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# Phenolic Compounds and Antioxidant Activity of Local Cultivar of Apple (*Malus domestica* Borkh) in East of Turkey

**Gülden Gökşen[1,a,*], Fevzi Keleş[2,b]**

[1]*Department of Food Engineering, Faculty of Engineering, Mersin University, 33110 Mersin, Turkey*
[2]*Department of Food Engineering, Faculty of Agriculture, Atatürk University, 25030 Erzurum, Turkey*
[*]*Corresponding author*

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ABSTRACT

Apples, rich in phenolic compounds, are widely grown in abundance and consumed as fruit which reduces the risk of many illnesses. In this study, physico-chemical properties old apple cultivars from east of Turkey were investigated. Identification and quantification of phenolic compounds of freeze dried fresh apple varieties (fruit, pulp and peel) were analysed by using the HPLC method. Total phenolic content and antioxidant capacity of samples was also found in 25.00-11.63 mg GAE/g DW and 49.86-86.12 µmol TE/g DW. The highest antioxidant activity was identified in the peel of the Kara Sakı apple whereas the lowest was identified in the pulp of Ak Sakı. Varieties of apple differ significantly in terms of ferulic acid and hidroxycinnamic acid. (-)-Epicatechin was found in the peel of Kara Sakı apple at the very most and p- coumaric acid was found in the pulp of Kara Sakı at the very least. Hidroxycinnamic acid was identified only in the peel. It is approved to these cultivars show unique long shelf life (unusual for apples) without any storage condition due to the significantly presence of ferulic acid.

[a] gulgok@mersin.edu.tr https://orcid.org/0000-0002-5432-7936 | [b] gulgok33@gmail.com https://orcid.org/0000-0002-5432-7936



## Introduction

Apple (*Malus domestica* Borkh.), belongs to Rosaceae family, is commonly consumed in nearly all countries because it is a tasty fruit (Çolak and Özoğul, 2020; Boyer and Liu, 2004). It produces approximately 3.7 million tons of apples in Turkey that ranks third in the world apple production (Anonymous, 2019). It has been reported to spread to different gene centers in 7 regions in the world (Abacı and Sevindik, 2014).

Apples are important dietary sources of a wide variety of pyhtochemicals including flavonoids and phenolic compounds that contribute to the colour, taste and flavour (Vondráková et al., 2020). The concentrations of polyphenols in apples are affected by many factors such as apple varieties, growing and storage conditions, sunlight, processing and the tissues of the fruit (Çolak and Özoğul, 2020; Drogoudi et al., 2008; Van der Sluis et al., 2001). So they are strongly associated with reducing the risk of age-related human diseases such as cardiovascular diseases, several types of cancer, immune system decline, asthma, and type 2 diabetes due to the presence of antioxidant compounds protecting the cells against stress factors (Boyer and Liu, 2004; Pirlak et al., 2017; Sevindik et al., 2017). The cultivar is important in controlling the polyphenol composition of apples. Apple cultivars such as Fuji, Catarina, Golden Delicious, Ida Red etc., and parts of apple exhibited differences of the chemical compositions and phenolic profiles. Moreover, the polyphenolic content is higher in the peel compared with the pulp of apples due to mostly localized in the peel (Tsao et al., 2005; Vieira et al., 2011; Du et al., 2020). Many regional cultivars of apples are available to find such as *M. domestica* 'Ak Sakı and Kara Sakı' are the only varieties of apple old cultivated in Erzincan, east of Turkey. The old cultivars have little commercial value because there are many unknown properties by consumers. These cultivars are also well-adapted to the climate and harvested in September. The Ak and Kara Sakı apples have to be stored at room temperature for seven months, to protect their characteristic flavor.

They have long shelf life because of a waxy skin and it is estimated that this layer keeps it until the end of the spring. These cultivars are identified due to colour and taste that Kara Sakı is red-light green; Ak Sakı is red-yellow. These pulps are high acid/sugar ratio so they supply a pleasantly acidulous and aromatic taste. Most fruits are medium in size with an oval shape and the skins are of medium thickness. The pulps of these native apple cultivars are more firm and crisp than others (D'Abrosca et al., 2007).

In this study: i) to evaluate the physico-chemical characteristics of the varieties ii) to determine the total phenolic content and antioxidant capacities at different methods (DPPH and FRAP) of peel, pulp and fruit of apple varieties and compare whether there is any relationship between these results or not iii) The peel, pulp and fruit of apple varieties are extracted to be analyzed by using high performance liquid chromatography, for identification and quantification of phenolic compounds.

## Materials and Methods

Ak and Kara Sakı old cultivars grown in Karatuş, Erzincan in East Turkey (39°35'48.9"N 39°40'45.4"E) and were harvested successively as they ripened from the second week of September. Apple peels, pulps and fruits were dried by freeze-drying (Labconco 117, MO, USA). The lyophilized samples were stored at -20°C.

The pH was measured, using a digital pHmeter (Inolab 720, WTW, Weilheim, Germany). The titratable acidity was determined by titration with 0.1 M sodium hydroxide using phenolphthalein as indicator pH 8.1. Acidity was expressed in percentage of equivalents of malic acid, dry weight basis (% of malic acid) (Anonymous, 1975). The soluble solid content (SSC) was determined using a refractometer and the results were expressed as % Brix at room temperature (Abbè Refractometer, Carl Zeiss, Germany).

Total sugar, reducing sugar and non-reducing sucrose were determined by the Lane-Eynon method. The sugar content was expressed as g/100 g (Cemeroğlu et al., 1992).

For the determination of the total phenolic content a slightly modified method described by Tsao et al. (2005) with Folin-Ciocalteu (FC) reagent was used. Results were expressed as gallic acid equivalent (GAE) in milligrams per gram of dry weight basis (mg/g dry weight – DW in the case of dry weight) from which the total phenolic contents were calculated.

### *Antioxidant Activity*

The ability of the extract to scavenge DPPH radicals were assessed spectrophotometrically (Brand-Williams et al., 1995). The principle of FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, coloured form in the presence of antioxidants and this method was carried out according to Vieira et al. (2011) with some modifications. Trolox solution was used as a reference standard, and all results are expressed in μmoles of Trolox per gram of dry weight (μmol TE/g DW).

### *HPLC Analysis*

The lyophilized samples were finely grinded with a hand blender. 0.5 g of powder so obtained was extracted in 10 mL of methanol and sonicated for 30 min. The mixture was centrifuged at 2500 rpm for 10 min (Hettich Mikro 22R, Buckinghamshire, UK), the supernatant was filtrated through 0.45 μm microfilter. The final filtrate was stored at -20°C before being analyzed polyphenols (Van der Sluis et al., 2001).

The analyses were carried out using a ChrometiSIL 120-5-C18 SH chromatographic column. Analyses of polyphenols were run on a Agillent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) performed using UV–Vis detector.

Elution conditions consisted in 10% acetic acid (solvent A) and acetonitrile (solvent B) gradient at a flow rate of 1.0 mL/min. A linear 20 min solvent gradient from 0 to 21% acetonitrile, with a 10 min hold at the final concentration, was used. The column was returned to the initial solvent composition over 1 min and equilibrated for 10 min before the next injection. Column temperature was at 35°C. The flow rate was 1.0 mL/min and the detection wavelength was between 280 and 350 nm. Phenolic compounds in samples were compared by their retention times and UV spectra for identification with those of commercial standards (Van der Sluis et al., 2001).

### *Statistical Analysis*

The results were expressed as means±standard deviation. For this purpose, SPSS 17.0 for Windows (SPSS Inc., Chicago, USA) software package program was used (two-tailed distribution). All results were carried out in triplicate. Differences at $P<0.05$ were considered to be significant.

(a) (b) (c)

Figure 1. Lyophilized samples (a) fruit, (b) peel, (c) pulp

## Results and Discussion

### *Physico-Chemical Analysis*

The results for the physico-chemical analysis were shown in Table 1. The Ak Sakı had higher values than Kara Sakı. Similar results have been demonstrated for apples of different genotypes harvested in Uşak, Turkey with total solid values between 9.8 and 17.9% (Çolak and Özoğul, 2020). The TSS content was 13.52 (Ak Sakı) and 13.51 (Kara Sakı) °Brix.

Kara Sakı for the titratable acidity had lower had a lower value and pH was higher indicating that Kara Sakı had a more sour taste. The TA of ten apple cultivars grown in Europe ranged from 1.5 to 5.4 g/kg TM (Rop et al., 2011). Abacı and Sevindik (2014) reported that 26 apple cultivars cultivated in Ardahan, Turkey and TA values were varied from 3.25 to 4.19%.

The ash contents of Ak and Kara Sakı were found as 0.2% and 0.28% respectively and Picouet et al. (2009) reported 0.19% for Granny Smith apples.

In addition, the TS content (g /100 g) varied from 12.8 (Ak Sakı) to 10.90 (Kara Sakı); the reducing sugar (RS) of Ak Sakı was 10.98% and Kara Sakı was 10.05%. Kara Sakı had a more sour taste according to data. TS values ranged between 12.82 and 19.15 (g/100 g) in nine apple cultivars harvested in Slovenia (Mikuliț Petkovšek et al., 2009). These cultivars were lower than standard and local apple cultivars (Vieira et al., 2011). The results could be dependent on geographical specific conditions of Erzincan. It is also an indication of the sour flavors due to a less amount of sugar content.

The difference obtained in these physico-chemical parameters could be explained by cultivars, climate conditions, mineral contents of soil, position of fruit and organic growing conditions. The two factors that are associated with the development of the fruit flavors are the ratio of acidity and TSS. In fact, it is also well known that the genotype is the main factor that strongly affects the chemical properties (Vieira et al., 2011). The results were explained that the cultivars with more acidic content which reduces the activity of microorganisms hence stored until late of April preserving quality parameters. In addition, these local cultivars were in existence for many years, and could be considered stable and consumed and grown out of season.

### *Total Phenolic Content and Antioxidant Capacity*

The total phenolic contents and antioxidant capacity of the fruit, pulp, and peel were reported in the freeze-dried of apple cultivars in Table 2.

In the peels, the total phenolic content (TPC) of Kara Sakı peel was higher at 25 mg GAE/g DW than Ak Sakı peel at 19.38 mg GAE/g DW. In the pulps, TPC of Ak Sakı pulp was lower at 11.36 mg GAE/g DW than Kara Sakı pulp at 12.63 mg GAE/g DW. TPC in the fruits were 13.5 and 20.63 mg GAE/g DW for Ak and Kara Sakı apples, respectively. According to results, TPC in the peels were the highest values in other parts of apples.

The eight apple varieties of TPC were reported between 0.78 and 2.01 mg GAE/g DW in the peels. TPC for four common varieties used for applesauce (Rome Beauty, Idared, Cortland and Golden Delicious) in the pulps were varied from 75.7 to 103.2 mg GAE/100g DW (Wolfe et al., 2003). It was published that the fruit of TPC of different cultivars were 46.9 and 112.2 mg GAE/100 g DW, respectively (Abacı and Sevindik, 2014). Our results were higher than this data. Pirlak et al. (2017) found that Galaxy Gala, Scarlet Spur, Fuji, Pink Lady and Granny Smith were determined TPC values between 18.29 and 26.83 mg GAE/g DW.

When the antioxidant activity was measured with the DPPH and FRAP methods, the freeze-dried fruits, pulps and peels under study showed similar values of antioxidant activity.

The freeze-dried peel with higher antioxidant activity corresponded to Kara Sakı peel (µmol TE/g DW) in comparison with fruit parts and varieties.

Our results are superior to those reported by Abacı and Sevindik (2014) that determined peel was as good source of antioxidants. Furthermore, the average of the total antioxidant capacity and total phenolic content of apple peels is over 1.5 and 2 respectively; fold more than the average of apple pulp values. These results showed that had a higher TPC and TAC compared to other studies (Vieira et al., 2011; Stratil et al., 2007; Tsao et al., 2005).

Table 1. Physical-chemical parameters of different apple cultivars

| | Ak Sakı | Kara Sakı |
|---|---|---|
| DW | 14.56±0.11 | 14.29±0.09 |
| TSS | 13.52±0.3 | 13.51±0.01 |
| pH | 3.42±0.02 | 3.36±0.01 |
| TA | 0.24±0.11 | 0.32±0.12 |
| Ash | 0.20±0.02 | 0.28±0.03 |
| RS | 10.98±0.04 | 10.05±0.02 |
| TS | 12.80±0.03 | 10.90±0.01 |

DW, Dry Weight (%); TSS, Total Soluble Solids(°Brix); TA, Titratable Acidity(%); Ash(%); RS, Reducing Sugar(g/100g); TS, Total Sugar(g/100g)

Table 2. Total phenolic (TPC) and antioxidant (TAC) contents of apple varieties

| | | Ak Sakı | Kara Sakı |
|---|---|---|---|
| Fruit | TPC* | 13.51±0.82 | 20.63±1.26 |
| | DPPH** | 65.79±0.94 | 80.57±0.69 |
| | FRAP** | 54.12±1.76 | 60.45±1.03 |
| Pulp | TPC* | 11.36±0.57 | 12.63±0.76 |
| | DPPH** | 60.74±1.11 | 63.19±2.04 |
| | FRAP** | 49.86±0.14 | 51.96±0.26 |
| Peel | TPC* | 19.38±1.96 | 25.00±1.67 |
| | DPPH** | 78.49±1.59 | 86.12±1.84 |
| | FRAP** | 57.36±1.28 | 64.84±1.71 |

*mg GAE/g DW ** µmol TE/g DW Values are expressed as means of three determinations± standard deviation

It was reported as being dependent upon different factors as cultivars, climatic conditions, growing years, agronomic practice and processing (Çolak and Özoğul, 2020; Van der Sluis et al., 2001). The peel was higher than other edible parts of fruit, which could be explained by more exposure to sunlight. In addition, the cultural attitudes to apple varieties against foreign varieties may adapt over time. Ak and Kara Sakı haven't grown orderly in the plant, in the presence of these apple varieties for

home garden so, more phenolic compounds can be synthesized. Due to the plants against stress conditions which are in the form of increasing compounds (Boyer and Liu, 2004; Abacı and Sevindik, 2014).

***Phenolic Compositions***

It was determined the seven phenolic compounds by using HPLC methods. (+)-catechin, (-)-epicatechin, catechol, vanillic acid, p-coumaric acid, ferulic acid and hydroxycinnamic acid were identified and quantified in fruit, pulp and peel in Figure 2.

Retention times of those extracts were matched with standards. The stardards retention times ranged from 6.39 ((+)-catechin) to 22.03 (hydroxycinnamic acid) min (Figure 2).

Table 3 shows comparison of phenolic compounds according apple varieties. Ak and Kara Sakı apples were reported to included almost high amounts of (-)-epicatechin and ferulic acid as important phenolic acid, were the most plentiful compounds. The hydroxycinnamic acid presented only in the peels of samples of Ak and Kara Sakı at 47.37 and 122.86 mg/kg DW, respectively ($P<0.05$). The highest concentrations of (-)-epicatechin (610.2 mg/kg DW) and (+)-catechin (405.57 mg/kg DW), the lowest concentrations of p-coumaric acid (50.21 mg/kg DW) and vanillic acid (124.71 mg/kg DW), were found in Ak Sakı fruit. The peel of Ak Sakı had the greatest polyphenols concentration of (-)-epicatechin (669.78 mg/kg DW) and ferulic acid (610.94 mg/kg DW) among of parts of Ak Sakı (fruit, pulp and peel). The (-)-epicatechin (558.94 mg/kg DW), p-coumaric acid (49.02 mg/kg DW), and ferulic acid (367.75 mg/kg DW) were the lowest concentration and vanillic acid (139.29 mg/kg DW) were the highest concentration in pulp of Ak Sakı compared to other parts. The major polyphenol found was ferulic acid in Kara Sakı peel (1100.3 mg/kg DW) and the fruit (612.24 mg/kg DW) and (-)-epicatechin (517.63 mg/kg DW) in pulp of Kara Sakı, were also reported. These two compounds were detected as the major phenolic compounds in the fruits, pulps and peels.

The fruit, pulp and peel of Kara Sakı contained a low concentration of p-coumaric acid from the other polyphenolic compounds, and were ranged from 46.75 to 61.98 mg/kg DW. The (+)-catechin (239.17 mg/kg DW), catechol (124.73 mg/kg DW) and ferulic acid (368.48 mg/kg DW) were the lowest amounts of phenols in pulp of Kara Sakı among the fruit and peel. Similar results of vanillic acid known as hydroxybenzoic acid were found in fruit and pulp of Kara Sakı at 120.11 and 120.15 mg/kg DW, respectively.

In the peel of apples, the amount of phenol compounds was higher when compared to the pulp. Similar results were reported by Drogoudi et al. (2008) and Vieira et al. (2011). The apple peels were determined to have high contents of phenolic compounds compared to other edible parts of the apple and its juice (Wolfe et al., 2003).

The phenolic composition of 67 varieties of apple cultivars (new and old varieties) was examined that (-)-epicatechin and (+)-catechin were changed 65.9-2760 and 10.1-720 mg/kg DW, respectively (Wojdyło et al., 2008). It is known that the different apple cultivars affect the phenolic compositions and varieties.

According to results of phenolic compounds, the highest in Kara Sakı ferulic acid and hydroxycinnamic acid were detected ($P<0.05$). Hydroxycinnamic acid were found in peels of four apple varieties (Gala, Topaz, Golden Delicious and Florina), and ranged from 0.03 to 0.18 mg/g FW (Kindt et al., 2007). This is confirmed by the study of our results. The ferulic acids were determined in peel (136.7 mg/kg FW) and fruit (119.8 mg/kg FW); p-coumaric acid in peel (523.1 mg/kg FW) and pulp (367.9 mg/kg FW) (Leontowicz et al., 2003). Fruits, pulps and peels of Ak and Kara Sakı were compared to the literature with regard to containing ferulic acid and p-coumaric acid ($P<0.05$).

Some parameters affected the high amount of phenolic compounds, depending on growing, organic conditions for these apples and exposed to various stress factors as diseases and mineral supplements (Mikulit Petkovšek et al., 2007; Vondráková et al., 2020). The phenolic compounds and compositions of Ak and Kara Sakı presented much higher amounts than the others; it might be a considered influence of these parameters.

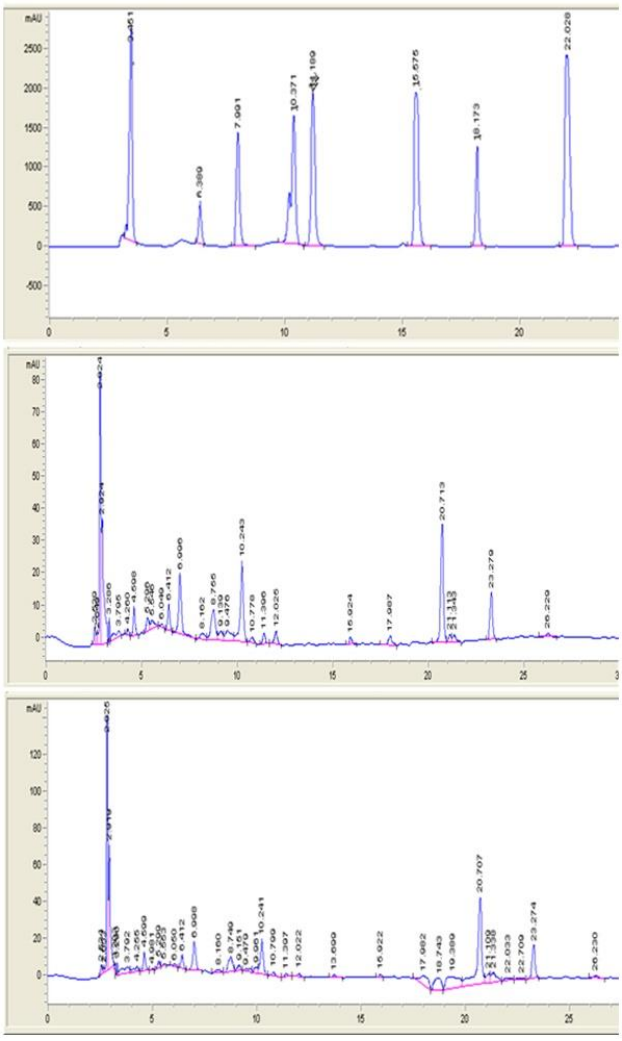

Figure 2. a) HPLC chromatogram of standart phenolic compounds. Peaks identification: 1, gallic acid; 2, (+)-catechin; 3, catechol; 4, (-)-epicatechin; 5, vanillic acid; 6, p-coumaric acid; 7, ferulic acid; 8, hydroxycinnamic acid. b) HPLC chromatogram of phenolic compounds of freeze-dried Ak Sakı and c) Kara Sakı fruits.

Table 3. Comparison of phenolic compounds (ppm) according to apple varieties

| Phenolic compounds | DF | Mean square | F value | P value |
|---|---|---|---|---|
| (+)-catechin | 1 | 6187.11 | 2.73 | 0.150 |
| (-)-epicatechin | 1 | 11.88 | 0.27 | 0.623 |
| catechol | 1 | 304.42 | 1.03 | 0.350 |
| vanilic acids | 1 | 224.99 | 0.61 | 0.464 |
| p-cumaric acids | 1 | 0.33 | 1.62 | 0.250 |
| ferulic acids | 1 | 166168.87 | 5716.47 | 0.000* |
| hydroxycinnamic acids | 1 | 1899.58 | 989.97 | 0.000* |
| TFM | 1 | 63.02 | 5.41 | 0.059 |

*P<0.001

Table 4. Comparison of phenolic compounds (ppm) according to fruit, pulp and peel

| Phenolic compounds | Total square | DF | Mean square | F value | P value |
|---|---|---|---|---|---|
| (+)-catechin | 29583.36 | 2 | 14791.68 | 6.51 | 0.031* |
| (-)-epicatechin | 1534.81 | 2 | 767.40 | 17342 | 0.003* |
| catechol | 62999.19 | 2 | 31499.59 | 106240 | 0.000** |
| vanilic acids | 1655.58 | 2 | 827.91 | 2.25 | 0.187 |
| p-cumaric acids | 353.79 | 2 | 167.79 | 879.36 | 0.000** |
| ferulic acids | 506200.58 | 2 | 253100.29 | 8707.09 | 0.000** |
| hydroxycinnamic acids | 19318.84 | 2 | 9859.42 | 5034.06 | 0.000** |
| TFM | 202.53 | 2 | 101.27 | 8.70 | 0.017* |

*P<0.05 **P<0.001

These included the larger amount of flavan-3-ols ((+)-catechin and (-)-epicatechin) than common apple cultivars in the literature (Le Bourvellec et al., 2011). This situation could be explained in that these have different genotypes. Due to the distribution of the different phenolic compounds in apple pulp and peel they are present in different amounts (Biedrzycka and Amarowicz, 2008; Vondráková et al., 2020).

Table 4 shows comparison of phenolic compounds according to fruit, pulp and peel. The synthesis of flavonoids needs sunlight, so usually were found in the leaves and peels. The flavonoids have two groups, flavonols and flavones, were present extensively parts in exposure of external impacts and green region (Boyer and Liu, 2004). There is a high amount of hydroxycinnamic acids and flavanols (catechin and epicatechin) in apple cultivars, is these are used as parameters of resistance (Drogoudi et al., 2008; Vondráková et al., 2020).

Phenolic derivatives can be oxidized and reacted with proteins, and thus there is a loss of enzyme functions and limits viability of attackers. It could be demonstrable that hydroxycinnamic acids were present only in peels (Wolfe et al., 2003).

It is known that the psycho-chemical contents and polyphenolic compounds of apple affect the sensory quality (Boyer and Liu, 2004). There are many volatile polyphenols such as hydroxycinnamic acids that contribute flavor vanillic acid is described as flavoring agent. The high amount of vanillic acid was found in these cultivars compared to the other standard cultivars of apples, were caused by ferulic acid. The vanillic acid is catabolism product of ferulic acid (Mathew and Abraham, 2004). The vanillic acid in pulps and fruits are closer to each other, but was higher than peels. The vanillic acid in Ak and Kara Sakı pulps presented the highest concentration; the results may be reason for these apples' distinctive taste and aroma.

Ferulic acid is located in cell walls of plants that play an important role in protecting plants against pathogen attacks (Mathew and Abraham, 2004). These apples are grown without use pesticide and the more amount of ferulic acid as secondary metabolite are synthesized due to exhibited the defense mechanisms against pathogens. Resulting of this is constituted difference in the distribution of phenolic compounds.

## Conclusions

This study highlighted the multipurpose properties of Ak and Kara Sakı that were shown the regional cultivars of apples. Ak Sakı had higher acidity and lower sugar content than Kara Sakı that was the least sweet. Furthermore, these cultivars are generally sourish when compared to cultivars cited in literature. Kara Sakı (fruit, pulp and peel) had a higher TPC and TAC than Ak Sakı but the peel of these cultivars indicated that they were the richest comparing to other tissues. For this reason, fruit should be consumed with peel that has healthy protective metabolites. The some phenolic compounds were the highest such as (+)-catechin in fruit, vanillic acids in pulp and ferulic acids in peel between tissues of Ak and Kara Sakı in addition to hydroxycinnamic acids which were determined only in peel of these cultivars. It was particularly demonstrated that these apples had the highest phenolic compounds. The apple cultivars contained significantly amount of ferulic acids. In terms of health, synthetic ferulic acids have noteworthy impact on the treatment of diseases such as cancer, infertility and Alzheimers.

Finally, it informs consumers about these cultivars which should be grown and consumed due to their very valuable nutritional qualities. It is contemplated to be a basis for further in-vitro research on Ak Sakı and Kara Sakı.

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