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Sugar and Biochemical Composition of Some Apple (*Malus* × *domestica* Borkh.) Cultivar Grown in the Middle Black Sea Region

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Abstract: Due to its different ecological conditions, Türkiye is one of the rare countries where the most of fruit species have spread widely throughout the country, both economically and naturally. In this study on apple, which is one of the important fruit species with economic importance, was aimed to determine the color characteristics, biochemical and sugar contents of 4 different commercial apple cultivars grown in the ecological conditions of Çarşamba district of Samsun province. The material of the study consisted of 6-7 years (2016 and 2017) old trees belonging to Granny Smith, Fuji, Galaxy Gala, and Royal Gala cultivars grafted on M9 clone rootstock. Wide variations occurred in all parameters evaluated in the study among the cultivars. The fructose content of the cultivars was from 14.22 (Royal Gala) to 34.40 g 100 ml^{-1} (Fuji), glucose content was from 1.30 (Galaxy Gala) to 4.34 g 100 ml^{-1} (Fuji), and sucrose content was varied from 2.01 (Fuji) to 4.52 g 100 ml^{-1} (Granny Smith). The highest value in total sugar content was determined in Fuji cultivar with 40.75 g 100 ml^{-1}. The highest total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP) were 423.7 mg 100 g^{-1} (Granny Smith), 96.1 mg 100 g^{-1} (Fuji), 250.8 µmol 100 g^{-1} (Granny Smith) and 952.9 µmol 100 g^{-1} (Granny Smith), respectively. In the principal component analysis results, the first component was associated with a*, b*, hue angle, total phenolics, total flavonoids and antioxidant activity (DPPH and FRAP) and explained 59.4% of the total variation. The second component explained 36.4% of the total variation of the obtained data and was determined to be related to L*, chroma, glucose, sucrose, fructose, and total sugar properties. As a result, Granny Smith cultivar gave remarkable results in terms of biochemical properties and Fuji cultivar in terms of sugar content. It is thought that the results obtained may guide the researches to be made about apples in this region.

Keywords: Antioxidant, cultivar, apple, phenolic, fructose

Orta Karadeniz Bölgesinde Yetiştirilen Bazı Elma (*Malus* × *domestica* Borkh.) Çeşitlerinin Şeker ve Biyokimyasal İçerikleri

Öz: Türkiye, sahip olduğu farklı ekolojik koşullar nedeniyle çoğu meyve türünün ülke genelinde hem ekonomik hem de doğal olarak yayılış gösterdiği ender ülkelerden biridir. Ekonomik öneme sahip önemli meyve türlerinden biri olan elma üzerine yapılan bu çalışmada Samsun ili Çarşamba ilçesi ekolojik koşullarında yetiştirilen 4 farklı ticari elma çeşidinin renk özellikleri, biyokimyasal ve şeker içeriklerinin belirlenmesi amaçlanmıştır. Çalışmanın materyalini M9 klon anacı üzerine aşılı Granny Smith, Fuji, Galaxy Gala ve Royal Gala çeşitlerine ait 6-7 (2016 ve 2017) yaşındaki ağaçlar oluşturmuştur. Çeşitler arasında incelenen bütün parametrelerde geniş varyasyonlar oluşmuştur. Çeşitlerin fruktoz içeriği 14.22 (Royal Gala)-34.40 g 100 ml^{-1} (Fuji), glikoz içeriği 1.30 (Galaxy Gala)-4.34 g 100 ml^{-1} (Fuji) ve sukroz içeriği 2.01 (Fuji)-4.52 g 100 ml^{-1} (Granny Smith) arasında değişim göstermiştir. Toplam şeker içeriğinde en yüksek değer 40.75 g 100 ml^{-1} ile Fuji çeşidinde belirlenmiştir. En yüksek toplam fenolik, toplam flavonoid ve antioksidan aktivitesi (DPPH ve FRAP) ise sırasıyla 423.7 mg 100 g^{-1} (Granny Smith), 96.1 mg 100 g^{-1} (Fuji), 250.8 µmol 100 g^{-1} (Granny Smith) ve 952.9 µmol 100 g^{-1} (Granny Smith) olarak tespit edilmiştir. Temel bileşen analizi sonuçlarına göre, 1. bileşen a*, b*, hue açısı, toplam fenolik, toplam flavonoid ve antioksidan aktivite (DPPH ve FRAP) ile ilişkili olup, toplam varyasyonun %59.4'ünü açıklamıştır. 2. bileşen ise elde edilen verilerin toplam varyasyonunun %36.4'ünü açıklamış ve L*, kroma, glikoz, sukroz, fruktoz ve toplam şeker özellikleri ile ilişkili olduğu belirlenmiştir. Sonuç olarak, biyokimyasal özellikler bakımından Granny Smith, şeker içerikleri bakımından ise Fuji çeşidi kayda değer sonuçlar vermiştir. Elde edilen sonuçların bu bölgede elma ile ilgili yapılacak araştırmalara yol gösterici nitelikte olabileceği düşünülmektedir.

Anahtar kelimeler: Antioksidan, çeşit, elma, fenolik, fruktoz

1. Introduction

Türkiye is considered among the countries with high agricultural potential due to its different geographical and ecological conditions (Ercisli, 2004). Apple, which is among these agricultural products, is grown throughout the country thanks to its wide adaptability (Yaman et al., 2021). A better understanding of the place and importance of apple fruit in human health and nutrition by the wider public (Khalil et al., 2019; Macit et al., 2021) has made apple cultivation and trade gain an important commercial dimension in the world and in Türkiye. While world apple production was 71,187,919 tons in 2010, it reached 86,442,716 tons in the 10-year production season, showing an increase of more than 20%. Türkiye's apple production reached 4,300,486 tons in 2020 with an increase of 60.45% from 2,600.00 tons in 2010, and it had an increase of 40% more than the proportional increase in world apple production. With this amount of production, Türkiye ranks 3rd after China and the USA (FAO, 2022).

In different studies conducted in recent years, specific biochemicals in fruit species including apple and their antioxidant effects on human health have been expressed positively (Uyar et al., 2013; Kasnak and Palamutoglu, 2015; Kolac et al., 2017; Balta et al., 2022). With its rich chemical content, apples reduce the risks of cardiovascular diseases, asthma and diabetes caused by oxidative stress. In laboratory experiments, it has been found that it has very strong antioxidant activity, inhibits cancer cell proliferation, and reduces fat oxidation and cholesterol (Lam et al., 2008). It has been determined that among all the commonly consumed fruit, it has the highest antioxidant activity after blueberry (Boyer and Liu, 2004).

Apple is a fruit specie, which rich in sugars, organic acids, phenolic compounds, and antioxidants. A significant source of dietary polyphenols, apples make up an important part of the human diet (Wu et al., 2007; Lata and Tomala, 2007). The positive effects of apples on human health have been reported in many studies (Vieria et al., 2011; Gundogdu et al., 2018; Celik et al., 2018; Balta et al., 2022). These positive effects of apple on human health are due to its antioxidants (Di Pietro et al. 2007). It has been stated that the bioactive compounds of apples vary depending on the cultivar, ecological conditions, maturity of the fruit and even fruit season (Lata, 2007; Drogoudi et al., 2008).

This study was aimed to determine the color characteristics, biochemical and sugar contents of 4 different commercial apple cultivars (Granny Smith, Fuji, Royal Gala, and Galaxy Gala) grown in the ecological conditions of Çarşamba county of Samsun province.

2. Materials and Methods

2.1. Materials

The research was conducted in 2016 and 2017 in the dwarf apple orchard established in 2011 in the town of Çarşamba, Samsun, which is in the Middle Black Sea region. The material of the study consisted of fruit of Granny Smith, Fuji, Galaxy Gala, and Royal Gala cultivars grafted on M9 clone rootstock.

The orchard was established at distances of 1.1 × 4.0 m and the central leader training system was applied to the trees. During the research, all cultural and technical applications (irrigation, fertilization, pruning, etc.) were performed regularly in the orchard.

2.2. Methods

The research was established according to the randomized design with 4 cultivars, 3 replications, and 5 trees in each replication. The harvesting process was carried out by considering the number of days from full flowering to harvest, fruit skin color, flesh firmness and starch index in the examined cultivars. During the harvest period, 20 fruit were collected from each of the 15 trees of each cultivar. Color characteristics, sugar contents and biochemical properties were investigated in harvested fruit.

2.2.1. Color characteristics

Fruit skin color was determined in terms of L*, a*, b*, chroma and hue angle using a colorimeter (Minolta, CR-400, Tokyo, Japan) (McGuire, 1992).

2.2.2. Sugar contents

To determine the sugar content, a slice from 10 fruit in each replication was sliced with a stainless knife and squeezed in a juicer (K 1583 SMS, Arcelik, Türkiye) to extract the juice. Sugar contents of the obtained fruit juices were determined using a reflectometer (RQFlex, Plus 10, Merck, Germany) device using glucose, sucrose and total sugar test kits. The readings are expressed as g 100 ml^{-1}. Glucose, sucrose, fructose, and total sugar were determined as sugar contents (Balta et al., 2021).

2.2.3. Biochemical properties

Total phenolics, total flavonoids and antioxidant activity [according to DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (The Ferric Reducing Ability of Plasma) assays] were determined as biochemical properties. These properties were determined using a spectrophotometer (UVmini-1240, Shimadzu, Japan). Total phenolics were detected according to the method defined by Aglar et al. (2019). Total flavonoids were measured according to the method defined by Zhishen et al. (1999). Total phenolics and flavonoids were expressed as mg GAE 100 g fw^{-1} and mg QE 100 g fw^{-1}, respectively.

Antioxidant activity was detected according to two different methods of DPPH assay described by Blois (1958) and FRAP assay defined by Benzie and Strain (1996). Antioxidant activity was expressed as μmol torolox 100 g fw^{-1}.

2.3. Statistical analysis

The data were evaluated by using Minitab 17 and JMP 10 (trial) statistical package programs. The difference between the means was determined at the 5% significance level using the Tukey multiple comparison method. Principle component and component biplot analysis were carried out using the color characteristics, sugar and biochemical content of the examined cultivars.

3. Results and Discussion

Fruit color is a significant quality parameter in apple that affects consumer's preference and the market value of the apples (Iglesias et al. 2008). Significant differences were found in color properties among the investigated apple cultivars ($p<0.05$). In the present study, Royal Gala cultivar produced higher results with L* value of 67.34 compared to other cultivars. The Galaxy Gala cultivar had the highest a* value with 19.77. On the other hand, Granny Smith was the most prominent cultivar in b*, chroma and hue angle values, and these values were determined as 40.19, 46.60 and 120.19, respectively (Table 1). Henriquez et al. (2010) used Granny Smith, Fuji and Royal Gala apple cultivars in a study. They reported that Granny Smith cultivar stands out in terms of L*, b*, chroma and hue angle values (63.8, 39.9, 44.3 and 115.6, respectively), and Royal Gala cultivar has the highest value in terms of a* value (24.6). In another study, the highest L* and b* values (58.0 and 32.0, respectively) were determined in Granny Smith cultivar, whereas the highest a* value (18.0) was determined in Fuji cultivar (Drogoudi et al., 2008). In the present research, the highest b*, chroma and hue angle values were determined in Granny Smith, while the highest L* and a* values were detected in Royal Gala and Galaxy Gala. It is seen that the results obtained in terms of color properties are compatible with the findings of researchers in the literature.

Table 1. Color characteristics of investigated apple cultivars
Çizelge1. *İncelenen elma çeşitlerinin renk özellikleri*

Cultivars	L*	a*	b*	Chroma	Hue angle
Granny Smith	63.67 b*	-23.48 c	40.19 a	46.60 a	120.19 a
Fuji	59.89 c	7.34 b	35.03 b	35.79 c	78.17 b
Royal Gala	67.34 a	17.11 a	32.62 c	42.23 b	66.62 c
Galaxy Gala	65.51 ab	19.77 a	32.22 c	41.97 b	61.06 d

* There is no difference among the averages shown with the same letter ($p<0.05$)

Soluble sugars are important components that affect the taste, aroma, and sensory quality of the fruit. The main soluble sugars in apples are fructose, sucrose, and glucose. Fructose, sucrose, and glucose differ significantly in sweetness in fruit (Borsani et al., 2009). Significant differences were found between the investigated apple cultivars in terms of sugar contents ($p<0.05$). The highest fructose and glucose content in the examined apple cultivars were determined in Fuji cultivar (34.40 and 4.34 g 100 ml^{-1}, respectively), while the lowest in Royal Gala and Galaxy Gala cultivars (14.22, 17.13 g 100 ml^{-1} and 1.39, 1.30 g 100 ml^{-1}, respectively) were determined. The highest sucrose content was determined in Granny Smith cultivar with 4.52 g 100 ml^{-1}, and the lowest in Fuji cultivar with 2.01 g 100 ml^{-1}. The total sugar content of these three sugar compositions varied between 18.74 and 40.75 g 100 ml^{-1} (Table 2). In a study, fructose, glucose, and sucrose contents in Granny Smith cultivar were 4.26, 2.66 and 2.09 g 100 ml^{-1}, respectively; it was reported as 6.45, 3.84 and 2.33 g 100 ml^{-1} in Fuji cultivar, respectively (Wu et al., 2007). In another study conducted on different apple cultivars, the sugar contents changed as follows; fructose content 0.11 to 5.85 g 100 ml^{-1}, glucose content 0.33 to 5.16 g 100 ml^{-1}, sucrose content 0.27 to 13.9 g 100 ml^{-1} and total sugar content 4.6 to 18.5 g 100 ml^{-1} (Ma et al., 2015). In the present research, the findings obtained in terms of sugar contents were generally like the findings of the researchers, while the fructose content was found to be high. Different researchers have reported that apples are rich in fructose content and make up 44-75% of total sugar (Wu et al., 2007; Zhu et al., 2013). The major sugar content of the examined apple cultivars was determined as fructose. It is thought that the differences in fructose content may be due to the cultivar, ecological conditions, and the maturity of the fruit.

Table 2. Sugar contents of investigated apple cultivars
Çizelge 2. *İncelenen elma çeşitlerinin şeker içerikleri*

Cultivars	Fructose (g 100 ml^{-1})	Glucose (g 100 ml^{-1})	Sucrose (g 100 ml^{-1})	Total sugar (g 100 ml^{-1})
Granny Smith	22.39 b*	2.58 b	4.52 a	29.49 b
Fuji	34.40 a	4.34 a	2.01 c	40.75 a
Royal Gala	14.22 c	1.39 c	3.01 b	18.74 c
Galaxy Gala	17.13 c	1.30 c	3.23 b	21.30 c

* There is no difference among the averages shown with the same letter (p<0.05)

Phenolic compounds constitute an important part of secondary metabolites and together make important contributions to antioxidants that promote human health. Apple is among the fruit species rich in phenolic compounds. Thanks to its phenolic compounds, the apple plays an important role in the prevention of many diseases such as cholesterol, heart diseases, cancer, and asthma (Da Silva, 2003; Wardlaw et al., 2004). Significant differences were detected among the investigated apple cultivars in total phenolics ($p<0.05$). The total phenolics of investigated apple cultivars were determined from 125.9 (Galaxy Gala) to 423.7 mg 100 g^{-1} (Granny Smith) (Table 3). Different researchers reported the total phenolic between 125 to 200 mg 100 g^{-1} in Granny Smith cultivar, 106.6 to 150.0 mg 100 g^{-1} in Fuji cultivar, and 125 to 190 mg 100 g^{-1} in Royal Gala cultivar (Henriquez et al., 2010; Quitral et al., 2013; Nedic-Tiban et al., 2017). Henriquez et al. (2010) and Quitral et al. (2013) determined the highest total phenolics in Granny Smith cultivar, while Nedic-Tiban et al. (2017) reported the highest in Fuji cultivar. In the present research, the highest total phenolics were detected in Granny Smith cultivar. It has been reported that the total phenolics in apples are affected by climate and soil characteristics, the maturity of the fruit, and cultural and technical applications (McGhie et al., 2005; Vieira et al., 2011; Yoon et al., 2020).

Table 3. Total phenolic, total flavonoids and antioxidant activity of investigated apple cultivars
Çizelge 3. *İncelenen elma çeşitlerinin toplam fenolik, toplam flavonoid ve antioksidan aktivitesi*

Cultivars	Total phenolics (mg 100 g^{-1})	Total flavonoids (mg 100 g^{-1})	Antioxidant activity (µmol 100 g^{-1})	
			DPPH	FRAP
Granny Smith	423.7 a*	91.5 a	250.8 a	952.9 a
Fuji	232.1 b	96.1 a	199.8 b	516.0 b
Royal Gala	188.3 c	82.7 b	168.7 c	408.1 c
Galaxy Gala	125.9 d	60.6 c	99.3 d	229.9 d

* There is no difference among the averages shown with the same letter (p<0.05)

Significant differences were detected among the investigated apple cultivars in total flavonoids ($p<0.05$). The highest total flavonoids were determined in Fuji cultivar (96.1 mg 100 g^{-1}) and the lowest in Galaxy Gala (60.6 mg 100 g^{-1}) cultivar. Granny Smith cultivar was in the same group as Fuji cultivar, which has the highest value in total flavonoids (Table 3). Lata et al. (2005) reported the highest total flavonoids in Granny Smith (102.8 mg 100 g^{-1}) cultivar and the lowest in Gala (27.0 mg 100 g^{-1}) cultivar in their study with different apple cultivars. The findings obtained in terms of total flavonoids are largely like the findings of the researchers.

Table 4. Principle component analysis of color characteristics, sugar and biochemical compositions of investigated apple cultivars
Çizelge 4. *İncelenen elma çeşitlerinin renk özellikleri, şeker ve biyokimyasal içeriklerinin temel bileşen analizleri*

Variable	Components	
	PC 1	PC 2
L*	-0.60958	**0.74223**
a*	**-0.92682**	-0.35832
b*	**0.95038**	0.28687
Chroma	0.19533	**0.97782**
Hue angle	**0.92542**	0.36990
Total phenolics	**0.93767**	0.34599
Total flavonoids	**0.81340**	-0.33085
DPPH	**0.95004**	0.07774
FRAP	**0.94564**	0.31714
Glucose	0.65821	**-0.75254**
Sucrose	0.36575	**0.90658**
Fructose	0.58738	**-0.79154**
Total sugar	0.66986	**-0.72550**
Eigenvalue	7.7	4.7
% of variance	59.4	36.4
Cumulative %	59.4	95.8

Factor loading ≥ |0.72| are marked in bold

Antioxidants play an important role in reducing the risk of diseases caused by reactive oxygen species (Valko et al., 2007). It has been reported that some antioxidants present in fruit are effective in preventing heart diseases, cancer, Alzheimer's disease, and arthritis (Kaur and Kapoor, 2001; Boyer and Liu, 2004; Ozdemir et al., 2022). The difference between the examined apple cultivars in terms of antioxidant activity was significant ($p<0.05$). The antioxidant activity of the cultivars was evaluated by both DPPH and FRAP methods, and the lowest data in both methods were found in Galaxy Gala cultivar (99.3 and 229.9 µmol 100 g^{-1}, respectively), while the highest results were found in Granny Smith cultivar (250.8 and 952.9 µmol 100 g^{-1}, respectively) were determined (Table 3). Like the results of the present study, Nedic-Tiban et al. (2017) reported the highest antioxidant activity in Granny Smith (2900 µmol 100 g^{-1}) cultivar and the lowest in Gala (1850 µmol 100 g^{-1}) cultivar according to the DPPH assay. In contrast, Henriquez et al. (2010) reported the highest antioxidant activity in Granny Smith (2934 µmol 100 g^{-}

1) cultivar and the lowest in Fuji (2243 µmol 100 g^{-1}) cultivar according to the FRAP assay. Again, Ozturk et al. (2017), in their research with Granny Smith, Mondial Gala and Red Chief apple cultivars, reported the highest antioxidant activity in Mondial Gala (309.1 and 285.3 µmol 100 g^{-1}, respectively) according to DPPH and ABTS assays. It has been reported that antioxidant activity in apples is affected by the cultivar (Lata et al., 2005; Yoon et al., 2020), ecological conditions, fruit maturity and growing season (McGhie et al., 2005).

Thirteen properties were used for principal component analysis. Out of the 13 components, first two components explained 95.8% of total variation in data. The first component was highly correlated with a*, b*, hue angle, total phenolics and total flavonoids, antioxidant activity (DPPH and FRAP) and explained 59.4% of the total variation. The second component was highly correlated with L*, chroma, glucose, sucrose, fructose, and total sugar. This component explained 36.4% of the total variation (Table 4). When evaluated based on cultivars, it was found that Granny Smith was distinguished from other cultivars by having higher total phenolics, antioxidant activity (FRAP and DPPH assays), sucrose, b*, chroma and hue angle values, while Fuji was distinguished by its higher total flavonoids, fructose, glucose, and total sugar values. Galaxy Gala and Royal Gala attracted attention with their higher with L* and a* color characteristics (Figure 1).

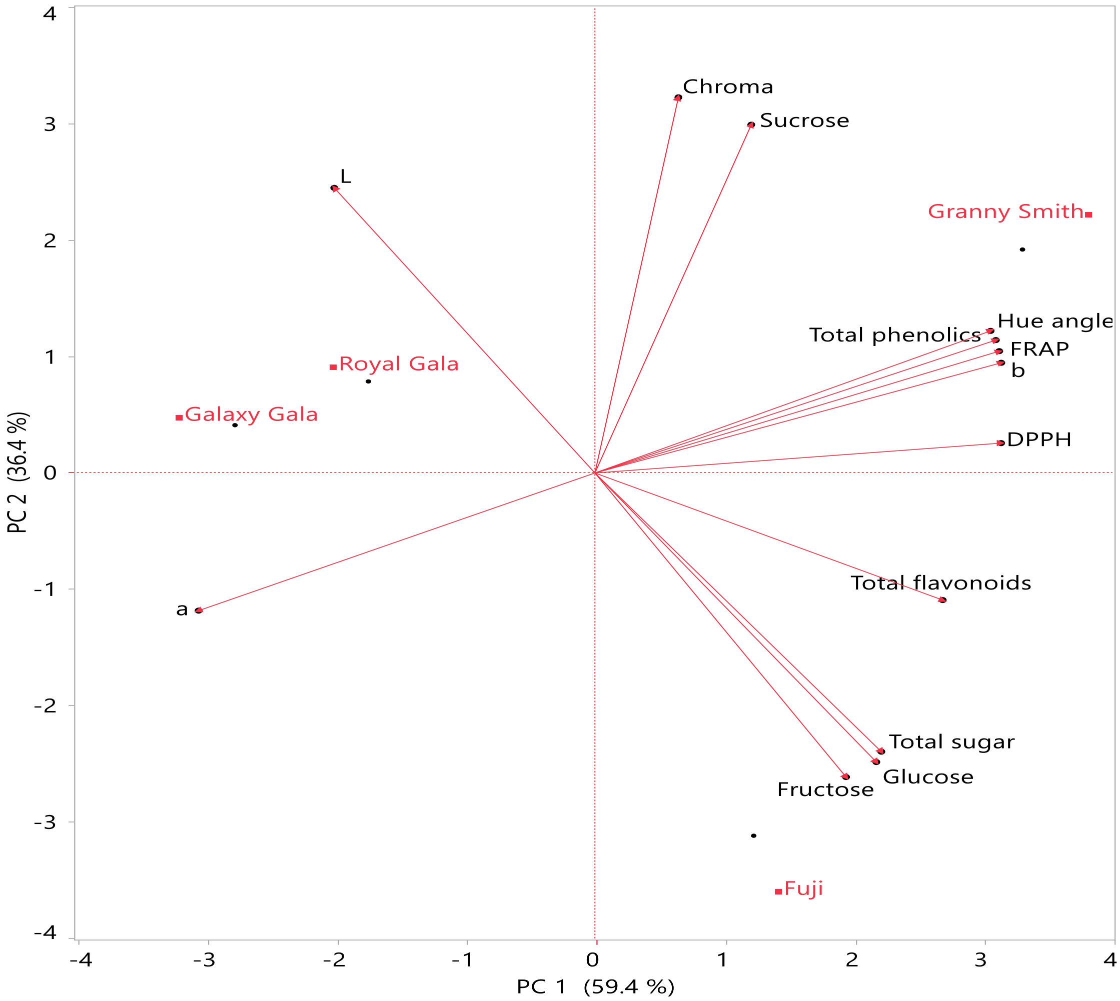

Figure 1. Component plot of the two principal components (PC1 and PC2) in the investigated apple cultivars based on color characteristics, sugar and biochemical compositions

Şekil 1. *Renk özellikleri, şeker ve biyokimyasal içeriklerine bağlı olarak incelenen elma çeşitlerinde iki temel bileşenin (PC1 ve PC2) bileşen grafiği*

4. Conclusion

Granny Smith cultivar stood out compared to other cultivars in total phenolics and antioxidant activity, and Fuji cultivar had higher values in sugar content. In terms of color characteristics, when compared with the findings of the researchers, it is seen that the examined cultivars have unique color characteristics in the ecology of the region. It is anticipated that the results of the study will provide guiding information to both producers and researchers in new studies to be carried out with apple species in the region. In addition, it is thought that producers can use this information for production and marketing purposes.

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