



Temporal shifts in phenolic composition and antioxidant responses of two Turkish grape cultivars ('Kureyş' and 'Tahannebi') during berry development

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Abstract

Phenolic compounds play a vital role in grape quality, antioxidant potential, and stress resilience; therefore, tracking their variation across developmental stages is essential for both viticultural practices and functional food production. The aim was to characterize variety-specific phenolic dynamics and antioxidant activity changes to guide optimized harvest timing and functional quality improvement. In this regard, present study evaluated the accumulation patterns of phenolic compounds and antioxidant activity across berry developmental stages from EL-27 (Eichhorn–Lorenz phenological scale) to EL-41 in two grape (*Vitis vinifera* L.) cultivars, 'Kureyş' and 'Tahannebi'. A total of 17 phenolic compounds were quantified by high-performance liquid chromatography (HPLC-DAD), and total phenolic content (TPC) and antioxidant activity were determined by Folin–Ciocalteu, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and DPPH ((1,1-diphenyl-2-picrylhydrazyl) assays. Significant differences ($p < 0.001$) were observed between varieties, phenological stages, and their interactions for all analyzed compounds. In general, phenolic concentrations peaked during the early stages (EL-27: K1, T1) and declined progressively through EL-31 (K2, T2), EL-33 (K3, T3), EL-35 (K4, T4), EL-36 (K5, T5), EL-38 (K6, T6), and EL-41 (K7, T7). 'Kureyş' consistently exhibited higher levels of gallic acid, catechin, chlorogenic acid, and caftaric acid, with chlorogenic acid decreasing by 98.8% from EL-27 to EL-41. In contrast, 'Tahannebi' showed lower initial concentrations but retained specific compounds like ellagic acid and *p*-coumaric acid longer. Multivariate analyses revealed distinct metabolic clustering. PCA explained 85% of total variance, with early-stage samples characterized by enriched antioxidant profiles. Correlation analysis indicated strong associations among TPC, antioxidant capacity (ABTS, and DPPH) assays, suggesting coordinated biosynthetic regulation. Hierarchical clustering clearly distinguished early high-phenolic samples (e.g., K1, T2) from late-stage samples (K7, T7) with reduced biochemical activity. Overall, this study highlights genotype-dependent differences in phenolic composition and antioxidant capacity during grape ripening, offering integrated insights into their physiological basis and implications for harvest optimization, nutritional enhancement, and breeding for functional quality.

Keywords Phenological stages · Flavonoid metabolism · Hydroxybenzoic acids · Ripening gradient

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Introduction

Among all plant developmental processes, fruit ripening attracts considerable attention because of its importance in plant biology and the nutritional value of fruit compounds for human health. Grapevine (*Vitis vinifera* L.) berries are particularly important, in this context, as they have been a fundamental part of human diet, culture, and economy throughout history. It also is one of the most widely cultivated fruit crops worldwide and plays a major role in agricultural economy through wine production, fresh consumption, and dried fruit processing [28, 30]. During ripening in grapes, berries undergo complex biochemical changes that directly affect the quality of grape products and their potential health benefits [10, 38]. Berry development for grape occurs in three main stages. The first stage involves rapid cell division and berry growth. During the second stage, growth slows down and berry hardening occurs. The third stage, called *véraison*, marks the onset of ripening when berries resume growth, soften, and accumulate sugars, resulting in increased sweetness [11]. The biochemical changes that happen during these stages are particularly important for polyphenol dynamics and antioxidant capacity [44]. In this context, the role of plant hormones becomes especially noteworthy. Although grapes are classified as non-climacteric fruits, they show some climacteric-like characteristics, including ethylene production [6]. However, ethylene activity occurs just before *véraison* rather than during the actual ripening period [9]. This unique ripening pattern suggests that our current understanding of berry ripening has been largely based on climacteric fruits, which are easier to study experimentally. As a result, the molecular mechanisms controlling ripening in non-climacteric fruits like grapes remain less well understood. This complexity is increased by spatial and temporal variations that occur at different levels - within berry tissues [8], grape clusters [37], and even between vines in the same vineyard [7]. These variations are influenced by many internal and external factors [27], showing that final berry composition results from complex interactions between genetic and environmental factors [13]. For this reason, the introduction has been structured to first address the physiological processes of berry development, followed by biochemical maturation, grape product characteristics, and finally, their implications for human health [12, 45]. Absciscic acid (ABA) plays a pivotal role in the onset of *véraison* and regulates sugar accumulation, color development, and phenolic metabolism in non-climacteric fruits such as grapes [27].

As known, changes in secondary metabolites like polyphenols and flavonoids are primary indicators of the biochemical shifts during ripening [43]. Polyphenols serve important functions in plants, including pigmentation, UV

protection, and pathogen defense [14]. These compounds, which include flavonoids and phenolic acids, have strong antioxidant properties and help prevent oxidative stress-related diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions [12, 45, 50]. These biochemical changes are not only crucial for plant developmental physiology but also directly influence the post-harvest nutritional value and potential health benefits for consumers [36]. Understanding polyphenol changes during grape ripening has both scientific and practical importance for improving fruit quality and maximizing health benefits. In early ripening stages, organic acids like tartaric and malic acids are high, contributing to the fruit's sour taste. As ripening progresses, these acids decrease while sugars such as glucose and fructose accumulate, increasing sweetness [35, 46]. Meanwhile, polyphenolic compounds like catechins, epicatechins, and resveratrol in the skin and seeds reach peak levels during early ripening and protect the fruit against environmental stress [49]. However, as berries approach full ripeness, polyphenol levels generally decline, corresponding with reduced antioxidant activity [34, 48]. On the other hand, the antioxidant capacity of grapes and their products depends largely on polyphenolic compound concentrations at harvest. Therefore, monitoring the chemical profile during ripening is crucial for agricultural efficiency and optimizing nutritional value of grape products [14]. Thus, the transition from physiological changes in the plant to their nutritional and functional implications for consumers forms a key link in understanding grape quality attributes. Natural antioxidants in grapes neutralize free radicals, protecting cells from oxidative damage and helping prevent chronic diseases like cancer, atherosclerosis, and Alzheimer's disease [22, 31, 36]. Growing consumer demand for functional foods has further emphasized the importance of tracking polyphenol dynamics in health-associated fruits like grapes.

Kureyş and Tahannebi grape varieties are grown in Turkey's Eastern Anatolia Region and have an important place in the local agricultural system. These varieties are used both as table grapes and for drying and play an important role in protecting regional agricultural diversity. However, scientific studies on the phenolic compound contents and antioxidant activities of these local varieties during ripening are quite limited. Evaluating local genetic resources is of great importance for sustainable agriculture and functional food production. In this regard, the objectives of our study were: (I) to determine the changes in polyphenol and the antioxidant activity content during ripening in Kureyş and Tahannebi grape varieties, (II), to assess the potential of these varieties for functional food production, (III) to contribute to the scientific knowledge about local Turkish grape varieties.

Materials and methods

Plant material and sampling

Grape variety selection and characteristics

The vines used in this study were 15 years old, grafted onto 1103 Paulsen rootstock, trained on a bilateral cordon system, and spaced at 2.5×3.0 m planting density. Kureys and Tahannebi grape varieties obtained from the vine collection vineyard of Apricot Research Institute (Malatya, Türkiye) were used as the primary materials. Regional adaptation capacity, economic value, and berry quality characteristics were considered as fundamental criteria in variety selection. The Tahannebi variety is characterized by thin-skinned, white-colored berries and is physiologically a female-flowered variety. Due to this characteristic, it requires a pollinator variety for effective fruit set. The vines of this variety exhibit vigorous growth, demonstrate moderate productivity levels, and are cultivated using mixed pruning methods, which in this case refers to a Guyot pruning system adapted to the variety's growth habit. The Kureys variety, on the other hand, is a late-ripening variety with round, thin-skinned berries. This variety is recognized for its high sugar content and small seeds, possessing a dense cluster structure. The variety was included in the research due to its widespread cultivation in the region, high economic value, and quality characteristics suitable for table consumption, including taste, total soluble solids content, and appearance.

Sampling strategy and berry development stages

Berry samples were collected within the framework of a systematic sampling program conducted between July and September. Stages were determined according to the Eichhorn-Lorenz [19] scale morphological descriptors, including berry size, firmness, skin color change, and juice soluble solids. The first sampling (Stage I, July 3, 2013) corresponded to the fruit set completion stage, representing the EL-27 stage (K1 and T1) where young clusters are visible and fully formed following successful pollination and fertilization. The second sampling (Stage II, July 26, 2013) was conducted 23 days after the initial sampling, capturing the early berry development phase corresponding to EL-31 (K2 and T2), where berries reach pea size and are small, hard, and green with active cell division. The third sampling (Stage III, August 3, 2013) represented the continued berry growth stage, aligning with the EL-33 stage (K3 and T3) where berries are continuing to grow through cell expansion while maintaining their green color and firm texture. The fourth sampling period (Stage IV, August 10, 2013) captured the berry softening initiation stage, corresponding to EL-35

(K4 and T4), where berries begin to soften as cell walls start to break down and physiological changes prepare the berry for ripening. The fifth sampling (Stage V, August 22, 2013) represented the *véraison* onset stage, corresponding to EL-36 (K5 and T5), where berries start color change from green to their varietal color, marking the beginning of the ripening process with initial sugar accumulation. The sixth sampling period (Stage VI, September 5, 2013) captured the active *véraison* stage, corresponding to EL-38 (K6 and T6), where *véraison* is continuing with approximately 50% color change throughout the cluster, accompanied by significant sugar development and acid reduction. The seventh sampling period (Stage VII, September 16, 2013) represented the advanced ripening stage, corresponding to EL-41 (K7 and T7), where berries are in the final ripening phase with continued sugar accumulation, color intensification, and flavor compound development approaching harvest maturity (Fig. 1). This comprehensive sampling program was designed to monitor the physiological and biochemical changes occurring from fruit set through advanced ripening, encompassing the critical developmental transitions that significantly impact phenolic compound accumulation, antioxidant activity, and overall berry quality parameters. During each sampling period, systematic sampling was conducted from ten different vines for each variety to ensure representative samples of the entire cultivar population under investigation.

Sample preparation and storage

Reagents

All solvents and reagents used in this study were of analytical or HPLC grade. Methanol, acetonitrile, formic acid, hydrochloric acid, Folin–Ciocalteu reagent, sodium carbonate, ABTS, DPPH, and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phenolic standards including gallic acid, protocatechuic acid, neochlorogenic acid, 4-hydroxybenzoic acid, catechin, vanillic acid, procyanidin B2, syringic acid, epicatechin, epicatechin gallate, caffeic acid, chlorogenic acid, p-coumaric acid, ellagic acid, rutin, myricetin, and naringin were obtained from Sigma-Aldrich or Fluka (Buchs, Switzerland). All reagents were stored and handled according to the manufacturer's instructions.

Lyophilization process

The collected grape berries were processed using freeze-drying (Christ Alpha 1–2 LD plus, Germany) methodology to maintain sample integrity throughout the analytical procedures. Grape berries of both Tahannebi and Kureys varieties were collected for lyophilization and stored at -80°C

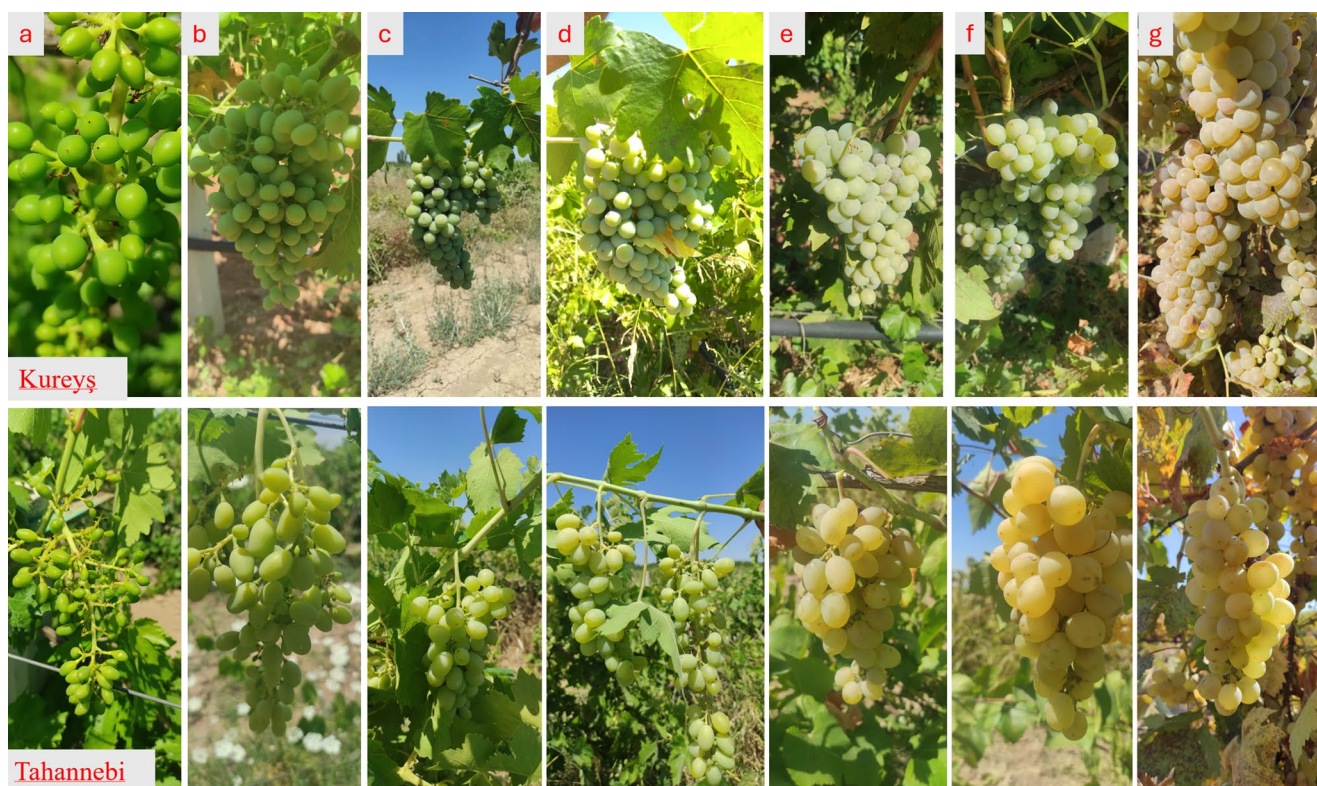


Fig. 1 Phenological stages of grape berry development in Kureys and Tahannebi cultivars from fruit set to ripening. **a** EL 27 - Fruit set complete, young clusters visible, **b** EL 31 - Berries pea-sized, **c** EL 33

- Berries continuing to grow, **d** EL 35 - Berries beginning to soften, **e** EL 36 - Berries start color change (*véraison* onset), **f** EL 38 - *Véraison* continuing, 50% color change, **g** EL 41 - Berries in ripening stage

prior to processing. Following the lyophilization process, the frozen samples were ground into fine powder using a Waring blender. The powdered samples were subsequently placed in polyethylene bottles, sealed, and stored at -20 °C until further analysis to prevent degradation of bioactive compounds.

Extraction procedures

Polyphenolic compounds from grape samples were extracted using an Accelerated Solvent Extraction (ASE) 200 system (Dionex, Sunnyvale, CA, USA), equipped with 11 mL stainless steel extraction cells and 60 mL collection vials. One gram of dry powder was loaded into each 11 mL extraction cell, with a cellulose paper filter (Dionex) placed at the bottom to prevent particulate matter from entering the extract. The cell was tightly sealed and placed into the ASE system for automated extraction. The extraction parameters were optimized with pressure set to 1500 psi, flush volume of 40%, and purge time of 60 s. Extractions were conducted for 15 min at 30 °C using a solvent mixture of methanol, water, and HCl (70:30:0.1, v/v/v) as described by Kennedy et al., [26].

Analytical methods

Quantitative analysis of total phenolic content and antioxidant activity

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method as described by Durmaz and Alpaslan [18]. The analytical procedure involved adding 950 µL of distilled water and 1 mL of Folin-Ciocalteu reagent to both 50 µL of extract. The mixture was allowed to stand for 3 min to ensure complete reaction, after which 1 mL of 2% Na₂CO₃ solution was added to neutralize the acidic conditions. Following a 10-min reaction period, the absorbance of the solutions was measured at 765 nm using a Shimadzu UV-2600 spectrophotometer with a 10 mm path-length quartz cuvette. A calibration curve was established using gallic acid standards, and results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) based on three replicates. Antioxidant activity was evaluated using two complementary assays: DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging activity and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). The DPPH radical scavenging assay was performed according to the method of Yen and Hung [47],

while the ABTS test was conducted following the protocol of Miller and Rice-Evans [33]. Both assays were standardized against trolox, and results were expressed as milligrams of trolox equivalent antioxidant activity per gram of dry weight (mg TEAC/g) to provide comparable antioxidant capacity measurements.

Quantitative determination of individual phenolic compounds

Individual phenolic compounds were identified and quantified using a Shimadzu LC-20AD Prominence HPLC system (Shimadzu LC-20AD Prominence, Shimadzu Corp., Kyoto, Japan) equipped with an SPD-M20A diode array detector, SIL-20 A HT autosampler, CTO-20 A column oven, and DGU-20A5 degassing unit. Chromatographic separations were performed using a C18 column (250 mm × 4.6 mm, 5 µm; GL Sciences, Kyoto, Japan) with detection at multiple wavelengths (280 nm, 320 nm, and 360 nm) to capture different classes of phenolic compounds. The mobile phase consisted of three different solvent mixtures: eluent A (water and formic acid, 98:2, v/v), eluent B (water, acetonitrile, and formic acid, 78:20:2, v/v/v), and eluent C (pure methanol, 100%). The analytical conditions were optimized with an injection volume of 20 µL, flow rate of 0.75 mL/min, and column temperature maintained at 28 °C. The chromatographic separation was achieved using a gradient elution program that began with 25% eluent B, which increased to 50% after 15 min, 75% after 20 min, and reached 100% eluent B after 25 min. The system was maintained at 100% eluent B for 52 min, followed by a 7-min passage of 100% methanol (eluent C) through the column for column cleaning. The system was then returned to initial conditions by passing eluent A for 6 min to ensure column equilibration for subsequent analyses. A total of 17 phenolic compounds were quantified in this study. Identification and quantification of phenolic compounds were performed by comparing retention times and UV spectra with authentic standards [26, 43]. All standards were purchased from Sigma–Aldrich or Merck (≥ 98% purity). Details of standard compounds, including supplier, purity, detection wavelength, retention time, and calibration curves, are provided in Supplementary Table S1; Supplementary Figures S1–S3.

Statistical analysis

All statistical analyses were performed using SPSS Statistics v25.0 (IBM Corp., Armonk, NY, USA) and figures were prepared in OriginPro 2022 (OriginLab Corp., Northampton, MA, USA). All measurements were performed in triplicate, and the results were expressed as means ± standard deviations (SD). The data were subjected to analysis

of variance (ANOVA) to determine statistical significance among treatments. Significant differences among applications were determined using the least significant difference (LSD) multiple comparison test at $p < 0.001$. Principal component analysis (PCA) was performed to identify patterns and relationships among variables and to reduce the dimensionality of the dataset while preserving maximum variance. Correlation analysis was conducted to examine the relationships between different parameters, and Pearson correlation coefficients were calculated to quantify the strength and direction of linear relationships among variables. A correlation matrix was constructed to visualize the interrelationships between all measured parameters. Heat map visualization was employed to graphically represent the correlation matrix and facilitate the interpretation of complex relationships among variables. The heat map was generated using color gradients to indicate the strength and direction of correlations, with color intensity corresponding to correlation coefficient values. All statistical analyses were performed using appropriate statistical software, and results were considered statistically significant when $p < 0.001$.

Results

Hydroxybenzoic acids

Our results revealed significant differences ($p < 0.001$) among grape varieties ('Kureys' and 'Tahannebi'), developmental stages (EL 27 to EL 41), and their interactions for hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, and antioxidant capacity (Tables 1 and 2; Supplementary Tables S2–S4). In general, the concentrations of phenolic compounds were highest during the early stages of berry development and declined progressively through ripening. The Kureys variety consistently exhibited higher phenolic content than Tahannebi throughout all developmental stages. Gallic acid exhibited its highest concentration at the K1 stage (EL 27 – fruit set complete, young clusters visible) with 18.84 mg/kg. Its levels decreased steadily in the following stages, reaching 1.32 mg/kg at K6 (EL 38) and 1.17 mg/kg at K7 (EL 41). Protocatechuic acid reached its peak during T3 (EL 33 – berries continuing to grow) at 25.05 mg/kg but was completely absent in multiple later stages, including K3–K7 and T5–T7. The Tahannebi variety generally accumulated lower levels of phenolic compounds compared to Kureys. At T1 (EL 27), gallic acid was 17.88 mg/kg, protocatechuic acid 8.54 mg/kg, and neochlorogenic acid 25.04 mg/kg. From EL 36 onward (T5–T7), these compounds were either very low or completely absent. Among other compounds, 4-hydroxy benzoic acid, catechin, and vanillic acid were highest at K1, with concentrations

Table 1 Changes in the contents of selected phenolic compounds (mg/kg) in different grape cultivars across ripening stages

C	RS	GA	PCA	NChA	4-HBA	CAT	VA	PB2	SA	EC	ECG	CFA
K	K1	18.84±0.62 ^{a*}	15.42±0.80 ^b	92.74±3.77 ^a	161.96±8.12 ^a	149.68±2.37 ^a	16.75±1.05 ^a	12.98±0.64 ^c	20.52±1.27 ^a	28.69±0.65 ^c	89.64±0.59 ^a	2839.96±15.64 ^a
	K2	17.39±0.32 ^b	8.84±0.16 ^d	47.47±0.20 ^b	94.42±2.01 ^b	83.41±1.82 ^e	7.60±0.05 ^c	7.26±0.15 ^d	9.05±0.40 ^b	15.69±0.55 ^e	52.15±0.36 ^b	2400.86±4.74 ^b
	K3	15.10±0.93 ^c	n.d	25.94±0.82 ^c	43.32±0.77 ^e	37.07±0.69 ^f	n.d	5.23±0.03 ^e	6.87±0.06 ^c	9.80±0.52 ^f	8.71±0.59 ^f	1285.62±0.87 ^c
	K4	6.07±0.69 ^e	n.d	16.09±0.91 ^e	32.12±0.88 ^g	37.48±1.52 ^f	n.d	4.05±0.16 ^f	5.19±0.01 ^d	8.75±0.23 ^g	2.60±0.07 ^g	506.32±12.41 ^g
	K5	5.31±0.31 ^e	n.d	5.10±0.17 ^g	12.04±0.87 ^h	9.42±0.07 ^g	n.d	3.45±0.29 ^g	1.15±0.06 ^g	n.d	n.d	248.28±2.33 ⁱ
	K6	1.32±0.04 ^f	n.d	0.35±0.33 ^h	11.84±0.88 ^h	9.67±1.29 ^g	n.d	2.43±0.20 ^h	n.d	n.d	n.d	42.01±0.72 ^j
	K7	1.17±0.06 ^f	n.d	n.d	8.49±0.55 ^h	6.29±0.32 ^{gh}	n.d	2.10±0.11 ^h	n.d	n.d	n.d	31.84±0.30 ^j
T	T1	17.88±0.35 ^{ab}	8.54±0.10 ^d	25.04±0.35 ^e	63.03±0.72 ^c	139.08±6.15 ^b	0.45±0.05 ^e	13.35±0.47 ^c	3.06±0.04 ^e	55.45±1.00 ^a	41.10±0.11 ^c	1196.08±17.77 ^d
	T2	11.14±1.85 ^d	9.70±0.29 ^e	13.78±1.45 ^f	55.87±1.38 ^d	124.14±2.24 ^c	3.33±0.02 ^d	19.56±0.08 ^a	2.38±0.11 ^f	35.65±0.60 ^b	29.67±0.61 ^e	821.05±17.49 ^e
	T3	15.99±0.61 ^e	25.05±0.89 ^a	19.05±0.47 ^d	52.91±0.40 ^d	105.02±1.61 ^d	8.45±0.14 ^b	14.51±0.05 ^b	n.d	26.72±1.41 ^d	30.78±0.18 ^d	563.77±5.23 ^f
	T4	4.85±1.14 ^e	6.99±0.39 ^e	14.15±1.53 ^{ef}	36.78±1.51 ^f	37.39±1.53 ^f	n.d	14.24±0.60 ^b	n.d	10.29±0.36 ^f	n.d	407.03±1.25 ^h
	T5	0.85±0.15 ^f	n.d	n.d	10.42±0.06 ^h	7.87±0.00 ^{gh}	n.d	n.d	n.d	n.d	n.d	15.19±0.53 ^k
	T6	0.80±0.02 ^f	n.d	n.d	10.54±0.27 ^h	5.50±0.36 ^h	n.d	n.d	n.d	n.d	n.d	14.06±0.22 ^k
	T7	0.84±0.05 ^f	n.d	n.d	8.62±0.54 ^h	5.25±0.04 ^h	n.d	n.d	n.d	n.d	n.d	9.82±0.32 ^k
C	F value	69.395	1196.046	1943.672	608.413	407.247	396.196	1504.940	2333.550	2834.144	6400.466	52101.164
	p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
RS	F value	647.419	1648.985	1856.343	1639.274	4052.680	928.012	2268.973	839.763	4824.914	45210.872	50629.737
	p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
CxRS	F value	19.075	1292.967	658.294	407.681	297.975	1050.200	842.349	440.890	619.961	8362.471	10088.850
	p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

*Different lowercase letters indicate differences between means based on 'Cultivars × Ripening stages' interaction according to Duncan multiple comparison test ($p < 0.05$). Data are presented as Mean ± Standard deviation (SD). C Cultivars, K Kureys, T Tahannebi, RS Ripening stage, GA Gallic acid, PCA Protocatechuic acid, NChA Neochlorogenic acid, 4-HBA 4-Hydroxy benzoic acid, CAT Catechin, VA Vanillic acid, PB2 Procyanidin B2, SA Syringic acid, EC Epicatechin, ECG Epicatechin gallate, CFA Caftaric acid. EL-27: (K1, T1), EL-31: (K2, T2), EL-33: (K3, T3), EL-35: (K4, T4), EL-36: (K5, T5), EL-38: (K6, T6), and EL-41 (K7, T7)

of 161.96, 149.68, and 16.75 mg/kg, respectively. Procyanidin B2 reached its maximum at T2 (EL 31 – berries pea-sized) with 19.56 mg/kg, then dropped to 2.10 mg/kg at K7. Vanillic acid was not detected from K3 through K7 and T4 through T7. The Kureys variety had higher values across all these compounds. In Tahannebi, T1 contained 63.03 mg/kg of 4-hydroxy benzoic acid, 139.08 mg/kg of catechin, 0.45 mg/kg of vanillic acid, and 13.35 mg/kg of procyanidin B2. However, in the ripening stages (T5–T7), these compounds dropped sharply, with 4-hydroxy benzoic acid below 11.00 mg/kg, catechin below 8.00 mg/kg, vanillic acid absent, and procyanidin B2 near zero.

Hydroxycinnamic acids

Neochlorogenic acid showed its highest value at K1 with 92.74 mg/kg and was undetectable by K7 and T5–T7, indicating a substantial reduction during ripening. The Tahannebi variety generally accumulated lower levels of phenolic compounds compared to Kureys. Caftaric acid had the overall highest concentration of all phenolics, reaching 2839.96 ± 15.64 mg/kg at K1. All of these compounds showed notable reductions in later stages, with syringic acid and epicatechin becoming undetectable at K6 and K7, and epicatechin gallate disappearing by K5. Caftaric acid declined drastically to 31.84 mg/kg at K7. In the Tahannebi variety, T1 showed caftaric acid at 1196.08 mg/kg. These values also declined throughout development, with caftaric acid falling to 9.82 mg/kg at T7. All three phenolic compounds (chlorogenic acid, caftaric acid, and p-coumaric acid) showed statistically significant differences between treatments, ripening stages, and their interactions (all $p < 0.001$). The treatment $\times$ ripening stage interactions indicated that the effect of treatments varied depending on the ripening stage for all compounds (Table 2). Chlorogenic acid demonstrated dramatic changes, with K1 treatment showing the highest concentration (1971.60 mg/kg), followed by a progressive decrease through K2 (1269.84 mg/kg), K3 (468.81 mg/kg), K4 (240.61 mg/kg), and K5 (94.03 mg/kg), reaching minimal levels in K6 (15.23 mg/kg) and complete elimination in K7. Tahannebi cultivar showed considerably lower concentrations overall, with T1 (18.32 mg/kg) and T2 (27.63 mg/kg) being the highest among ripening stages. Caftaric acid followed a similar pattern with K1 showing the highest concentration (3.92 mg/kg), decreasing to K2 (3.64 mg/kg) and K3 (1.74 mg/kg), followed by a sharp decline to K4 (1.04 mg/kg) and complete elimination from K5–K7 treatments. Among T treatments, T3 showed the highest concentration (6.09 mg/kg).

Flavonoids

In our study, rutin, myricetin, EGCG and naringin contents showed notable variations depending on phenological stages (EL 27–41) and cultivars ('Kureys' – K1–K7, and 'Tahannebi' – T1–T7). The highest values were recorded in 'Tahannebi', while undetectable levels were observed in 'Kureys'. A similar sharp decrease was observed in rutin, which was highest at EL 27 (K1) with 125.60 mg/kg, then dropped progressively, reaching 6.51 mg/kg at K7 (EL 38). Myricetin also declined from 11.89 mg/kg at K1 to 0.51 mg/kg at K6, with a complete absence at K7. Naringin content was highest at K1 (39.24 mg/kg) and showed a consistent decline, falling to 4.16 mg/kg by K6. Rutin showed moderate levels, peaking at T2 (EL 31) with 23.08 mg/kg and dropping to 1.98 mg/kg at T7. Myricetin content was 4.44 mg/kg at T4 (EL 35) but it was not detected at T6–T7 (EL 38–41). Naringin was relatively high at T3 (23.57 mg/kg) and declined to 2.91 mg/kg at T7. In the Kureys cultivar (K1–K7), EGCG content peaked at K1 (EL 27) with 16.62 mg/kg, while the lowest value was 2.46 mg/kg at K7 (EL 41), reflecting a decline of over 90%. For Tahannebi (T1–T7), the highest EGCG was found at T2 (EL 31) with 36.29 mg/kg, and the lowest at T6 (EL 38) with 2.39 mg/kg. TEAC levels ranged from 14.19 mg TEAC/g at T3 to 0.85 mg TEAC/g at T5.

Antioxidant capacity

ABTS was highest at K1 (34.47 mg TEAC/g) and dropped sharply to 1.10 mg TEAC/g at K7. DPPH values followed a similar trend, declining from 16.77 mg TEAC/g at K1 to 0.98 mg TEAC/g at K7. TPC values also showed a pronounced reduction, with a high of 11.18 ± 0.29 mg GAE/g at K1, decreasing to 0.45 mg GAE/g at K7. DPPH values decreased from 8.62 mg TEAC/g (T3) to 1.80 mg TEAC/g (T7). TPC reached a maximum of 5.21 mg GAE/g at T3 (EL 33), while the lowest was 0.42 mg GAE/g at T7 (EL 41). In both cultivars, all antioxidant-related parameters (ABTS, DPPH and TPC) decreased substantially toward the ripening stage (EL 41), with earlier stages showing markedly higher values.

Multivariate analysis

Our results demonstrated that PCA analysis revealed distinct groupings of samples based on their phenolic compound profiles. PC1 explained 68.43% of the variance while PC2 explained 16.53%, together representing 85% of the total variance (Fig. 2). Samples T1, T2, and T3 positioned in the upper right region showed strong positive correlations with ellagic acid and p-coumaric acid. In the upper left region, compounds including epicatechin, procatechuic

Table 2 Variations in the contents of selected polyphenolic compounds, and antioxidant parameters (ABTS, DPPH, TPC) in grape cultivars at different ripening stages

C	RS	CGA (mg/kg)	CAF (mg/kg)	p-COU (mg/kg)	EA (mg/kg)	RUT (mg/kg)	MYR (mg/kg)	NAR (mg/kg)	EGCG (mg/kg)	ABTS (mg TEAC/g)	DPPH (mg TEAC/g)	TPC (mg GAE/g)
K	K1	1971.60±40.36 ^a	3.92±0.35 ^b	4.53±0.32 ^b	1.32±0.07 ^f	125.60±4.07 ^a	11.89±0.57 ^a	39.24±0.17 ^a	16.62±0.70 ^c	34.47±0.75 ^a	16.77±2.40 ^a	11.18±0.29 ^a
	K2	1269.84±12.00 ^b	3.64±0.23 ^c	3.77±0.04 ^c	1.24±0.04 ^f	90.39±0.25 ^b	3.76±0.16 ^c	17.05±0.01 ^c	10.32±0.16 ^d	23.28±0.40 ^b	16.51±0.85 ^a	8.79±0.06 ^b
	K3	468.81±3.92 ^c	1.74±0.19 ^d	3.60±0.01 ^{c-e}	1.10±0.05 ^f	66.29±0.10 ^c	3.86±0.13 ^c	15.16±0.06 ^d	8.08±0.03 ^e	11.87±0.16 ^d	9.98±0.12 ^b	4.54±0.02 ^d
	K4	240.61±3.01 ^d	1.04±0.06 ^e	3.41±0.04 ^{d-f}	1.10±0.03 ^f	37.13±1.99 ^d	2.70±0.10 ^d	10.86±0.12 ^e	6.10±0.10 ^{fg}	8.59±0.16 ^f	6.97±2.51 ^{cd}	4.10±0.18 ^e
	K5	94.03±5.70 ^e	n.d	3.41±0.01 ^{d-f}	1.07±0.01 ^f	27.49±0.67 ^e	2.33±0.25 ^e	10.71±0.04 ^e	5.66±0.14 ^g	3.59±0.34 ^g	5.03±0.59 ^{ef}	1.36±0.19 ⁱ
	K6	15.23±1.48 ^{gh}	n.d	3.37±0.00 ^f	n.d	7.86±0.03 ⁱ	0.96±0.09 ^f	4.55±0.19 ^h	4.45±0.33 ^h	1.56±0.04 ^{ij}	1.03±0.34 ^h	0.61±0.01 ^j
	K7	n.d	n.d	3.38±0.01 ^f	n.d	6.51±0.15 ⁱ	0.51±0.04 ^g	4.16±0.03 ⁱ	2.46±0.15 ⁱ	1.10±0.08 ^{ij}	0.98±0.14 ^h	0.45±0.04 ^{jk}
T	T1	18.32±0.45 ^{gh}	n.d	7.42±0.03 ^a	9.58±0.16 ^c	13.94±0.38 ^{gh}	n.d	7.98±0.27 ^g	9.76±0.25 ^d	9.69±1.40 ^e	6.40±1.30 ^{de}	3.87±0.01 ^f
	T2	27.63±0.98 ^{fg}	n.d	3.62±0.21 ^{cd}	19.70±0.12 ^b	23.08±1.50 ^f	1.20±0.19 ^f	17.26±0.43 ^c	36.29±1.42 ^a	9.72±1.10 ^e	7.28±0.36 ^{cd}	3.46±0.26 ^g
	T3	44.64±2.44 ^f	n.d	3.69±0.19 ^c	25.08±0.98 ^a	15.43±0.30 ^g	3.53±0.02 ^c	23.57±0.42 ^b	18.30±0.05 ^b	14.19±0.39 ^c	8.62±0.12 ^{bc}	5.21±0.15 ^c
	T4	9.07±0.22 ^{gh}	n.d	3.38±0.02 ^{ef}	4.59±0.27 ^d	13.01±0.74 ^h	4.44±0.28 ^b	10.12±0.06 ^f	6.54±0.21 ^f	2.73±0.47 ^{gh}	3.36±0.41 ^{fg}	1.62±0.03 ^h
	T5	n.d	n.d	3.43±0.00 ^{d-f}	2.46±0.16 ^e	2.57±0.24 ^j	n.d	3.48±0.11 ^j	2.45±0.15 ⁱ	0.85±0.02 ^j	1.36±0.06 ^h	0.29±0.01 ^k
	T6	n.d	n.d	3.39±0.02 ^{ef}	2.56±0.13 ^c	2.30±0.07 ^j	n.d	2.96±0.15 ^k	2.39±0.14 ⁱ	1.95±0.00 ^{hi}	2.08±0.47 ^{gh}	0.66±0.00 ^j
	T7	n.d	n.d	3.45±0.06 ^{d-f}	2.44±0.07 ^e	1.98±0.04 ^j	n.d	2.91±0.05 ^k	2.64±0.21 ⁱ	1.39±0.05 ^{ij}	1.80±0.40 ^{gh}	0.42±0.08 ^{jk}
C	F value	25589.105	147.566	139.371	9815.139	10388.255	1522.373	6117.982	637.791	1312.956	133.201	2878.251
	p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
RS	F value	6673.533	500.721	410.206	1730.703	2288.060	634.236	9722.404	1676.093	1312.469	112.581	2797.054
	p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
CXRS	F value	6395.553	439.483	137.137	1518.982	1293.699	732.476	6022.866	921.432	452.304	27.628	774.072
	p-value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

*Different lowercase letters indicate differences between means based on 'Cultivars × Ripening stages' interaction according to Duncan multiple comparison test ($p < 0.05$). Data are presented as Mean ± Standard deviation (SD). C Cultivars, K Kureş, T Tahannebi, RS Ripening stage, CGA Chlorogenic acid, CAF Caffeic acid, p-COU-p-Coumaric acid, EA Ellagic acid, RUT Rutin, MYR Myricetin, NAR Naringin, EGCG Epigallocatechin gallate; ABTS, DPPH, TPC Total phenolic content. EL-27: (K1, T1), EL-31: (K2, T2), EL-33: (K3, T3), EL-35: (K4, T4), EL-36: (K5, T5), EL-38: (K6, T6), and EL-41 (K7, T7)

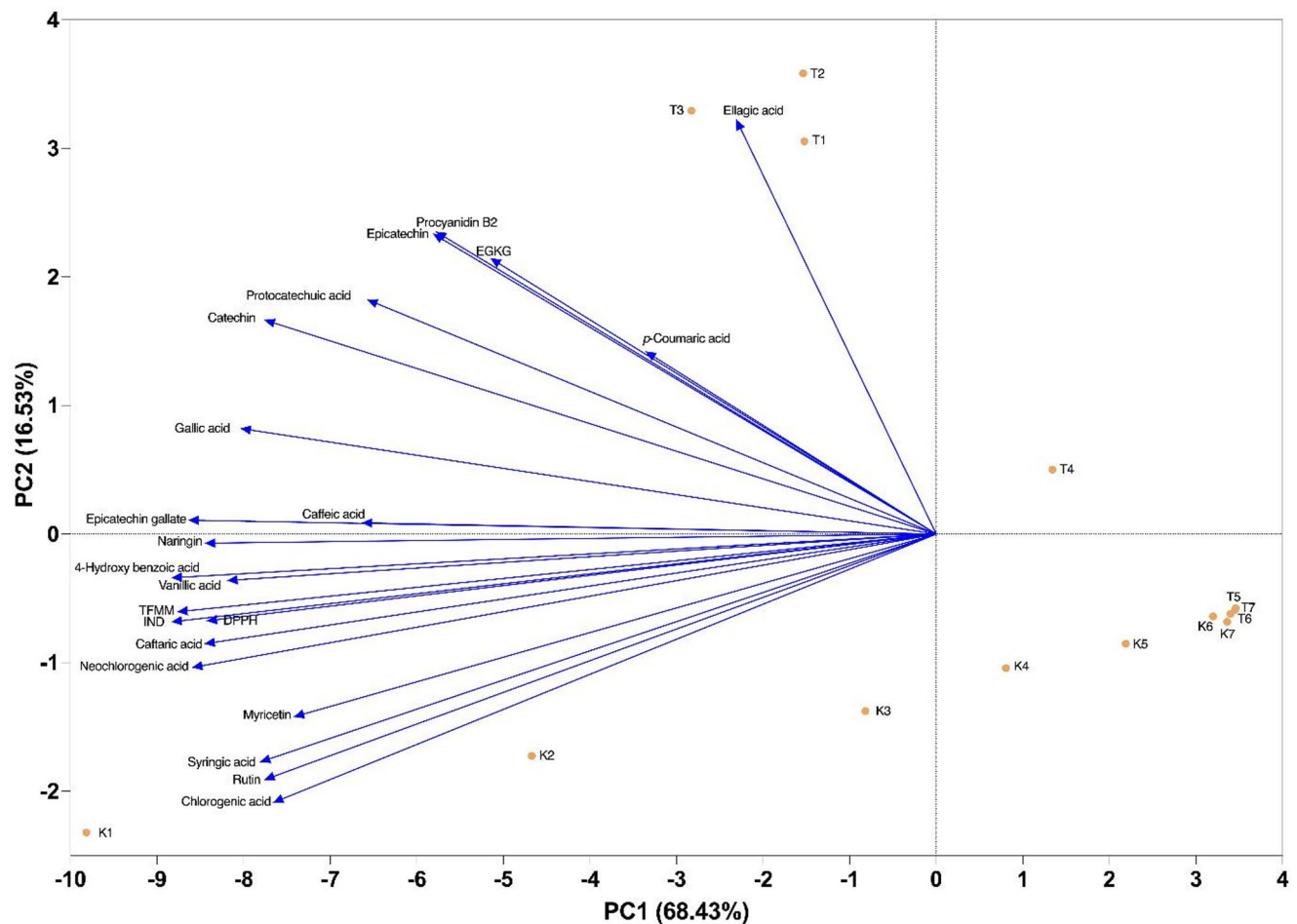


Fig. 2 Principal component analysis (PCA) biplot of phenolic compounds and samples showing the relationship between different phenolic metabolites and sample groups (T1–T7 and K1–K6) based on their chemical profiles. EL-27: (K1, T1), EL-31: (K2, T2), EL-33: (K3, T3), EL-35: (K4, T4), EL-36: (K5, T5), EL-38: (K6, T6), and EL-41 (K7, T7), EGKG: EGCG: Epigallocatechin gallate, IND: ABTS: The capac-

ity of scavenging ABTS radicals, TFMM: TPC: Total phenolic content. K1–K7 and T1–T7 represent the sequential phenological stages (Eichhorn–Lorenz scale) of ‘Kureys’ and ‘Tahannebi’ grapes, respectively, with colors corresponding to developmental progression from early growth to full maturity

acid, catechin, and gallic acid clustered together, showing negative correlation with PC1 and positive relationships among themselves. The lower region contained samples K2 and K3 along with chlorogenic acid, syringic acid, rutin, and myricetin, characterized by flavonoids and phenolcarboxylic acids. Samples K4, K5, K6, K7, T4, T5, T6, and T7 clustered in the lower right region generally possessed low phenolic compound content, while sample K1 was distinctly separated from all other samples, displaying a unique phenolic profile. Overall, T samples exhibited more heterogeneous distribution whereas K samples (except K1) formed a more homogeneous group in terms of their phenolic compound composition. Our results revealed significant correlations among phenolic compounds and antioxidant activity parameters through correlation matrix analysis (Fig. 3). Strong positive correlations ($r > 0.8$) were observed among several phenolic compounds, particularly between

gallic acid, protocatechuic acid, neochlorogenic acid, and 4-hydroxy benzoic acid. Catechin, vanillic acid, and procyanidin B2 also showed high correlations. Epicatechin and epicatechin gallate demonstrated moderate to strong correlations with most phenolic compounds, while ellagic acid showed weaker correlations with other compounds. Among antioxidant activity parameters, DPPH and TPC showed strong positive correlations ($r > 0.9$). The correlation analysis also revealed that certain phenolic compounds such as gallic acid, protocatechuic acid, and catechin showed stronger associations with antioxidant activities compared to others like ellagic acid and p-coumaric acid. Our results demonstrated clear clustering patterns through hierarchical cluster analysis, revealing distinct sample groupings based on their phenolic compound profiles and antioxidant activities (Fig. 4). The dendrogram identified two main clusters: Group 1 consisting primarily of K samples (K1, K2, K3,

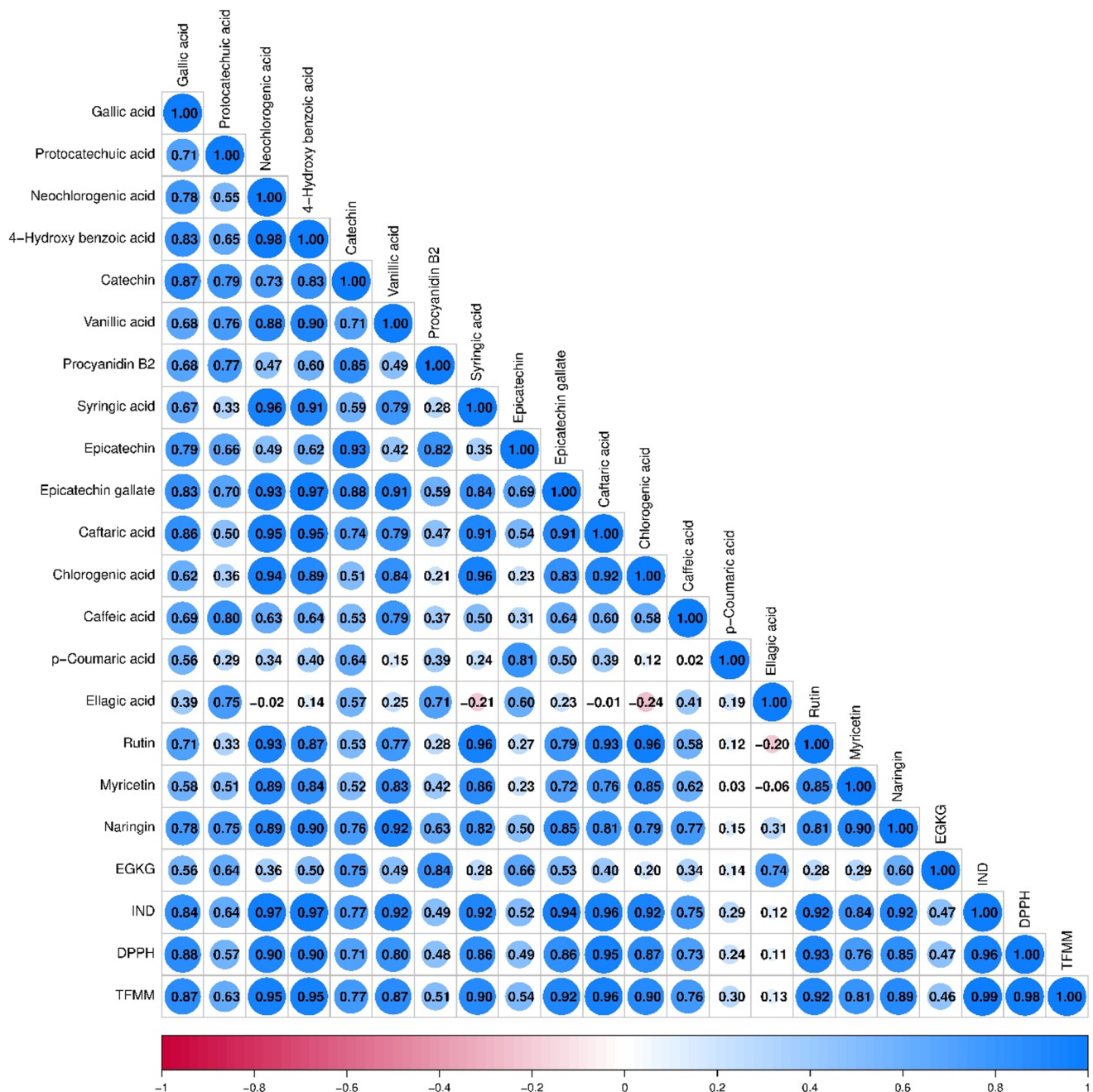


Fig. 3 Correlation matrix heatmap showing Pearson correlation coefficients between phenolic compounds and antioxidant activity parameters. EL-27: (K1, T1), EL-31: (K2, T2), EL-33: (K3, T3), EL-35: (K4,

T4), EL-36: (K5, T5), EL-38: (K6, T6), and EL-41 (K7, T7), EGKG: EGKG: Epigallocatechin gallate, IND: ABTS: ABTS radical scavenging activity, TFMM: TPC: Total phenolic content

K4, K5, T6, T7, T5, K6, K7) and Group 2 containing most T samples (T2, T3, T4, T1). Sample K1 showed the highest concentrations of most phenolic compounds, displaying intense red coloration for gallic acid, protocatechuic acid, neochlorogenic acid, catechin, and antioxidant activity parameters. Samples T2 and T3 exhibited elevated levels of specific compounds including ellagic acid, p-coumaric acid, and procyanidin B2, distinguishing them from other

samples. The lower cluster samples (K5, T6, T7, T5, K6, K7) generally showed reduced phenolic compound concentrations, represented by blue coloration across most parameters. The hierarchical clustering effectively separated samples with high phenolic content and antioxidant activity from those with lower concentrations, providing a clear visualization of the biochemical diversity among the sample groups.

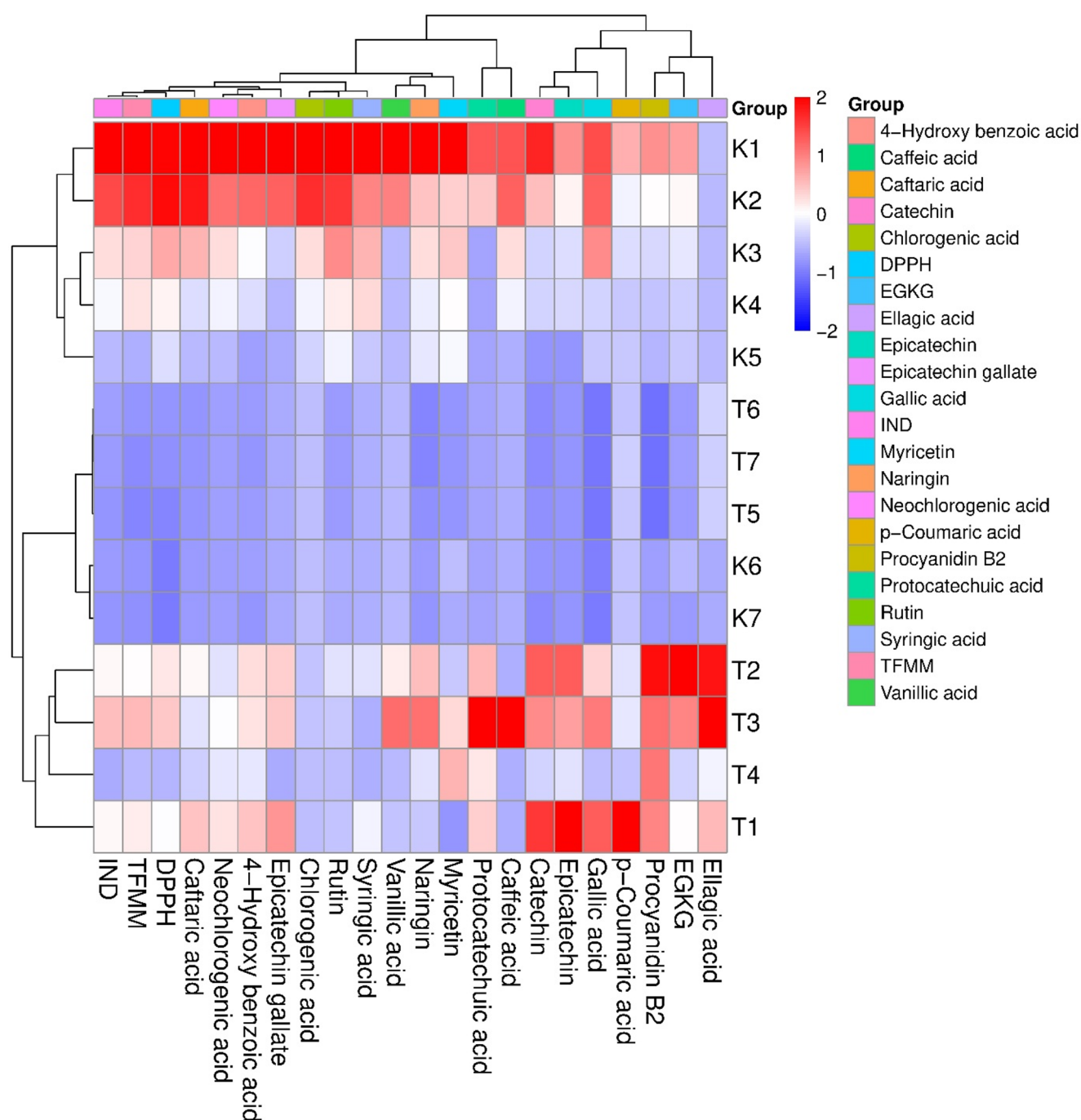


Fig. 4 Hierarchical cluster analysis heatmap showing the distribution and clustering patterns of phenolic compounds and antioxidant activities across sample groups. EL-27: (K1, T1), EL-31: (K2, T2), EL-33: (K3, T3), EL-35: (K4, T4), EL-36: (K5, T5), EL-38: (K6, T6),

and EL-41 (K7, T7), EGKG: EGCG: Epigallocatechin gallate, IND: ABTS: The capacity of scavenging ABTS radicals, TFMM: TPC: Total phenolic content

Discussion

Phenolic compound changes during ripening

In our results, the significant differences observed among grape varieties (Kureys and Tahannebi), developmental

stages (EL 27 to EL 41), and their interactions for all phenolic compounds align with established patterns in grape ripening research [3, 17, 21, 25, 48]. The consistently higher phenolic content in Kureys variety compared to Tahannebi throughout all developmental stages reflects genetic control over polyphenolic biosynthesis, as genotype highly affects

the polyphenolic composition in grapes and, consequently, the nutritional properties. The progressive decline in gallic acid from 18.84 mg/kg at K1 to 1.17 mg/kg at K7 mirrors findings in other grape varieties. Similar reductions have been documented in Maratheftiko grapes, where polyphenolic levels, particularly flavonoids (i.e., catechin and rutin) and phenolic acids, drop considerably during final ripening stages [41, 42]. The complete absence of protocatechuic acid in later stages (K3–K7 and T5–T7) corroborates research on Plavac Mali and Trnjak varieties, where dramatic polyphenol declines were attributed to enzymatic breakdown and oxidative processes during grape maturation [1, 24]. The substantial reduction in neochlorogenic acid from 92.74 mg/kg at K1 to undetectable levels by K7 in Kureys suggests active metabolic conversion rather than dilution effects. The timing of compound-specific disappearance provides insights into metabolic priorities, with vanillic acid disappearing earlier (from K3 onwards) compared to flavonoids persisting until K6–K7, suggesting differential metabolic fates for distinct phenolic classes. The highest concentrations of 4-hydroxy benzoic acid, catechin, and vanillic acid at K1 (161.96, 149.68, and 16.75 mg/kg, respectively) support the concept that early ripening stages represent peak biosynthetic activity. The decline in seed catechin and epicatechin occurs concomitantly with seed browning, and the amount of flavan-3-ols remaining in seeds at harvest appears to depend on the variety, which directly explains our observations of catechin levels dropping to below 8.00 mg/kg in later Tahannebi stages. The exceptional caftaric acid concentration of 2839.96 mg/kg at K1 in Kureys, declining drastically to 31.84 mg/kg at K7, represents one of the most dramatic phenolic reductions observed. Similar trends have been reported in other grape cultivars. For instance, Roufas et al. [41] observed an 85–90% reduction in catechin and epicatechin contents and over 95% decline in caffeic acid from early berry development to harvest in *Vitis vinifera* cv. Maratheftiko. Likewise, Asproudi et al. [2] documented marked decreases in proanthocyanidins during ripening of Nebbiolo and Barbera grapes. These reductions are comparable to those observed in our study, such as the 98.8% decrease in chlorogenic acid content from EL-27 to EL-41 in the Kureys cultivar. This pattern has been documented across diverse cultivars including Nebbiolo and Barbera varieties, where similar phenolic degradation patterns occur during ripening [2]. We propose that this massive caftaric acid decline reflects its conversion to other phenolic compounds or its utilization in lignification processes during berry development. Phenolic compounds, while important for human nutrition, play critical physiological roles in plants, including UV protection, pathogen defense, and regulation of tissue development [36, 49].

Chlorogenic acid, caftaric acid, and p-coumaric acid dynamics

These compositional changes are associated with shifts in phenylpropanoid pathway regulation and the activity of enzymes such as phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO), which are developmentally regulated during berry ripening [16, 40]. The complete reduction in chlorogenic acid from K1 to K7 represents one of the most dramatic phenolic changes documented in our study. This substantial decline exceeds reductions reported in many other grape varieties, suggesting that Kureys may possess particularly active chlorogenic acid catabolism during ripening. The lower initial concentrations in Tahannebi (18.32 mg/kg at T1) further support the existence of variety-specific differences in hydroxycinnamic acid metabolism. Similar trends in chlorogenic acid decline have been observed during wine aging and grape development. For instance, Laqui-Estaña et al. [29] reported marked reductions in chlorogenic and related phenolic acids during oak aging in Carménère and Cabernet Sauvignon wines, suggesting enzymatic oxidation or transformation during phenolic maturation. Although their study focused on post-harvest transformations, the observed decline aligns with our hypothesis that metabolic pathways redirect chlorogenic acid toward other secondary metabolites as ripening progresses. The complete elimination of caftaric acid from K5–K7 treatments, parallels trends observed in Mediterranean varieties and in fermentation-driven systems. Lisov et al. [32] described a similar depletion of caftaric acid during Cabernet Sauvignon fermentation, attributing this trend to polymerization reactions or enzymatic transformations that lead to the formation of more complex phenolics such as flavonoids or tannins. In our study, the temporal accumulation of caftaric acid peaking at T3 (6.09 mg/kg) in Tahannebi supports the view that this compound may act as a transient precursor during early berry development, participating in stress defense or biosynthetic pathways. p-Coumaric acid dynamics also followed a declining pattern, consistent with the findings of Qiang et al. [39], who observed developmental stage-dependent reductions in hydroxycinnamic acids in Cabernet Franc grapes subjected to chitoooligosaccharide treatments. Their study highlights the role of elicitors in modulating phenolic biosynthesis, but the natural decline seen in our untreated samples suggests intrinsic developmental regulation, possibly involving phenylpropanoid pathway reallocation. Together, these trends indicate that hydroxycinnamic acid derivatives undergo enzymatic conversion, likely contributing to structural development (e.g., lignin formation) or the synthesis of complex defense compounds. The synchronized decline of chlorogenic, caffeic, and p-coumaric acids with key developmental transitions

supports the hypothesis of active metabolic channeling rather than passive degradation.

Flavonoid compound variations and antioxidant activity parameters

The notable variations in ellagic acid, rutin, myricetin, and naringin contents across phenological stages showed the intricate regulation of flavonoid biosynthesis during grape development. The peak ellagic acid concentration observed in Tahannebi at EL 33 (T3, 25.08 mg/kg), in contrast to the comparatively lower levels in Kureys, suggests genotype-specific optimization strategies for flavonoid accumulation. Such variability aligns with the findings of Kamah et al. [23], who reported varietal differences in flavonoid compound profiles across ripening stages in Algerian grape cultivars, highlighting distinct genetic regulation of phenolic metabolism. The progressive decline in rutin from 125.60 mg/kg at K1 to 6.51 mg/kg at K7 in Kureys follows well-established ripening trends. Awad et al. [4] demonstrated a similar decline in rutin and other flavonols in ‘El-Bayadi’ grapes, attributing it to the hydrolysis of glycosylated flavonoids and subsequent conversion into downstream phenolic derivatives. Furthermore, the near-complete disappearance of myricetin at K7 and the gradual reduction in naringin content throughout ripening indicate coordinated catabolic processes likely modulated by flavonoid-specific oxidases and glycosidases. These dynamics reflect changing physiological priorities as berries shift from active defense and growth to sugar accumulation and seed maturation. Qin et al. [40] observed similar developmental-stage-dependent shifts in flavonoid accumulation in grape berries grown under different climates, suggesting that both environmental and genetic factors modulate biosynthetic rates. In our study, the differences in timing, Kureys having higher early-stage concentrations, and Tahannebi showing intermediate-stage peaks, may represent evolutionary divergences in balancing photoprotection, oxidative defense, and reproductive investment.

The marked decline in epigallocatechin gallate (EGCG) content, over 90% from K1 to K7 in Kureys, illustrates the susceptibility of gallate esters to enzymatic or oxidative degradation during late ripening. This is consistent with the findings of Benbouguerra et al. [5], who documented similar reductions in galloylated catechins in Merlot, Tannat, and Syrah varieties as berries approached harvest maturity. Interestingly, in Tahannebi, the EGCG peak at T2 (36.29 mg/kg) contrasts with the earlier K1 peak in Kureys, highlighting genotype-specific temporal shifts in flavanol accumulation. These differences could be linked to varietal variation in galloyltransferase activity or differential susceptibility to oxidative enzymes such as peroxidases and

polyphenol oxidases [16]. The parallel decline observed in antioxidant activity parameters, ABTS, DPPH, and TPC, across both varieties toward EL 41 further supports a direct relationship between phenolic content and radical scavenging capacity. The decrease in ABTS activity from 34.47 to 1.10 mg TEAC/g in Kureys mirrors patterns reported by Genova et al. [20, 40], where antioxidant potential consistently declined with berry ripening in multiple *Vitis vinifera* cultivars. These consistent patterns suggest a universal physiological shift in berry metabolism from defense-oriented phenolic synthesis to processes supporting ripeness and palatability. The strong correlations observed between antioxidant parameters and key flavonoids such as rutin, EGCG, emphasize the health-promoting potential of early-harvested grapes. However, this must be carefully weighed against enological considerations, as premature harvest may compromise sugar accumulation and flavor development. Based on our findings and consistent with metabolomic observations by Darwish et al. [15], we propose that the coordinated decline in antioxidant-related compounds reflects a fundamental reprogramming of berry metabolism, from oxidative stress protection to resource allocation for seed viability and dispersal.

Multivariate analyses

Principal component analysis (PCA), correlation matrices, and hierarchical clustering jointly revealed clear varietal and developmental distinctions in phenolic metabolism. The PCA results, explaining 85% of the total variance through PC1 and PC2, demonstrated distinct metabolic clustering, particularly the outlier status of K1, which exhibited exceptional phenolic richness (notably gallic acid, catechin, and antioxidant activity). This suggests intense early biosynthetic activity in Kureys, a finding consistent with reports by Kamah et al. [23] and Benbouguerra et al. [5], who noted similar early-stage phenolic accumulations. Conversely, Tahannebi samples (T1–T3) clustered around compounds such as ellagic and p-coumaric acids, indicating prolonged retention of certain phenolics during mid-ripening, a pattern previously reported in Mediterranean cultivars [40]. This differential metabolic pacing may reflect varietal adaptations in phenylpropanoid pathway regulation, possibly influenced by environmental or genotypic factors (Darwish et al. 2021). These results highlight the shared biosynthetic regulation of key antioxidant-related phenolics, as also demonstrated in previous grape studies [20, 32]. In contrast, ellagic acid showed weaker correlations, implying a more distinct biosynthetic or compartmental behavior. Hierarchical clustering further emphasized varietal differences: Kureys samples grouped more tightly, reflecting uniform phenolic degradation patterns, while Tahannebi exhibited

greater variability. Despite these early differences, late-stage samples from both varieties converged into a biochemically similar cluster, marked by low phenolic content, supporting the concept of ripening-driven metabolic convergence [5, 29]. Altogether, these multivariate analyses suggest that early ripening stages are critical for phenolic accumulation and antioxidant potential, with genotype-specific timing and regulation shaping the dynamics. Monitoring key compounds such as gallic acid, catechin, and ellagic acid could serve as practical indicators for optimal harvest timing and grape quality management tailored to varietal profiles and end-use goals. The observed phenolic dynamics are direct indicators of physiological reprogramming during ripening, marking the shift from defense-related secondary metabolism to resource allocation for seed maturation [5].

Conclusion

This paper has highlighted the importance of understanding variety-specific phenolic dynamics and antioxidant responses during grape ripening. We have succeeded in characterizing the temporal shifts in key flavonoid and phenolic acids, confirming that Kureys and Tahannebi grapes follow distinct metabolic strategies. Our multivariate approach has provided comprehensive results demonstrating that Kureys shows intense early phenolic accumulation, while Tahannebi exhibits a more gradual and prolonged retention of certain compounds. We have devised an effective analytical framework combining PCA, correlation analysis, and hierarchical clustering, which offers a powerful tool for interpreting phenolic metabolism in relation to developmental stages. Considerable insight has been gained into how specific compounds, such as gallic acid, catechin, and ellagic acid, contribute differently to overall antioxidant potential. Taken together, these findings suggest that harvest timing and variety selection should be aligned with specific quality targets, such as maximizing antioxidant capacity or tailoring phenolic profiles for functional products. Our research provides a basis for future work aimed at optimizing grape quality through targeted phenolic monitoring. Although the current study is limited to two varieties, we believe our methodology and findings can be generalized to broader grape breeding and viticultural strategies. Further research is already underway to validate these insights under different climatic and agronomic conditions. In summary, ‘Kureys’ and ‘Tahannebi’ exhibited distinct phenolic accumulation patterns across berry development, with practical implications for harvest timing and cultivar-specific processing strategies. These findings can guide viticultural practices aiming to optimize both nutritional value and market quality.

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Author contributions Author Contributions S.A. and Y.U. contributed to the study conceptualization, experimental design, and data collection. N.D. and R.Z. were responsible for laboratory analyses and data acquisition. T.D. and S.E. conducted the formal statistical analyses, supervised and data interpretation. H.H.V. provided critical revision of the manuscript content. S.E. contributed to the methodology development and experimental investigation. O.K. performed additional statistical analyses, and the writing, review, and editing process. All authors contributed to the preparation of the original manuscript draft, have read and approved the final version, and agree to be accountable for all aspects of the work.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflict of interest.

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