize towards the last stages [41]. Concentrations of total and individual polyphenolics in fruits could change during fruit development. As reported in previous studies, lower phenolic levels have been determined in apples at the late stages of the ripening [43,39]. While concentrations of some phenolics increase, decreases in the levels of others could occur. However, these changes show differences according to factors such as fruit species and cultivars [39]. As a result of decreases in phenolic concentrations towards the last stages of ripening, antioxidant activities of crab apples may be decreased during ripening.

## CONCLUSION

Findings of this study indicates that the crab apples used in this study show differences in terms of the concentrations of sugars, organic acids, anthocyanin, tannin and phenolic compounds. Peels of all species exhibited approximately 2-2.5 fold higher antioxidant activity than their fleshes. The highest amounts of total phenolic and tannin were found in the *M. evereste*. Anthocyanin accumulation was highest in *M. floribunda* among the fleshes of all species. While the antioxidant activity, concentration of phenolic compounds, anthocyanin, tannin, malic acid were decreased towards last stages, increases in the amounts of fructose, glucose, sucrose, soluble solids were observed. Considering the changes in phenolic compounds and antioxidant activity, it can be concluded that the fruits of all species could be harvested at the first two weeks of September.

**Conflicts of Interest:** The authors declare no conflict of interest.

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