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Fruit Quality Parameters, Sugars, Vitamin C, Antioxidant Activity, Organic Acids, and Phenolic Compounds for a New Endemic Apple Variety, "Long Apple"

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Abstract: The aim of this study was to determine the quality characteristics and bioactive components of the local variety "Long Apple". Although it is a very delicious and popular old apple variety, the knowledge about "Long Apple" is very insufficient. In this study, fruit quality parameters, organic acids, vitamin C, sugar components, phenolic compounds, and antioxidant capacity of the endemic apple "Long Apple" were determined as follows: chlorogenic acid, catechin, syringic acid, and o—coumaric acids, 117.68, 35.11, 22.71, and 15.54 mg kg^{-1}fw, respectively; vitamin c 135.67 mg L^{-1}, total sugar 196.29 g L^{-1}; malic, succinic, and citric acid 10.50, 2.88, and 2.13 g $100g^{-1}$, respectively; fruit weight 139.11 g, hardness 8.27 kg cm^{-2}, shape index 1.42, and soluble solid content 16.70%; antioxidant capacity 3.30 µmol g^{-1} TE were the highest. Except for fruit size and shape, the "Long Apple" genotypes outperformed Starking Delicious based on quality parameters and bioactive properties. The genotypes of "Long Apple" are separated by different parameters, but the genotypes 36K093, 36K094, and 36K106 are more prominent than others. In addition to its health benefits, the "Long Apple" has valuable properties for researchers. Therefore, increased research on "Long Apple" has been recommended.

Keywords: Apple genetic resources; genetic diversity; bioactive components; Kağızman; clonal selection; *Malus domestica* Borkh

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1. Introduction

Apples (*Malus domestica* Borkh.) are, after citrus fruits and bananas, the most important fruit produced in the world. With their high adaptability, apples can be economically cultivated under different environmental conditions, from high altitudes to subtropical regions [1,2]. The fact that apples can be dried and processed into many products such as fruit juice, jam, etc., their high genetic variation, excellent taste, and flavour, and their ability to be consumed fresh throughout the year have given apples high economic value [3,4]. Approximately 93 million tons of apples are produced in the world, of which 4.5 million tons are produced in Türkiye, the second largest apple producing country in the world after China [5]. Türkiye is also an important country in terms of biodiversity due to its location at the intersection of different gene centres, and it is especially important to preserve many local apple genotypes that are facing disappearance [3]. Anatolia has a complex geological structure and special climatic conditions from sea level to altitudes above 3000 m. These characteristics make it home to a rich flora and a wide variety of species. As the homeland of many horticultural plants, Anatolia, which has a great number of apple varieties, may be one of the origins and natural spreading areas of apples. Türkiye's wide biodiversity richness emphasizes its global importance [6–8].

Apple fruit, which contain many sugars, vitamins, organic acids, phenolic components, and other antioxidants, is a popular and beneficial fruit for human health and represent a significant part of the daily diet of much of the world [9,10]. Phenological and morphological properties, phenolic content, sugars, vitamins, and antioxidants in apples may vary according to genetic feature of cultivars, climatic conditions, tissue type, phenological stage, cultural practices, orchard management, and abiotic–biotic stress [11–13]. Recent studies have revealed that bioactive compounds in apples show significant differences, but the major contributor is associated with genetic variability between different varieties [14]. In recent years, the use of the same commercial apple cultivars as the source for apple breeding efforts has resulted in severely limiting the genetic diversity of cultivated apples. For example, Jonathan, Cox Orange, Macintosh, Red Delicious, and Golden Delicious apple cultivars have been reported to be the main parents of 281 cultivated apples that are still being cultivated [15]. The decreasing genetic diversity, which has accelerated in recent years, has led to limitations in the nutritional value and processing capacity of fruit species. Therefore, researchers are interested in new apple genotypes with unique and originally characterized traits to be included in new breeding efforts [16]. As a result of the dependence of apple cultivation on limited commercial varieties, old and local varieties have almost disappeared, whereas local varieties were generally higher in organic acids, sugar content, and total phenolic matter content than commercial varieties [13,17].

Phenolics, sugars, vitamins, antioxidant capacity, and other pomological characters have been extensively characterized in such commercially major cultivars as "Red Delicious", "Golden Delicious", "Fuji", and "Granny Smith", but little data are available for cultivars native to a small production area such as "Long Apple". The "Long Apple" variety is planted and produced only in the Kağızman district [18], which has a microclimate that is relatively temperate compared to the colder Eastern Anatolian climatic conditions. In addition to its ellipsoid and oblong fruit shape combined with its red blush colour that distinguishes it from all other apples, it is locally known for its very prominent features such as easy propagation, high fruit setting rate, medium growth power, semi-upright tree character, keeping fresh for months under ordinary storage conditions, and being resistant to packaging and transportation. Unfortunately, there are almost no scientific studies on these characters of Kağızman "Long Apple". This research focused on the analysis of the physiochemical characteristics of the "Long Apple" cultivar, an endemic, old, and local apple cultivar grown in Kağızman, and its comparison with the Starking Delicious cultivar produced at the same location.

2. Materials and Methods

2.1. Research Area

Kağızman district (Iğdır, Türkiye), where the research was conducted, is also located in the Aras Valley, and is known as the only area where "Long Apple" is widely grown [19]. The Aras Valley, a heat area in the centre of the extreme climate of eastern Anatolia, extends from Kağızman to Iğdır through the Aras River [20]. This temperate effect gradually increases from Kağızman to the Iğdır Plain. Some of the areas where apples are grown are around the Aras River, and a significant part is spread along the hillside land including the district centre. The altitude of the location where the Aras River enters the district borders of Kağızman is around 1500 m and the exit location is around 1000 m. The altitude along the valley including the district centre varies between 1150–1650 m [21].

2.2. Plant Material

"Long Apple" trees planted in Kağızman district were the study material. Trees were selected from orchards in different parts of the district to minimize differences due to altitude and cultural practices. Harvest maturity fruits were taken from the selected trees for two years. After the measurement of pomological characteristics, the fruit juice was obtained. The juice was preserved at −80 °C for biochemical analysis.

2.3. Reagents and Standards

Purified water was obtained using the Water Story Mini Pure system (Mini Pure II, MDM, Gyeonggi-do, Republic of Korea). Acetonitrile, methanol (MeOH), and ethanol (EtOH) were obtained from Merc Millipore (Darmstadt, Germany). Toroloxfor bioactive compounds and standards of malic acid, succinic acid, oxalic acid, citric acid, and tartaric acid were purchased from Sigma-Aldrich (Darmstadt, Germany). The standards of chlorogenic acid (LGC-Dr. Ehrenstorfer Standards GmbH C 11415750), caffeic acid (LGC-Dr. Ehrenstorfer Standards GmbH C 10934700), rutin hydrate (Sigma R5143-50G), o–coumaric acid (Aldrich H22809-5G), myricetin (sigma 70050-25mg), p–coumaric acid (Fluka 55823-50 mg), syringic acid (Chem Service NG-17689-1G), gallic acid (Chem Service N-12105-2G), quercetin (Chem Service NG-BS100-1G), and catechin (Fluka 43412-10mg) were supplied.

2.4. Fruit Quality Parameters

Standard quality parameters were determined using 20 fruits for each genotype. Fruit weight was determined with an electronic scale (0.01 g accuracy) (Radwag, Radom, Poland). Fruit flesh firmness was measured using a manual penetrometer. The fruits were peeled enough for the penetration of the penetrometer probe and treated. A penetrometer tip with a diameter of 11 mm was used for the measurements [22]. The average of the values was recorded as fruit flesh firmness (kg cm^{-2}). Fruit diameter and length were measured using a digital calliper (0.01 mm accuracy) (Mitutoyo, Japan). Fruit shape index was calculated according to the formula "Fruit shape index = fruit length/fruit diameter". Titratable acidity (TA), pH, and soluble solids content (SSC) were assessed in juice obtained from the whole fruit. TA was determined in 10 mL fruit juice by diluting with 10 mL distilled water and titrating with 0.1 N NaOH to pH 8.1 [23] and expressed as g malic acid 100 mL^{-1}. A digital table refractometer (WAY–2S, Seoul, Republic of Korea) was used for SSC assessment, and data given as °Brix. The pH of fruit juice was determined using a portable pH meter (Jenco Instruments Inc., San Diego, CA, USA).

2.5. Antioxidant Activity

The assay of Trolox equivalent antioxidant capacity (TEAC) was performed according to Re et al. (1999) with minor modifications [24]. According to the method, 7 mM ABTS salt is dissolved in water and oxidized with 2.45 mM potassium persulfate. This mixture is kept at dark room temperature for 14–16 h before use. This solution is then diluted with ethanol or buffer (pH 7.4) to an absorbance of 0.7 at 734 nm [25]. Then 30 µL of sample is mixed with 3 mL of $ABTS^{+}$. solution and allowed to stand at room temperature for 6 min, after which the absorbance is measured. The results are expressed in terms of µmol Trolox equivalent antioxidant capacity per gram of sample (fresh weight).

2.6. Organic Acids, and Phenolic Compounds

2.6.1. Apparatus

A high-performance liquid chromatography (HPLC) system involving an LC–20 AT pump, CTO–20A column oven, and SPDM20A prominence diode-array detector equipped with SIL–20A HT auto sampler (Shimadzu Co., Kyoto, Japan) was used. LabSolutions LC (Shimadzu) software was used for data collection and processing.

2.6.2. Extraction and Analysis of Organic Acids

Organic acid extraction and determination method by Bevilacgua and Califano (1989) was used by some modifications. About 5 g fractionated sample was added into centrifuge tubes [26]. The samples were added with a 10 mL of 0.005 N H_2SO_4 and were homogenized and then were mixed for an hour with a shaker and centrifuged at 15,000× *g* for 15 min centrifuge. The process was performed using an Agilent Hi–Plex H (8 µm, 300 mm × 7.7 mm i.d.) column (Agilent Technologies, Santa Clara, CA, USA). Column oven temperature was set to 55 °C. The mobile phase was filtered through membrane filter (0.45 µm) and was sonicated for 10 min in an ultrasonic bath. The injection volume was

20 µL and target compounds were detected at 210 nm. Organic acids (oxalic acid, citric acid, tartaric acid, succinic acid, and malic acid) were quantified from regression curves calculated for authentic standards purchased from Sigma–Aldrich (Steinheim, Germany). All calibration curves exhibited a good linear relationship with correlation coefficients above 0.999. The amounts of organic acids are expressed as 100 g^{-1} fresh weight (fw).

2.6.3. Extraction and Analysis of Phenolic Compounds in Fruit Samples

Phenolic compounds were extracted according to the method described by Aaby et al. (2007) [27] with some minor modifications. Approximately 150 g of fruit was diced, and 5 g was weighed and sonicated for 10 min in 10 mL 80% (*v*/*v*) acetone. The extract was centrifuged at 15,000 rpm for 10 min at 4 °C and the supernatant was collected. In the case of insoluble material, it was re-extracted a second time in 10 mL of 80% acetone and the supernatants were pooled. Residual acetone was removed under reduced pressure in a rotary evaporator (Heidolph, Schwabach, Germany) at 37 °C for 4 min at 67 rpm. The remaining acetone-free sample solution was made up to 25 mL with purified water. Chromatographic data obtained by reading at 273 and 370 nm were collected and processed. Each extract was filtered through a 0.45 µm nylon filter (Millipore Corp., Billerica, MA, USA) prior to injection. Chromatographic separations were performed on an Inertsil® ODS–3V column (250 mm × 4.6 mm i.d., 5 µm particle size) (GL Sciences, Tokyo, Japan). The column temperature was 40 °C, and 20 µL of each extract was used as the injection volume. The mobile phases were (A) acetic acid/water (2:98, *v*/*v*), (B) 50% aqueous acetonitrile/0.5% aqueous acetic acid (1:1, *v*/*v*), and (C) acetonitrile. The phenolic compounds (gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, o–coumaric acid, p–coumaric acid, myricetin, quercetin, andrutin) were separated under gradient conditions at a flow rate of 1.2 mL min^{-1}. The gradient was implemented as follows; 0.00–4.99 min 95% A and 5% B, 5.00–7.99 min 95% A and 5% B, 8.00–9.99 min 80% A and 20% B, 10.00–16.99 min 78% A and 22% B, 17.00–18.99 min 75% A and 25% B, 19.00–29.99 min 73% A and 27% B, 30.00–34.99 min 60% A and 40% B, 35.00–39. 99 min 55% A and 45% B, 40.00–44.99 min 35% A and 65% B, 45.00–49.99 min 10% B and 90% C, 50.00–51.99 min 100% C, and 52.00–60.99 min 95% A and 5% B. All calibration curves showed a good linear relationship with correlation coefficients above 0.999. The concentration of phenolics is expressed as mg kg^{-1}fw.

2.7. Vitamin C and Sugar Component

Sample preparation: Fruit juices of the genotypes were extracted and filtered. The samples were processed as follows and vitamin C, glucose, fructose, sucrose, and total sugar contents were determined. Vitamin C content was measured with a reflectometer (RQflex 10 plus, Merck, Germany) using a 25–450 mg L^{-1} measuring range for ascorbic acid. The method consists of reducing yellow molybdophosphoric acid to molybdenum blue by the action of ascorbic acid. The results were interpreted as mg L^{-1} of fruit juice. Glucose content: After diluting the prepared juice with distilled water (1:100), the test strip (Cat. No. 116720, Reflectoquant, Glucose test, Merck, Germany) was plunged into this solution for 2 s and the surplus liquid was removed. After 1 min, the test strip was placed in the strip holder of the reflectometer (RQflex 10 plus, Merck, Germany) and measured. The reading was expressed as g L^{-1} multiplied by the dilution coefficient. Sucrose content: The juice was diluted with distilled water (1:100). To 1 mL of the resulting solution, 10 mL of distilled water and five drops of Sa–1 reagent were added. The test strip (Cat. No. 116141, Reflectoquant, Sucrose test, Merck, Germany) was then immersed in the prepared solution for 2 s. The surplus liquid on the test strip was removed and after waiting for 5 min, the test strip was placed in the strip adapter of the reflectometer (RQflex 10 plus, Merck, Germany) and measured. The reading was multiplied by the dilution coefficient and expressed as g L^{-1}. Fructose content was calculated according to the formula (Fructose = Total sugar-Glucose) specified in the total sugar package insert and expressed in g L^{-1}. Total sugar content: After dilution with distilled water (1:100), 1 mL of juice was separated, 10 mL of distilled water and five drops of Ts–1 reagent were added. The test strip (Cat. No. 116136, Reflectoquant, Total sugar test, Merck, Germany) was incubated in the prepared solution for 2 s and the surplus liquid was removed. After

10 min, the test strip was placed in the strip adapter of a reflectometer (RQflex 10 plus, Merck, Germany) and measured. The reading was multiplied by the dilution coefficient and expressed as g L^{-1}.

2.8. Statistical Analyses

The study was conducted as three repetitions. Data set was tested using two-way ANOVA with the JMP 17 program package (Trial), and averages were allocated by the Fishers' least significant difference test at $p \leq 0.05$. The correlations between studied parameters and factors were expressed by using principal component analysis (PCA).

3. Results and Discussion

Nineteen genotypes were selected for this study from the numerous "Long Apple" trees, which are probably an endemic apple variety grown in Kağızman district. Samples taken from the standard apple variety Starking Delicious trees (growing in Kağızman District) were included in the study for a comparison.

3.1. Fruit Quality Parameters

Some quality parameters were presented in this study included in 19 "Long Apple" genotypes (Figure 1) and Starking Delicious apple variety. There was a statistically significant difference ($p \leq 0.05$) in all fruit quality parameters among the genotypes.

Figure 1. "Long Apple" genotypes selected from Kağızman district (original).

Genotype 36K011 had the highest fruit weight (139.11 g), followed by genotypes 36K094 (129.82 g), 36K098 (127.43 g), and 36K090 (127.30 g). The lowest fruit weight was recorded for genotype 36K007 (87.21 g). The "Long Apple" was "medium size" compared to the Starking Delicious (187.70 g) and met the apple standards. Fruit length (75.38 mm–67.37 mm), fruit diameter (59.34 mm–51.79 mm), and fruit shape index (1.42–1.18) were also significantly different ($p \leq 0.05$) between the genotypes. The longest fruits were obtained from genotype 36K083, and the widest fruits were obtained from genotype 36K011 (Table 1).

Table 1. Some fruit quality parameters of selected "Long Apple" clones.

Genotype	FWT		FRM		FLN		FDM		SHI		pH		SSC		TA		TEAC	
36K006	121.35	efg	6.47	j	72.50	b–f	58.02	bcd	1.25	efg	3.67	b	12.20	hi	0.60	de	2.08	hij
36K007	87.21	n	8.27	a	73.67	a–d	57.55	bcd	1.28	b–f	3.65	bc	11.63	jk	0.49	j	2.35	fg
36K009	118.42	fgh	7.51	ef	70.64	e–h	57.15	cd	1.24	fgh	3.52	def	13.00	ef	0.54	gh	3.30	a
36K011	139.11	b	7.34	fg	74.48	ab	59.34	b	1.26	def	3.67	b	11.30	k	0.47	kl	2.55	def
36K014	117.44	ghi	7.09	ghi	73.15	a–d	57.51	bcd	1.27	c–f	3.52	def	12.20	hi	0.52	i	2.27	ghi
36K025	122.23	ef	8.08	abc	73.03	a–e	57.04	cd	1.28	b–e	3.58	b–e	14.90	b	0.56	f	2.09	hij
36K031	114.90	hij	7.90	c	73.79	abc	56.93	cd	1.30	bcd	3.61	b–e	12.90	fg	0.66	a	2.94	bc
36K043	113.70	ijk	6.85	i	67.53	I	57.38	bcd	1.18	i	3.50	ef	13.40	de	0.65	ab	2.87	bc
36K044	122.64	ef	7.62	de	70.20	fgh	57.94	bcd	1.21	ghi	3.62	bcd	11.80	ij	0.54	ghi	1.98	jk
36K045	112.66	jkl	8.18	ab	71.38	c–g	54.28	ef	1.32	b	3.50	ef	12.50	gh	0.59	e	2.75	cd
36K083	109.42	lm	7.59	ef	75.38	a	53.22	fg	1.42	a	3.53	c–f	16.70	a	0.54	ghi	1.80	kl
36K090	127.30	cd	7.85	cd	74.34	ab	58.45	bc	1.27	c–f	3.43	f	13.80	d	0.62	cd	2.71	cde
36K093	106.08	m	7.05	hi	72.96	a–e	56.22	de	1.30	bc	3.52	def	13.60	d	0.63	bc	3.11	ab
36K094	129.82	c	7.61	de	67.37	i	51.79	g	1.30	bc	3.60	b–e	13.80	d	0.66	a	2.85	c
36K097	116.11	hij	7.91	c	73.50	a–d	57.60	bcd	1.28	b–f	3.54	c–f	12.90	fg	0.46	l	1.72	l
36K098	127.43	cd	7.17	gh	71.22	d–g	59.31	b	1.20	hi	3.59	b–e	14.40	c	0.55	fg	2.06	ij
36K099	109.61	klm	7.94	bc	69.47	ghi	56.15	de	1.24	fgh	3.49	ef	13.10	ef	0.48	jk	2.46	efg
36K104	110.50	kl	7.03	hi	68.72	hi	58.34	bc	1.18	i	3.54	c–f	12.80	fg	0.64	bc	2.09	hij
36K106	124.31	de	7.86	cd	68.66	hi	57.94	bcd	1.19	i	3.58	b–e	13.10	ef	0.53	hi	2.32	fgh
Stark.D *	187.70	a	8.28	a	60.03	j	70.15	a	0.86	j	3.81	a	12.00	ij	0.39	m	1.71	l
P.St.Dev. ×	2.569		0.157		1.487		1.213		0.026		0.075		0.273		0.012		0.156	
F-Value	164.93	***	31.30	***	16.98	***	25.15	***	50.78	***	3.85	***	62.75	***	128.77	***	27.20	***

* Starking Delicious cv | × Pooled Standard Deviation | *** Mean values followed by a different letter within the same column are significantly different at $p \leq 0.05$ according to LSD test | FWT: Fruit weight (g), FRM: Firmness (kg cm^{-2}), FLN: Fruit length (mm), FDM: Fruit diameter (mm), SHI: Shape index, SSC: Soluble solid content (%), TA: Titratable acidity (%), and TEAC: Trolox equivalent antioxidant capacity (µmol g^{-1}).

The highest and lowest flesh firmness was measured in genotypes 36K007 (8.27 kg cm^{-2}) and 36K006 (6.47 kg cm^{-2}), respectively. Under the ecological conditions of Kağızman, the Starking Delicious cultivar showed harder flesh with a value of 8.28 kg cm^{-2} than all investigated "Long Apple" genotypes. There were significant differences in pH, soluble solid content (SSC), and titratable acidity (TA) parameters among the 19 selected "Long Apple" genotypes. The pH value was 3.67–3.43, the SSC value was 16.70–11.30% and the TA value was 0.66–0.46% in the "Long Apple" genotypes. These values were 3.81, 12%, 0.39% in Starking Delicious cv, respectively. Compared to previous studies [2,4,8,28–31], our results are both suitable for the genus *Malus* and agree with previous findings. However, all quality parameters, the variation among genotypes was found to be significantly different and the parameter values were distributed over a wide scale. Especially for fruit length parameter, all genotypes, differed from the results of other studies including Starking Delicious cv investigated in this study. In terms of shape index, the longest fruits were obtained from genotypes 36K083 with a ratio of 1.42 and 36K045 with a ratio of 1.32, while the shortest or nearer to oval fruits were obtained from genotypes 36K043–36K104–36K106 (Table 1). In this study, the shape index value of the Starking Delicious apple cultivar from the same region was 0.86, indicating an oval shape. The genotypes of "Long Apple", on the other hand, were grouped far away from this value and appeared to be ellipsoidal and oblong in shape (Figure 1). It is known that environmental factors [32], tree age [33] have effects on fruit shape and other quality parameters. However, since the present study was conducted in a homogeneous and limited geographical and ecological environment, we consider that genetic factors are the more important components. Chang et al. (2014) [34], stated that apple fruit size and shape are under independent genetic control. Perhaps the special fruit shape may have resulted from the high adaptability of the "Long Apple" variety to the ecological conditions of Kağızman.

3.2. Antioxidant Activity

The highest Trolox equivalent antioxidant capacity (TEAC) was 3.30 µmol g^{-1}fw (36K009) and the lowest was 1.72 (36K097) µmol g^{-1}fw in "Long Apple" genotypes. The

TEAC of the standard variety Starking Delicious was 1.71 µmol g^{-1}fw, almost half that of the "Long Apple" highest genotype. There was a statistically significant difference between all the genotypes for TEAC. Previous studies have reported results consistent with our findings [35,36]. Scalzo et al. (2005) [37], reported that genotype AN89.4.1.211 had the highest TEAC (2.58 µmol TE g^{-1}fw), which was at least two–fold higher than the two currently most widely grown cultivars Golden Delicious and Fuji (0.94 and 0.89 µmol TE g^{-1}fw, respectively). Zucoloto et al. (2015) [14], analyzed five apple cultivars and found the highest ABTS level in Gold Rush apple (as TE mM g^{-1} of dry weight) and Schempp et al. (2016) [30], found at highest in Baujade apple (13.3 mmol L^{-1} fruit juice). Murathan et al. (2022) [19], found that ABTS (%), DPPH (%), and FRAP (µmol Fe–II g^{-1}) were 66.86, 70.97, and 870.81, respectively. It is hypothesized that the different results found in previous studies are due to altitude, genetic traits, environment–genotype interactions, and cultural practices. In this study, the higher and lower antioxidant capacity of the genotype grown in Kağızman region, where there are different altitude levels include in 1200–1500 m approximately, is in accordance with the results of Bahukhandi et al. (2018) [38].

3.3. Organic Acids, Sugars, and Vitamin C

The results of 19 "Long Apple" genotypes and Starking Delicious apple cultivars investigated in this study are presented in Table 2. These results show that there are statistically significant differences between the "Long Apple" genotypes. Vitamin C 66.67–135.67 mg L^{-1}, glucose 13.67–28.00 g L^{-1}, sucrose 7.57–20.13 g L^{-1}, fructose 28.09–165.83 g L^{-1}, total sugars 58.28–196.29 g L^{-1} were found in the "Long Apple" genotypes. Oxalic acid 0.02–1.20 g 100 g^{-1}, citric acid 0.98–2.13 g 100 g^{-1}, tartaric acid 0.31–3.46 g 100 g^{-1}, malic acid 4.34–10.50 g 100 g^{-1} and succinic acid 0.79–2.88 g 100 g^{-1} were found agree with previous study [8]. Genotype 36K025 (135.67 mg L^{-1}) gave the highest results in vitamin C content and genotype 36K093 (196.29 g L^{-1}) in total sugar content.

Table 2. Contents of organic acids (g 100g^{-1}fw), Vitamin C (mg L^{-1}fw), and sugar components (g L^{-1}fw) for selected "Long Apple" clones.

Genotype	OXA		CIT		TAR		MAL		SUC		VIT-C		GLK		SCR		FRU		TOT-S	
36K006	0.51	h	0.98	i	0.31	j	4.34	kl	1.19	j	66.67	n	24.00	b–e	16.80	c	57.53	Gh	96.17	h
36K007	0.82	c	ND		0.57	gh	5.92	ef	1.46	hi	117.33	f	22.67	b–f	12.80	f	80.51	D	115.08	e
36K009	1.16	b	ND		0.84	d	6.38	de	2.17	d	124.67	cde	23.67	b–e	10.90	g	55.72	H	86.90	i
36K011	0.76	de	ND		0.68	ef	5.28	ghi	1.84	ef	109.00	gh	28.00	ab	12.77	f	70.62	F	111.89	ef
36K014	0.25	l	1.33	g	0.35	ij	4.90	ij	1.20	j	98.33	jk	22.33	b–g	11.20	g	53.33	i	87.07	i
36K025	0.57	g	1.66	c	0.60	fg	6.10	def	1.48	gh	135.67	a	27.33	abc	7.57	h	48.55	j	85.06	ij
36K031	0.78	cd	1.55	d	0.49	h	7.23	c	2.51	bc	101.00	ijk	26.00	bcd	14.67	d	103.48	c	143.69	c
36K043	0.35	ij	ND		0.73	e	5.77	fg	2.25	d	127.33	bc	23.67	b–e	10.37	g	74.11	e	105.05	g
36K044	0.22	l	1.05	hi	0.40	i	5.60	fgh	0.79	k	118.67	def	16.67	gh	7.93	h	131.83	b	155.13	b
36K045	0.02	m	2.13	a	1.11	b	8.42	b	2.88	a	134.00	ab	22.67	b–f	8.37	h	58.98	g	79.88	j
36K083	0.25	l	1.09	h	0.63	fg	6.59	d	1.57	gh	76.00	m	21.67	c–g	14.70	d	47.27	j	83.67	ij
36K090	0.73	e	1.37	fg	0.40	i	5.29	ghi	1.78	f	118.33	ef	21.33	d–g	20.13	b	41.16	k	81.73	ij
36K093	0.04	m	2.04	a	3.46	a	8.34	b	2.42	c	128.67	abc	23.33	b–f	12.80	f	165.83	a	196.29	a
36K094	0.02	m	1.77	b	0.72	e	7.15	c	1.89	ef	125.67	cd	27.00	a–d	12.77	f	74.75	e	111.59	ef
36K097	0.62	f	1.52	de	0.33	ij	5.19	hi	1.43	hi	105.00	hij	17.67	fgh	13.63	def	28.09	m	58.28	k
36K098	0.30	k	1.44	ef	0.55	gh	4.36	kl	1.42	hi	115.67	fg	21.33	d–g	13.27	ef	80.34	d	114.52	e
36K099	0.31	jk	1.15	h	0.40	i	4.57	jk	1.32	ij	95.00	k	23.00	b–f	14.47	de	73.90	e	106.02	fg
36K104	1.20	a	ND		0.99	c	10.50	a	2.65	b	84.33	l	19.00	e–h	12.57	f	73.39	e	103.10	g
36K106	0.34	ijk	1.34	fg	0.92	cd	6.05	ef	1.95	e	105.67	hi	13.67	h	8.23	h	37.23	l	60.46	k
Stark.D *	0.38	i	1.13	h	0.61	fg	3.93	l	1.63	g	108.00	hi	32.33	a	22.47	a	79.77	d	133.75	d
P.St.Dev. ×	0.028		0.063		0.051		0.304		0.091		4.291		3.505		0.777		1.241		3.688	
F-Value	468.62	***	376.18	***	525.28	***	84.51	***	107.86	***	57.93	***	4.33	***	72.61	***	2033.49	***	236.40	***

ND: Not detected | * Starking Delicious cv | × Pooled Standard Deviation | *** Mean values followed by a different letter within the same column are significantly different at $p \leq 0.05$ according to LSD test | OXA: Oxalic acid (g 100 g^{-1}), CIT: Citric acid (g 100 g^{-1}), TAR: Tartaric acid (g 100g^{-1}), MAL: Malic acid (g 100g^{-1}), SUC: Succinic acid (g 100 g^{-1}), VIT-C: Vitamin C (mg L^{-1}), GLK: Glucose (g L^{-1}), SCR: Sucrose (g L^{-1}), FRU: Fructose (g L^{-1}), and TOT-S: Total sugar (g L^{-1}).

For the "Long Apple" and Starking Delicious genotypes grown in Kağızman, malic acid was the major organic acid and fructose was the major sugar component. However, the ratio of organic acids, glucose, and sucrose varied from one variety to another.

Gökmen et al. (2001) [39], found that malic acid (4.918 mg L^{-1}) was the major component for Golden Delicious apple variety. Zucoloto et al. (2015) [14], reported that malic acid and fructose were the major components for five apple cultivars, in agreement with our results. Coklar et al. (2018) [40], stated that malic acid (25.394 g kg^{-1}fw) was the main organic acid and amounts of sucrose, glucose, and fructose were found 0.497, 0.504, and 4.334 g 100 g^{-1}fw, respectively. In the analysis of some apple accessions in Türkiye, malic acid (4.62–2.06 mg 100 mL^{-1}) was found to be the major organic acid followed by others [41]. Four local apple varieties were analysed in a study of apples from the Van Lake Basin; it was found that fructose (12.315–16.495 g L^{-1}) was the dominant sugar followed by glucose and sucrose, malic acid (3.195–4.613 mg 100 mL^{-1}) was major organic acid [42]. According to Kim et al. (2020) [43], total sugar content ranged from 71.2 to 134.4 g kg^{-1} fresh weight, fructose was the major sugar, major organic acid in apple juice was malic acid, which ranged from 413 to 2984 mg kg^{-1}fw, followed by citric acid and shikimic acid for 24 apple cultivars. Karatas et al. (2021) [29], reported that apple fruits are known for their low vitamin C concentration and found low vitamin C content ranging from 5.6 to 9.3 mg 100 g^{-1} in 20 summer apple genotypes compared to our study. Macit et al. (2021) [7], found that vitamin C varied from 2.31 to 7.66 mg 100 g^{-1} for investigated 8 local apple varieties. Średnicka-Tober et al. (2020) [44], stated that vitamin C content in fruits was highly dependent on differences in fruit growing conditions. In this study, organic acid, vitamin C, and sugar values were generally consistent with previous studies, some "Long Apple" genotypes produced different values; therefore, it can be considered that genotype 36K093 can be consumed both as table and dried due to its favourable sugar and acid content.

3.4. Phenolic Compounds

Table 3 summarizes the amount of some phenolics in 19 "Long Apple" genotypes and the standard cultivar Starking Delicious. There was a statistically significant difference ($p \leq 0.05$) in phenolics among the genotypes. According to these results, chlorogenic acid is the major phenolic component, followed by catechin and syringic acid. However, the content of phenolic compounds varied from one genotype to another, including Starking Delicious.

Table 3. In Eastern Türkiye, contents of some phenolics (mg kg^{-1}fw) for selected "Long Apple" clones in 'Kağızman district' from Aras Basin.

Genotype	GAL		CAT		CHG		CAF		SYR		P-CA		RUT		O-CA		MYR		QRS	
36K006	ND		24.49	ef	81.68	f	2.99	cde	17.74	e	0.81	bc	1.40	ab	11.80	de	1.73	gh	1.26	def
36K007	1.42	c	33.32	ab	85.35	f	3.30	b	21.61	b	0.76	de	1.46	A	9.65	h	1.76	e–h	2.74	a
36K009	1.64	a	31.38	b	91.62	de	3.09	c	22.23	a	0.82	b	1.27	c–f	13.64	b	1.86	c	1.25	d–h
36K011	1.56	b	15.84	ij	64.88	i	2.88	efg	13.61	h	0.76	de	1.11	jkl	9.50	h	1.78	efg	1.26	d–g
36K014	1.46	c	14.63	ijk	70.91	gh	2.74	gh	9.60	i	0.77	de	1.16	hij	9.69	h	1.74	fgh	1.24	e–h
36K025	ND		16.06	ij	86.63	ef	2.21	ij	14.77	g	0.74	e	1.33	bc	12.48	c	1.73	gh	1.23	fgh
36K031	ND		30.45	bc	101.60	b	2.35	i	15.34	fg	0.81	b	1.23	efg	9.37	h	1.79	def	1.22	fgh
36K043	1.34	d	24.55	ef	82.63	f	3.34	b	19.87	d	ND		1.22	e–h	15.54	a	1.80	de	1.22	fgh
36K044	1.09	g	22.72	fg	70.66	gh	2.35	i	17.41	e	ND		1.08	klm	12.35	c	1.72	h	1.23	fgh
36K045	1.64	a	26.55	de	71.01	gh	2.88	efg	20.89	c	ND		1.18	ghi	12.19	cd	1.76	e–h	1.22	gh
36K083	1.08	g	12.62	k	35.75	j	1.67	k	4.94	l	0.77	de	1.20	f–i	4.26	l	1.70	h	1.28	de
36K090	1.53	b	27.90	cd	101.97	b	2.82	fgh	20.75	c	0.76	de	1.10	jkl	11.62	ef	1.84	cd	1.28	d
36K093	1.21	f	35.04	a	98.19	bc	2.93	def	22.45	a	0.76	de	1.14	ijk	11.23	f	1.95	b	2.52	b
36K094	ND		35.11	a	95.10	cd	3.58	a	22.71	a	ND		1.06	lm	11.26	f	1.86	c	1.26	d–g
36K097	1.22	ef	19.68	gh	70.59	gh	2.72	h	13.23	h	0.74	e	1.31	cd	8.46	i	1.75	e–h	1.25	d–h
36K098	ND		24.73	ef	93.68	cd	2.88	efg	17.15	e	0.78	cd	1.25	d–g	9.67	h	1.73	gh	1.21	h
36K099	1.56	b	14.02	jk	82.28	f	3.06	cd	15.71	f	ND		1.28	cde	11.88	de	1.73	gh	1.25	d–h
36K104	1.29	de	14.59	ijk	74.03	g	2.27	i	8.76	j	ND		1.22	e–h	7.59	j	1.74	fgh	1.26	def
36K106	ND		25.68	def	117.68	a	2.30	i	15.79	f	0.87	a	1.22	e–h	10.67	g	1.75	e–h	1.28	d
Stark.D *	1.08	g	17.48	hi	66.93	hi	2.08	j	7.55	k	0.76	de	1.03	M	6.46	k	3.06	a	1.76	c
P.St.Dev. ×	0.041		1.873		3.415		0.091		0.363		0.021		0.040		0.277		0.036		0.025	
F-Value	781.40		46.97		80.94		83.41		631.40		950.06		22.60		254.65		202.81		880.77	
	***		***		***		***		***		***		***		***		***		***	

ND: Not detected | * Starking Delicious cv | × Pooled Standard Deviation | *** Mean values followed by a different letter within the same column are significantly different at $p \leq 0.05$according to LSD test | GAL: Gallic acid, CAT: Catechin, CHG: Chlorogenic acid, CAF: Caffeic acid, SYR: Syringic acid, P–CA: P–coumaric acid, RUT: Rutin, O–CA: O–coumaric acid, MYR: Myricetin, and QRS: Quercetin.

Genotype 36K106 had the highest chlorogenic acid (117.68 mg kg^{-1}fw), followed by genotypes 36K090 (101.97 mg kg^{-1}fw), 36K031 (101.60 mg kg^{-1}fw), and 36K093 (98.19 mg kg^{-1}fw). The lowest chlorogenic acid was recorded for genotype 36K083 (35.75 mg kg^{-1}fw). Standard cultivar Starking Delicious contained 66.93 mg kg^{-1}fw chlorogenic acid. Other phenolics were detected between 22.71–4.94 syringic acid, 35.11–12.62 catechin, 15.54–4.26 o–coumaric acid, 3.58–1.67 caffeic acid, 1.95–1.70 myricetin, 2.74–1.21 quercetin, 1.64–1.08 gallic acid, 1.46–1.06 rutin, and 0.87–0.74 p–coumaric acid, highest and lowest at 'mg kg^{-1}fw'. The results of "Long Apple" genotypes and standard cultivar Starking Delicious in this study agreed with those of previous studies [43–46]. The results of FotirićAkšić et al. (2022) [47], showed that the apple peel is richer compared to the pulp, in the range of 1.2-fold (for chlorogenic acid) to 114-fold (quercetin 3–O–galactoside), the amount of chlorogenic acid as a major phenolic component in fruit pulp is between 60.56–21.39 mg kg^{-1} which is lower compared to our "Long Apple" genotypes but is compliance with Starking Delicious grown in Kağızman. In addition, Średnicka-Tober et al. (2020) [44], and FotirićAkšić et al. (2022) [47], concluded that there is a significant effect of horticultural management on the amounts of phenolics, but it is still much lower than variety effects.

3.5. Principal Component, Heatmap and Correlation Analysis

Twenty-seven properties were used for principal components analysis (PCA). Nineteen PCs were formed, and eight PCs had an eigenvalue above 1.0. The first three PCs explained 51.3% of total variation. PC 1 explained 24.5% of total variation and is mainly related to catechin, chlorogenic acid, syringic acid, o–coumaric acid, myricetin, sucrose, antioxidant activity, malic acid, fruit weight, fruit diameter, shape index, pH, and TA. PC 2 was related to vitamin C, fructose, total sugar, and fruit length, and explained 17.1% of total variation. PC 3 explained 9.7% of total variation, is related to caffeic acid, rutin, tartaric acid, and SSC (Table 4; Figure 2). Similarly, the first two components were explained 69.2% of total variation in the different apple genotypes, and organic acids clustered in distinct points [8]. Celik et al. (2018) [42], reported that the first two components explained 85.1% of total variation in the different apple genotypes, and catechin, chlorogenic acid, syringic acid, o–coumaric acid, and sucrose clustered in the same point. Mignard et al. (2022) [48], reported that the first two components explained 55.5% of total variation in the different apple genotypes. They also noted that fructose and total sugar clustered in the same points, while glucose clustered in distinct point. Macit et al. (2021) [7], reported that the first two components explained 51.1% of total variation in the apple germplasm, and fruit weight, fruit diameter, and pH clustered in same points.

Table 4. Principal component of "Long Apple" genotypes.

Variables	PC 1	PC 2	PC 3
Gallic acid	0.0023	0.0166	−0.1022
Catechin	0.5953 *	0.5606	−0.1797
Chlorogenic acid	0.4470 *	0.3921	−0.3201
Caffeic acid	0.5228	0.3282	−0.5641 *
Syringic acid	0.7247 *	0.4520	−0.4074
p-coumaric acid	−0.2737	-0.0940	−0.1025
Rutin	0.2112	-0.4324	−0.4444 *
o-coumaric acid	0.5935 *	0.2760	−0.5108
Myricetin	−0.6992 *	0.6353	0.0937
Quercetin	0.0784	0.4831	0.0459
Vitamin C	0.3985	0.5272 *	−0.1329
Glucose	−0.3222	0.4687	0.0012
Sucrose	−0.5540 *	0.2125	0.0193
Fructose	0.2249	0.6463 *	0.3777
Total sugar	0.0780	0.6959 *	0.3652
Antioxidant activity	0.7164 *	0.4403	−0.1396
Oxalic acid	−0.0855	-0.1913	−0.4254
Citric acid	0.1372	0.0948	0.4909
Tartaric acid	0.4179	0.5093	0.5013 *
Malic acid	0.5269 *	0.0500	0.5040

Table 4. *Cont.*

Variables	PC 1	PC 2	PC 3
Succinic acid	0.4230	0.2964	0.2736
Fruit weight	−0.7583 *	0.4119	0.0204
Firmness	−0.2179	0.0855	−0.0383
Fruit length	0.4601	−0.5560 *	0.0451
Fruit diameter	−0.8038 *	0.4105	−0.1886
Shape index	0.6968 *	−0.5345	0.1952
pH	−0.6735 *	0.3690	−0.0883
SSC	0.1717	−0.3772	0.5019 *
TA	0.7349 *	0.0710	0.2459
Eigenvalue	7.1	4.9	2.8
Percent	24.5	17.1	9.7
Cumulative Percent	24.5	41.6	51.3

* Factor loading ≥ |0.44|.

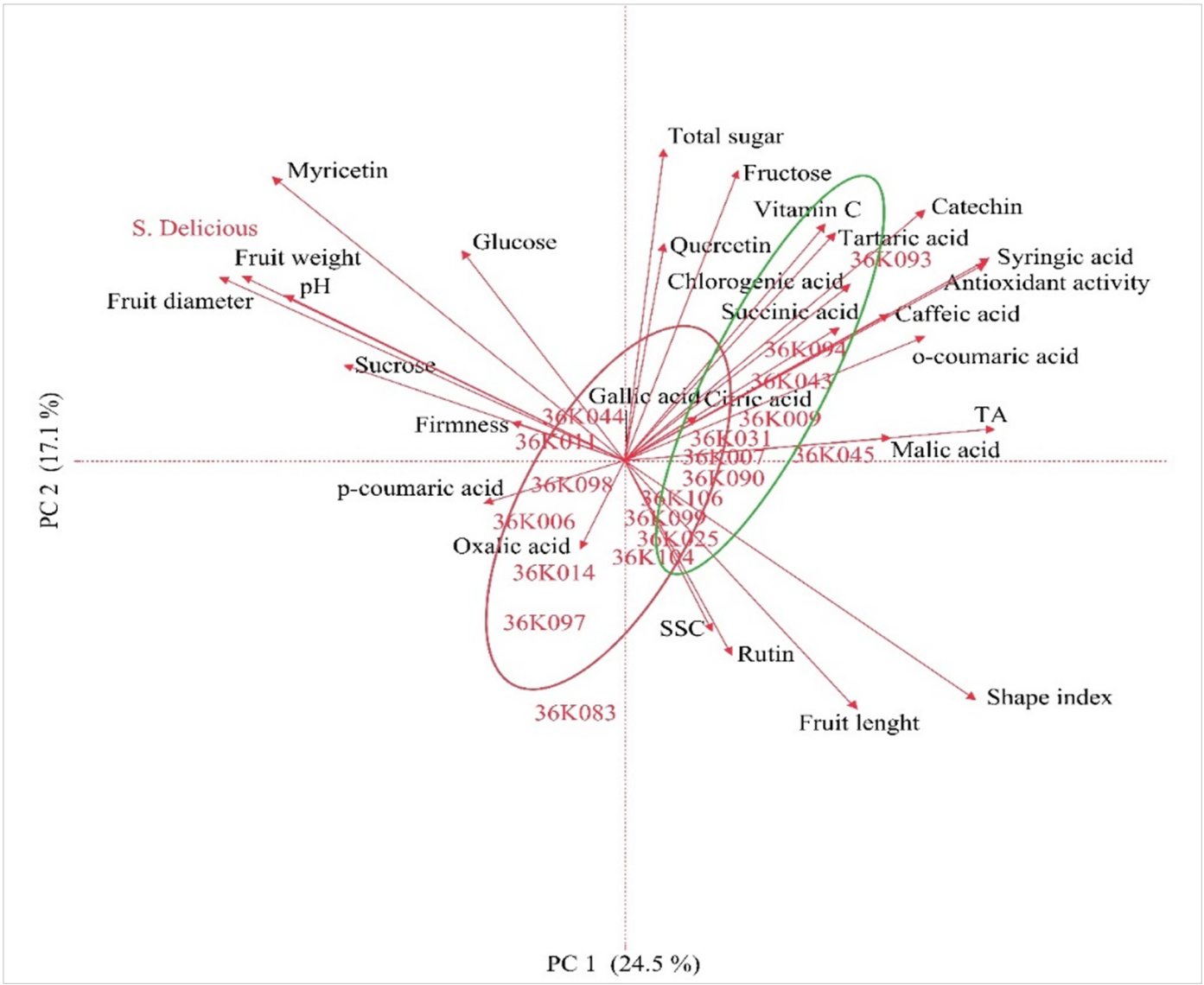

Figure 2. Biplot graph of the first two principal components in the "Long Apple" genotypes.

According to PCA results, "Long Apple" genotypes and Starking Delicious cultivar were clustered in different places on the dendrogram and formed two main group. The first main group (blue) included Starking Delicious cultivar. The second main group (red

and green) was divided into two sub-groups. The first sub-group (green) consisted of five "Long Apple" genotypes (36K009, 36K043, 36K045, 36K094, and 36K093). The second sub-group (red) included fourteen "Long Apple" genotypes (36K006, 36K011, 36K014, 36K097, 36K099, 36K044, 36K007, 36K025, 36K098, 36K106, 36K031, 36K090, 36K083, and 36K104) (Figure 3).

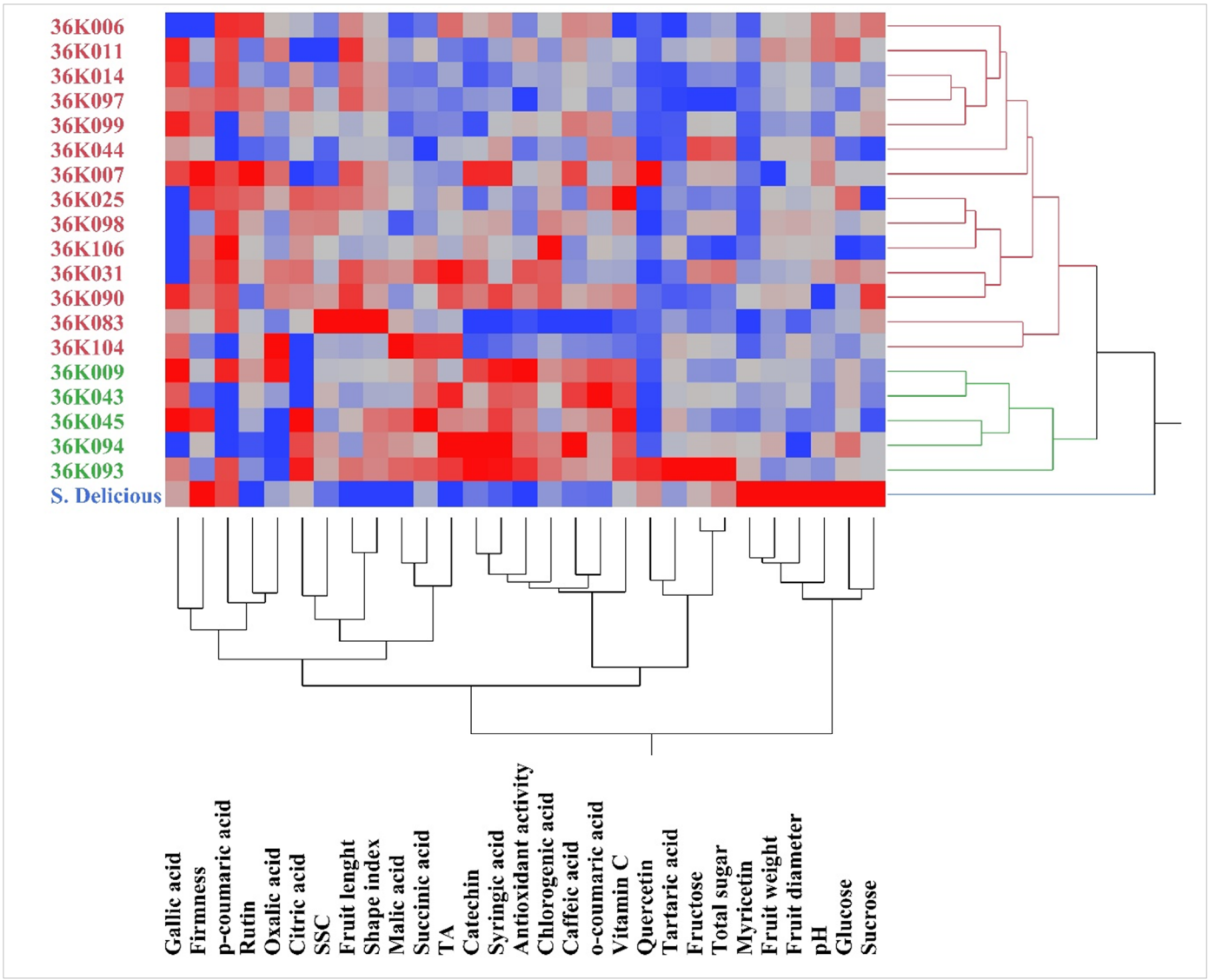

Figure 3. Heatmap analysis and grouping of "Long Apple" genotypes based on properties investigated.

Catechin showed a strong positive correlation with syringic acid (r = 0.85 ***) (Figure 4). It also had a moderate positive correlation chlorogenic acid (r = 0.66 **) and antioxidant activity (r = 0.66 **). A moderate positive correlation was determined between caffeic acid and o–coumaric acid (r = 0.63 **). Syringic acid showed a strong positive correlation with caffeic acid (r = 0.78 ***), o–coumaric acid (r = 0.77 ***), and antioxidant activity (r = 0.71 ***), while a moderate correlation with chlorogenic acid (r = 0.63 **) and vitamin C (r = 0.62 **). Myricetin acid showed a strong positive correlation with fruit weight (r = 0.81 ***), and fruit diameter (r = 0.80 ***). Celik et al. (2018) [42], reported that a strong positive correlation between catechin and chlorogenic acid in apple genotypes. On the contrary, they noted that a weak correlation was noted between catechin, caffeic acid, o–coumaric acid, and chlorogenic acid with syringic acid.

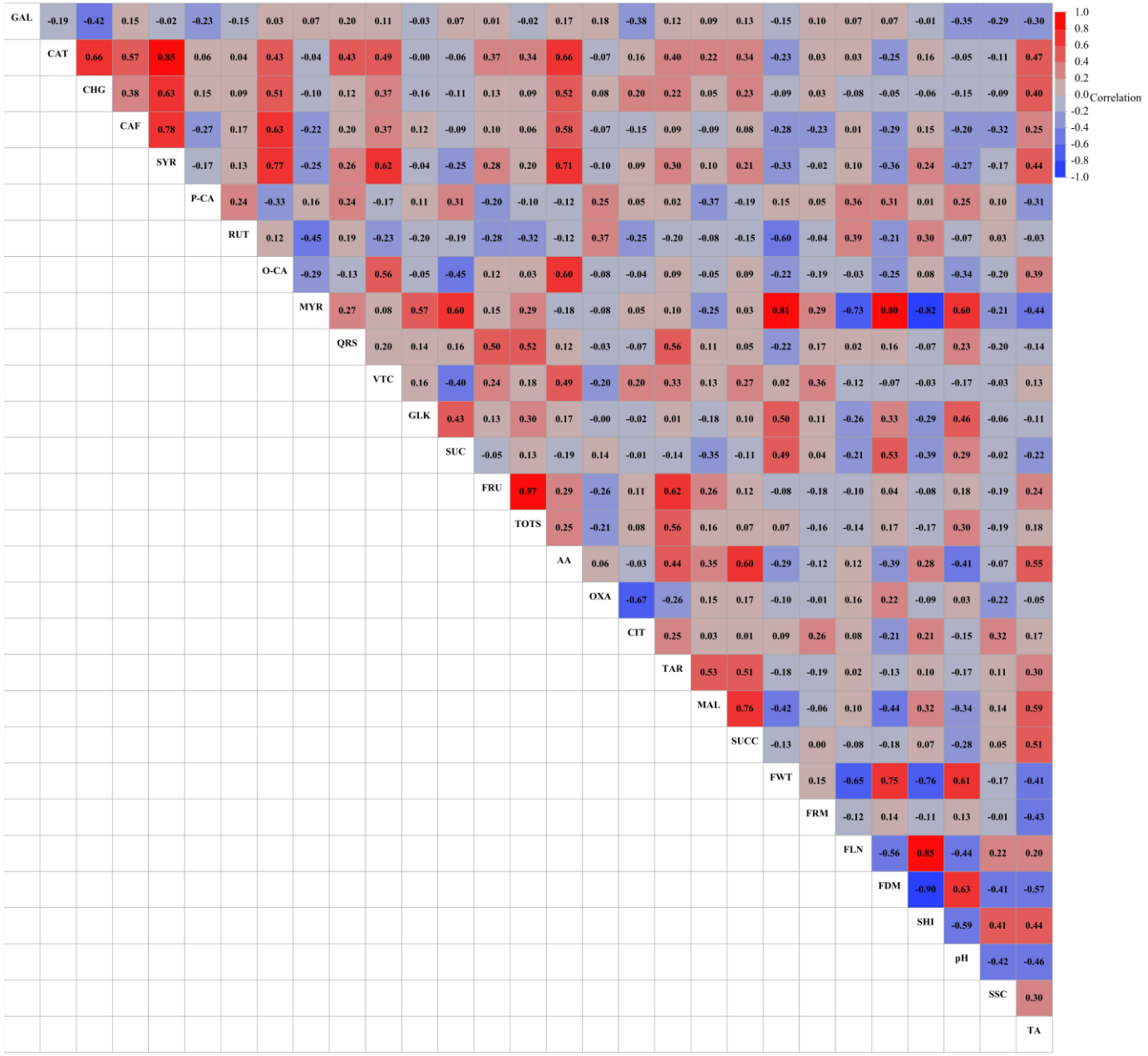

Figure 4. Correlations the among investigated properties of "Long Apple" genotypes.

A strong positive correlation was determined between fructose and total sugar (r = 0.97 ***) (Figure 4). Oxalic acid showed a negative correlation with citric acid (r = −0.67**). A strong positive correlation was determined between malic acid and succinic acid (r = 0.76 ***). Similarly, Mignard et al. (2022) [48], recorded that a strong positive correlation between fructose and total sugar in apple germplasm. On the contrary, they reported that a weak correlation between oxalic and citric, and malic acid and succinic acid. Ma et al. (2015) [49], recorded similar results in terms of sugar contents. Fruit diameter showed a strong positive correlation with fruit weight (r = 0.75 ***), while a moderate positive correlation with pH (r = 0.63 **). A strong positive correlation was determined between fruit length and shape index (r = 0.85 ***). Similarly, Macit et al. (2021) [7], reported that a moderate positive correlation between fruit weight and pH with fruit diameter in apple genetic resources. In accordance with our results, the correlation between the total phenolic content and the antioxidant capacity of the fruits was found to be significant at the $p \leq 0.05$ level, and it was also found that there was a positive and

significant correlation between the concentration of all bioactive compounds in the fruits and the antioxidant capacity [50].

4. Conclusions

The "Long Apple" variety has been cultivated only in the Kağızman region of Anatolia. Due to a combination of social practices and ecological conditions this has created an endemic character for the "Long Apple" variety. The most important characteristics that distinguish it from all other apples are the ellipsoid and oblong fruit shape, combined with its red blush colour. According to the results of this study, "Long Apple" genotypes are separated from one another according to different parameters, but genotypes 36K093, 36K094, and 36K106 are more prominent than others. "Long Apple" is known locally as a medium-sized apple, harvested in late September, consumed as a table fruit, and very suitable for storage, as a result of its waxy skin structure. This study's findings showed that "Long Apple" is delicious with excellent sugar/acid balance and is very helpful for the health of urban people due to its favourable quality characteristics, high antioxidant capacity, and phenolic content. Besides its absolute nutritional value in the human diet, "Long Apple" has very important properties for producers and researchers. It has been recommended that research on "Long Apple", which has thus far been extremely inadequate, should be increased. In future studies, agronomic characteristics of "Long Apple", different propagation techniques, compatibility levels with standard rootstocks, effects of cultural practices on yield and quality parameters, disease and pest tolerance levels, cross-pollinator capacity with standard apple varieties, post-harvest storage conditions, and marketing strategies will be the main research topics.

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