

Article

Growing Site and Cultivar Effects on Fruit Quality, Phenology and Antioxidant Capacity of Sweet Cherry (*Prunus avium* L.) Cultivars in Eskişehir, Türkiye

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Abstract

This study compared four sweet cherry cultivars—Regina, Sweetheart, Kordia and 0900 Ziraat—at two sites of contrasting altitude in Eskişehir province, Türkiye: Eskişehir (792 m) and Sarıcakaya (220 m). Across two growing seasons (2016–2017; $n = 64$), phenological stages were recorded and fruit were assessed for eighteen pomological traits and three phytochemical measures—total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging activity. Location affected almost every pomological trait measured: fruit from the cooler Eskişehir site were heavier and more intensely red, whereas fruit from the warmer Sarıcakaya site had higher soluble solids content and acidity, and reached bud break and bloom roughly 13 days earlier. TPC, TFC and DPPH activity were all higher at Eskişehir than at Sarıcakaya (TPC: 1071.0 vs. 823.3 mg GAE kg⁻¹ FW; TFC: 344.3 vs. 234.6 mg CE kg⁻¹ FW; DPPH: 45.5% vs. 22.5% inhibition; $p < 0.001$). Kordia stood out with the highest antioxidant activity and was also among the cultivars richest in phenolics and flavonoids. TPC and TFC were strongly correlated ($r = 0.93$), and both tracked with skin redness and lower titratable acidity. Overall, growing location, rather than cultivar, was the strongest factor behind the pomological, phenological and phytochemical differences observed over the two study years.

Keywords: *Prunus avium*; altitude; genotype-by-environment interaction; total phenolic content; antioxidant capacity; phenology



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1. Introduction

Sweet cherry (*Prunus avium* L.) ranks among the economically important temperate fruit species, grown for the fresh market and, increasingly, valued for its phenolic and antioxidant content, properties that have been associated with a range of health benefits [1]; interest has extended beyond the fresh fruit itself to bioactive compounds recovered from processing by-products such as pomace and kernel oil [2,3]. Türkiye leads global sweet cherry production [4], and breeding trials that bring foreign genotypes such as Regina, Sweetheart and Kordia together with the widely grown domestic cultivar 0900 Ziraat have become routine across Turkish orchards, as growers seek cultivars offering high yield alongside large fruit size and strong eating and nutritional quality [5,6].

Fruit size, colour, ripening time and phytochemical content are shaped by the growing environment as much as by genotype. Temperature during dormancy and flowering governs the timing of bud break, bloom and harvest [7,8], while temperature and light after bloom affect fruit growth, sugar accumulation, colour and phenolic and flavonoid

production [1]. Altitude is a convenient natural gradient for studying these effects together, since it changes mean temperature, the day–night temperature range and solar radiation all at once, giving a useful way to explore how genotype and environment interact in fruit crops. In Lebanon, sweet cherry grown at different altitudes showed total phenolic content and reducing power that changed with growing site, whereas radical scavenging activity depended more on cultivar [9]. An earlier Greek study similarly found up to 85% more total phenolics and antioxidant capacity in fruit from higher, cooler orchards than from lower ones [10]. Cultivar trials in Türkiye have repeatedly identified Kordia as one of the most phenolic and antioxidant-rich commercial cultivars [5,11], while cultivar and rootstock together have also been shown to affect fruit weight and total phenolics [12,13]—a reminder that cultivars need testing at more than one site before being recommended for a region.

Despite the growing number of Turkish cultivar trials, few studies have combined a detailed, multi-year pomological and phenological record with phytochemical characterisation of the same trees at more than one growing site. We addressed this gap using Regina, Sweetheart, Kordia and 0900 Ziraat at two Eskişehir province sites differing mainly in altitude, tracking two complete growing seasons of pomological data—fruit size, colour and stone characteristics—together with a detailed phenological record for each cultivar at each site and phytochemical assays (total phenolic content, total flavonoid content and DPPH antioxidant activity) covering both the 2016 and 2017 harvests. Bringing these three strands together allows fruit quality, ripening time and phytochemical content to be considered together for the same cultivars and sites. Turkish trials to date have generally examined either phenology, pomological quality or phytochemical content in isolation, or have compared cultivars within a single site; what remains largely untested is whether the same trees, followed for more than one season, show a consistent pattern across all three strands at two markedly different growing sites.

A recent study at these same two Eskişehir province orchards examined how site-related climatic differences shape the concentration of individual organic and phenolic acids in the same four cultivars, using molecular docking to model how malic acid and chlorogenic acid might bind the proprotein convertase furin [14]. That study drew fruit from trees allocated independently of the present trial, with no trees, fruit lots, dataset or authorship shared between the two studies. It focused on a narrow set of individual organic and phenolic acids and an *in silico* binding mechanism, rather than on the pomological, phenological and total phenolic, total flavonoid and antioxidant profile addressed here across two complete growing seasons; the two studies ask different questions of the same growing environment and are complementary rather than overlapping.

This study had three objectives: to measure how location, growing season and cultivar affected 18 pomological traits of Regina, Sweetheart, Kordia and 0900 Ziraat sweet cherry over 2016 and 2017; to describe the phenological calendar of the four cultivars at both sites in relation to recorded weather conditions; and to quantify total phenolic content, total flavonoid content and DPPH radical scavenging activity in the same cultivars at both sites, linking these phytochemical traits to fruit pomological and colour characteristics.

2. Materials and Methods

2.1. Plant Material and Study Sites

Fieldwork spanned the 2016 and 2017 growing seasons at two Eskişehir province sites, Türkiye, separated by roughly 572 m in altitude: Eskişehir (Odunpazarı district, 792 m; 39°45′36.12″ N, 30°27′57.71″ E) and Sarıcakaya (220 m; 40°3′27.55″ N, 30°43′55.65″ E) (Table 1, Figure 1). The trial included four sweet cherry cultivars—Regina, Sweetheart, Kordia and 0900 Ziraat. Trees at both sites were planted in 2011, grafted on Maxma 14

rootstock, trained to a modified leader system, and irrigated and fertilised through a drip system under similar routine annual orchard management. Maxma 14 (*Prunus avium* × *Prunus mahaleb*) is a semi-vigorous interspecific hybrid rootstock that has performed well with sweet cherry, including the 0900 Ziraat cultivar used here, under Turkish growing conditions [15], which supported its choice for both orchards in this trial. Fruit for the present trial were sampled from trees allocated independently of those used in a related organic-acid and molecular-docking study conducted at the same two orchards [14]; no trees, fruit lots or dataset were shared between the two studies. Since both orchards were established in the same year, on the same rootstock and training system, and received the same irrigation, fertilisation and routine care, orchard age and management are unlikely to explain the differences found between the two sites; however, because each location is represented by a single orchard, altitude and its associated climate cannot be fully separated from other unmeasured site-specific factors—soil properties, water source or local topography—that may also differ between Eskişehir and Sarıcakaya. At each location, eight trees per cultivar were sampled in four replicates of two trees each, giving a balanced design of 4 cultivars, 2 locations and 2 years ($n = 64$) for the pomological and phenological data. Ten fruit were sampled per tree at full commercial maturity, placed in ice-filled coolers and transported to the laboratory for measurement. Additional fruit destined for phytochemical analysis (total phenolic content, total flavonoid content and antioxidant activity) came from the same orchards and harvest dates in 2016 and 2017: samples for total phenolic and total flavonoid content were pooled into three replicate groups of two trees each per cultivar, location and year and analysed once each, whereas samples for DPPH radical scavenging activity were pooled into four replicate groups of two trees each per cultivar and location, with every extract measured twice and the pair of readings averaged.

Table 1. Characteristics of the two study sites in Eskişehir province, Türkiye.

Site	District	Altitude (m)	Notes
Eskişehir	Odunpazarı, Eskişehir	792	Central Anatolian plateau. Semi-arid continental climate with cold winters and hot, dry summers.
Sarıcakaya	Sarıcakaya, Eskişehir	220	Sheltered valley about 60 km from the Eskişehir city centre, surrounded by higher terrain. Known locally for a warmer microclimate, roughly 570 m lower than the Eskişehir site.



Figure 1. Location of the study area within Türkiye (a) and the two sweet cherry study sites in Eskişehir province (b): Eskişehir (Odunpazarı, 792 m a.s.l.) and Sarıcakaya (220 m a.s.l.).

Representative ripe-fruit cross-sections of the four cultivars used in this trial are shown in Figure 2.

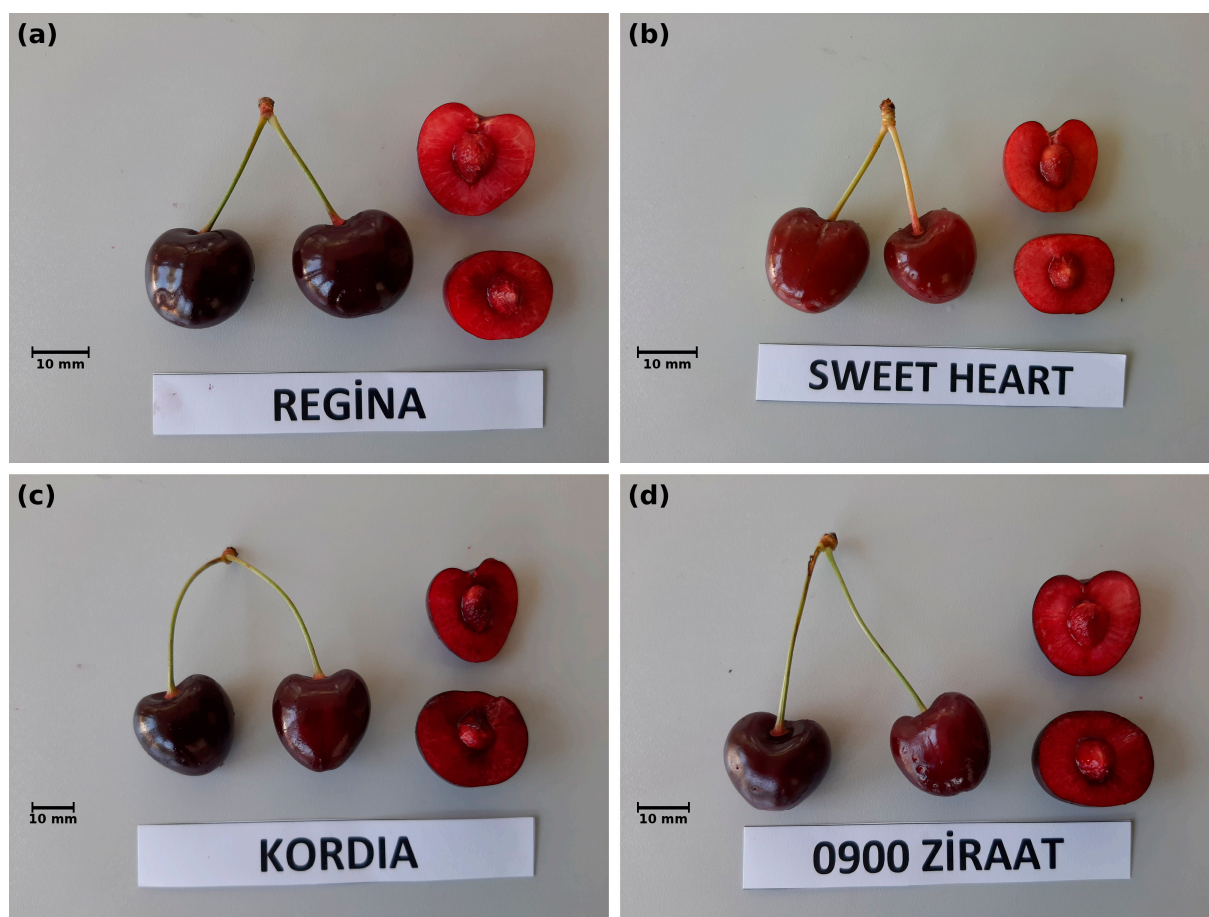


Figure 2. Representative ripe-fruit cross-sections of four sweet cherry cultivars: (a) Regina; (b) Sweet-heart; (c) Kordia; (d) 0900 Ziraat. Scale bars = 10 mm.

2.2. Meteorological Data

We obtained daily records of maximum, minimum and mean air temperature, along with mean relative humidity and total precipitation, for 2016 and 2017 from the Turkish State Meteorological Service (T.C. Ministry of Environment, Urbanisation and Climate Change, General Directorate of Meteorology), covering the Eskişehir Bölge station (no. 17126) and the Sarıcakaya station (no. 18091). Daily records were combined into monthly means of temperature and relative humidity, and monthly totals of precipitation, at both stations and in both years, in order to relate weather conditions to phenological timing and fruit quality (Table 2, Figure 3).

Table 2. Monthly mean air temperature and total precipitation at the Eskişehir and Sarıcakaya meteorological stations, 2016 to 2017.

Month	Eskişehir 2016	Eskişehir 2017	Sarıcakaya 2016	Sarıcakaya 2017
January	0.5 °C, 78.9 mm	−1.7 °C, 29.0 mm	3.7 °C, 58.2 mm	2.5 °C, 23.0 mm
February	7.6 °C, 26.3 mm	2.8 °C, 3.5 mm	9.9 °C, 43.0 mm	6.0 °C, 3.1 mm
March	8.3 °C, 46.1 mm	8.5 °C, 16.6 mm	11.1 °C, 31.3 mm	11.0 °C, 31.7 mm
April	14.4 °C, 25.6 mm	10.8 °C, 52.7 mm	16.5 °C, 38.0 mm	14.2 °C, 60.4 mm
May	15.3 °C, 45.8 mm	15.4 °C, 48.8 mm	17.8 °C, 75.9 mm	19.1 °C, 90.4 mm
June	22.1 °C, 14.2 mm	20.1 °C, 38.1 mm	24.9 °C, 30.3 mm	23.6 °C, 46.8 mm

Table 2. Cont.

Month	Eskişehir 2016	Eskişehir 2017	Sarıcakaya 2016	Sarıcakaya 2017
July	23.4 °C, 1.7 mm	23.7 °C, 13.5 mm	27.0 °C, 0.4 mm	26.7 °C, 2.3 mm
August	23.3 °C, 41.9 mm	22.4 °C, 27.5 mm	27.1 °C, 15.9 mm	26.8 °C, 49.4 mm
September	18.6 °C, 28.1 mm	20.9 °C, 3.7 mm	21.8 °C, 52.5 mm	25.1 °C, 2.0 mm
October	13.6 °C, 3.8 mm	11.9 °C, 46.1 mm	16.1 °C, 2.6 mm	15.3 °C, 40.8 mm
November	6.9 °C, 23.6 mm	6.7 °C, 12.9 mm	9.1 °C, 26.6 mm	10.4 °C, 22.8 mm
December	−0.3 °C, 32.7 mm	4.5 °C, 36.2 mm	2.0 °C, 24.4 mm	8.6 °C, 37.6 mm

Source: Turkish State Meteorological Service, stations Eskişehir Bölge (17126) and Sarıcakaya (18091). Values are monthly means of daily mean temperature and monthly sums of daily precipitation.

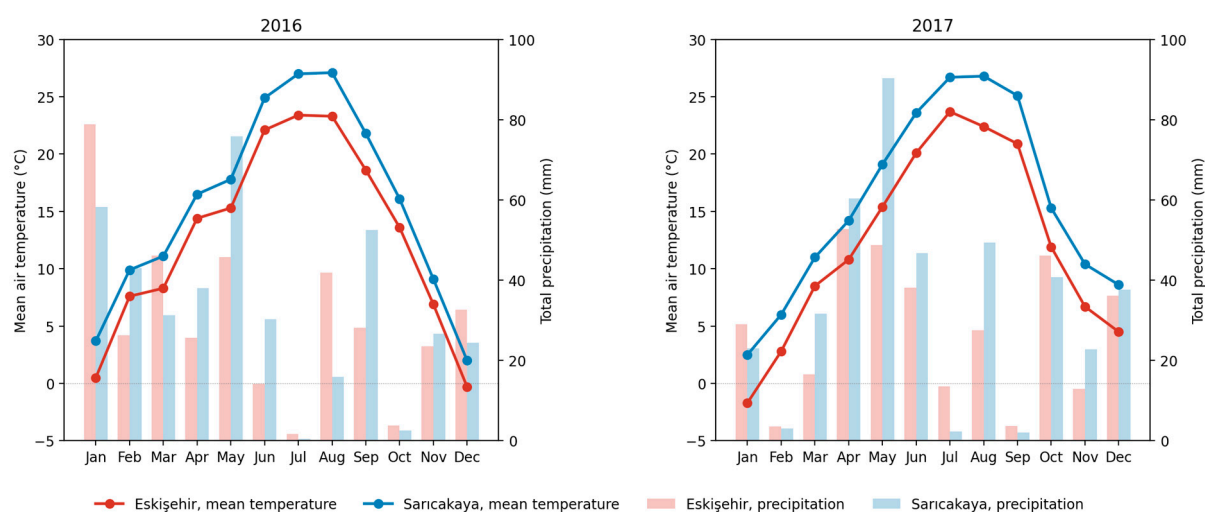


Figure 3. Monthly mean air temperature (lines, left axis) and total precipitation (bars, right axis) at the Eskişehir (792 m) and Sarıcakaya (220 m) meteorological stations, shown as separate panels for 2016 and 2017.

2.3. Phenological Observations

At both locations, in both years, the following stages were recorded in the field for each cultivar: bud swelling, beginning of bloom, full bloom, onset of veraison (colour change and pit hardening, locally called *ben düşme*), and harvest maturity. Stage dates were converted to day of year for analysis. The bloom-to-maturity interval, from beginning of bloom to harvest maturity, was calculated for each cultivar, location and year combination (Table 3). Harvest maturity was judged independently at each site and in each year according to standard commercial practice for each cultivar, based on skin colour and fruit firmness assessed by the orchard management team, and pomological and phytochemical samples were both taken on the day this stage was reached; consequently, the maturity dates in Table 3 differ between locations and cultivars by design, and the standardisation applied is to physiological ripeness rather than to a fixed calendar date.

To relate the monthly weather records presented above (Table 2, Figure 3) more directly to bud development, we additionally computed chill accumulation and growing degree days (GDD) for each site and year from the daily temperature records. Hourly temperatures were reconstructed from the daily maximum and minimum using the single sine-curve interpolation of Baskerville and Emin [16], and chill accumulation was expressed as the number of hours at or below 7.2 °C from 1 November of the preceding year to the observed bud-swelling date at each site. GDD were accumulated from bud swelling to full bloom

using a base temperature of 4.5 °C and the daily mean method, following Campillay-Llanos et al. [7]. Results are summarised in Table 4.

Table 3. Phenological stage dates (day month) and the derived bloom-to-maturity interval (days) of four sweet cherry cultivars at two locations in Eskişehir province, 2016 to 2017.

Year	Location	Cultivar	Bud Swelling	Bloom Start	Full Bloom	Veraison Onset	Maturity	Bloom to Maturity (Days)
2016	Eskişehir	0900 Ziraat	25.03	12.04	14.04	26.05	17.06	66
2016	Eskişehir	Kordia	20.03	12.04	16.04	26.05	15.06	64
2016	Eskişehir	Regina	18.03	10.04	15.04	28.05	18.06	69
2016	Eskişehir	Sweetheart	28.03	15.04	19.04	30.05	20.06	66
2016	Sarıcakaya	0900 Ziraat	10.03	06.04	11.04	26.05	12.06	67
2016	Sarıcakaya	Kordia	08.03	06.04	11.04	26.05	10.06	65
2016	Sarıcakaya	Regina	12.03	07.04	12.04	28.05	14.06	68
2016	Sarıcakaya	Sweetheart	12.03	07.04	15.04	30.05	14.06	68
2017	Eskişehir	0900 Ziraat	01.04	17.04	21.04	04.06	22.06	66
2017	Eskişehir	Kordia	01.04	14.04	19.04	30.05	24.06	71
2017	Eskişehir	Regina	31.03	17.04	21.04	31.05	26.06	70
2017	Eskişehir	Sweetheart	25.03	17.04	24.04	06.06	28.06	72
2017	Sarıcakaya	0900 Ziraat	16.03	08.04	12.04	24.05	10.06	63
2017	Sarıcakaya	Kordia	15.03	07.04	12.04	22.05	08.06	62
2017	Sarıcakaya	Regina	19.03	08.04	14.04	25.05	12.06	65
2017	Sarıcakaya	Sweetheart	18.03	09.04	14.04	27.05	14.06	66

Table 4. Chill accumulation (hours ≤ 7.2 °C, 1 November to bud swelling) and growing degree days (base 4.5 °C, bud swelling to full bloom) at the two study sites, 2016 to 2017, averaged across the four cultivars.

Year	Location	Chill Hours (≤ 7.2 °C)	GDD, Bud Swelling to Bloom (Base 4.5 °C)
2016	Eskişehir	2069.8	188.1
2016	Sarıcakaya	1432.6	269.0
2017	Eskişehir	2570.2	136.0
2017	Sarıcakaya	1989.4	218.0

Chill hours and GDD were computed from hourly temperatures reconstructed from daily maxima and minima by the sine-curve method of Baskerville and Emin [16]; values are means of the four cultivars at each site and year.

2.4. Pomological Measurements

At commercial maturity, four fruit replicate samples per cultivar, location and year were used to measure: fruit weight; fruit thickness, width and length; peduncle length; skin and flesh colour as CIE L^* , a^* and b^* values, measured with a Minolta CR-400 chromametre (Konica Minolta Sensing, Inc., Osaka, Japan); stone fresh weight, thickness, width and length; soluble solids content (SSC, in °Brix), read with a hand-held digital refractometer (ATAGO PAL-1, Atago Co., Ltd., Tokyo, Japan); titratable acidity, given as the percentage of citric acid and determined by titrating the juice with 0.1 N NaOH to pH 8.1; and juice pH, read directly with a digital pH metre (HI 2211 pH meter, Hanna Instruments, Woonsocket, RI, USA). Eighteen traits were analysed in total.

2.5. Total Phenolic Content

All chemicals and reagents used in this study were of analytical grade and were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). We quantified total phenolic content (TPC) with the Folin–Ciocalteu colorimetric assay, based on the procedure of Cemeroğlu [17]. Fruit samples were first homogenised and centrifuged (4000 rpm, 10 min), after which the supernatant was filtered and diluted 20-fold with distilled water. A 2.5 mL aliquot of 10% Folin–Ciocalteu reagent together with 20% sodium carbonate solution was added to the diluted juice, and the reaction mixture stood in the dark at room temperature for 2 h prior to absorbance measurement at 750 nm on a Shimadzu UV-1800 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan), with a matching reagent blank run alongside each batch. Concentrations were derived from a five-point gallic acid calibration curve (20–100 mg/L), built by serially diluting a 500 mg/L ethanolic stock solution and carrying each standard through the identical reaction sequence as the samples; results are reported as mg gallic acid equivalents (GAE) per kg fresh weight (FW), based on three replicate fruit groups per cultivar, location and year across the 2016 and 2017 harvests.

2.6. Total Flavonoid Content

We estimated total flavonoid content (TFC) using the aluminium chloride colorimetric assay described by Shams Ardekani et al. [18]. A 1 mL portion of juice supernatant was combined with 4 mL distilled water and 0.3 mL of 5% sodium nitrite (NaNO_2); five minutes later, 0.3 mL of 10% aluminium chloride (AlCl_3) was introduced, followed after a further minute by 2 mL of 1 M sodium hydroxide (NaOH), and the final volume was brought to 10 mL with distilled water. Absorbance was measured at 510 nm on a Shimadzu UV-1800 UV-Vis spectrophotometer relative to an identically prepared reagent blank. A four-point catechin calibration curve (50, 100, 200 and 400 mg/L) was used to convert absorbance into mg catechin equivalents (CE) per kg fresh weight (FW). Measurements were performed in triplicate on three replicate fruit groups per cultivar, location and year across the 2016 and 2017 harvests.

2.7. DPPH Radical Scavenging Activity

Free-radical scavenging capacity was assessed with the DPPH assay, adapted from Brand-Williams et al. [19]. Diluted juice supernatant was combined with a 0.1 mM DPPH solution in methanol, and the resulting mixture was left to stand in the dark at room temperature for 30 min; absorbance was then recorded at 515 nm on a Shimadzu UV-1800 UV-Vis spectrophotometer against a methanol blank. The extent of radical scavenging was derived from the drop in absorbance relative to a DPPH-only control lacking extract and is reported as percent inhibition of the DPPH radical. Four replicate fruit groups were assayed per cultivar, location and year in the 2016 and 2017 harvests, each extract measured in duplicate and the pair of readings averaged.

2.8. Statistical Analysis

Pomological traits, total phenolic content, total flavonoid content and DPPH radical scavenging activity were analysed using two-way ANOVA (location and cultivar as fixed factors), pooling the two growing seasons as additional replicates. For pomological traits, Location tests used 1 and Cultivar tests 3 numerator degrees of freedom, against 56 error degrees of freedom ($n = 64$). For total phenolic and total flavonoid content, based on three replicate fruit groups per cultivar, location and year, the error term had 40 degrees of freedom ($n = 48$); for DPPH radical scavenging activity, based on four replicate fruit groups per cultivar, location and year, the error term had 56 degrees of freedom ($n = 64$). Because location is represented by a single orchard at each altitude, the error term in these models

captures variation among trees and replicate groups within an orchard, not variation among independently replicated orchards; the Location tests should therefore be interpreted as differences between the two orchards studied rather than as a formally replicated test of altitude per se.

A separate three-way analysis including year as a factor was also run for all traits. For pomological traits, year had a significant main effect ($p < 0.05$) for fifteen of the eighteen traits, interacting significantly with cultivar for most traits and with location for about half of them. For total phenolic content, total flavonoid content and DPPH radical scavenging activity, year had a significant main effect on total phenolic content ($p = 0.018$) and total flavonoid content ($p < 0.001$), with a significant location-by-year interaction for total flavonoid content only ($p = 0.044$), but no significant year effect on DPPH radical scavenging activity ($p = 0.70$). In every case, however, the direction of the location effect was consistent between the two years, which supports the pooled two-year reporting used throughout the Results section. Pooling year as an additional replicate, rather than fitting it as a random effect in a mixed model, treats year-to-year variation as part of the residual error; we chose this simpler approach because it matches common practice in comparable multi-year cultivar trials, though a mixed-model analysis with year as a random effect would be a worthwhile refinement for future trials at these sites. We treat this pooled, two-way analysis as the primary result and the supplementary three-way analysis including year as a factor as a robustness check on the pooling decision, rather than as a formal hierarchical model of year effects.

Cultivar means and the two locations were separated using Tukey's HSD test ($p < 0.05$). We also computed Pearson correlation coefficients linking phytochemical traits to selected pomological and colour traits, and ran a principal component analysis (PCA) on fourteen standardised pomological, colour and phytochemical variables at the finer cultivar-by-location-by-year level ($n = 16$) to capture the overall pattern of variation among cultivars, locations and years. Because this analysis is based on only sixteen cultivar-by-location-by-year combinations, it should be regarded as an exploratory, descriptive summary of the pattern already established by the analyses of variance and correlations reported in the Results section, rather than as an independent statistical test.

Statistical computations were performed with JMP 17 (SAS Institute Inc., Cary, NC, USA), and graphics were generated in OriginPro 2026 (OriginLab Corporation, Northampton, MA, USA); effects were considered statistically significant at $p < 0.05$.

3. Results

3.1. Meteorological Conditions During the Study Period

The two sites showed a consistently different thermal regime in both years (Table 2, Figure 3). Annual mean air temperature averaged 12.5 °C at Eskişehir versus 15.7 °C at Sarıcakaya—about 3.2 °C higher at the lower site in both years, close to what would be expected from the 572 m difference in altitude. The gap was already apparent before bloom: mean February–April temperature was 2.4 °C higher at Sarıcakaya in 2016 and 3.0 °C higher in 2017. Total annual precipitation was similar at both sites (369–410 mm), though its monthly distribution differed, with more rain falling at Sarıcakaya in April and May of both years.

3.2. Phenological Development

Dormancy release was consistently earlier at Sarıcakaya than at Eskişehir (Table 3). Mean bud swelling occurred on day of year 73.3, about 14 March, at Sarıcakaya, and on day of year 85.9, about 27 March, at Eskişehir, an advance of 12.6 days at the lower, warmer site. This matches the higher late winter and early spring temperatures recorded there

(Section 3.1). The bloom-to-maturity interval, used here as a simple measure of the fruit development period, averaged 65.5 days at Sarıcakaya and 68.0 days at Eskişehir, about 2.5 days shorter at the warmer site, and differed little between years (66.6 days in 2016 vs. 66.9 days in 2017). Among cultivars, Kordia and 0900 Ziraat tended to reach beginning of bloom slightly earlier than Sweetheart, which was consistently the last cultivar to flower at both sites.

Chill and heat-unit accumulation differed consistently between the two sites in both years (Table 4). Sarıcakaya, the warmer, lower-altitude site, reached bud swelling after accumulating markedly fewer chill hours at or below 7.2 °C than Eskişehir (1432.6 vs. 2069.8 h in 2016; 1989.4 vs. 2570.2 h in 2017), consistent with its earlier bud-swelling dates (Table 3). From bud swelling to full bloom, however, Sarıcakaya accumulated more growing degree days than Eskişehir in both years (269.0 vs. 188.1 °C·d in 2016; 218.0 vs. 136.0 °C·d in 2017). Taken together, these patterns suggest that the earlier phenology recorded at Sarıcakaya (Table 3) reflects faster progress toward its chilling requirement at the warmer site, rather than a lower heat-unit requirement for the subsequent transition from bud swelling to bloom; a formal chilling-model comparison was beyond the scope of the present daily temperature dataset but would be a useful next step to test this interpretation.

3.3. Pomological Characteristics

Results for fruit size and peduncle length are given in Table 5. Location and cultivar both had a significant effect on all five traits. Fruit from Eskişehir were heavier and had a longer peduncle than fruit from Sarıcakaya, while fruit width, length and peduncle length also differed between cultivars. Sweetheart produced the smallest fruit and the shortest peduncle of the four cultivars, while Kordia had the longest peduncle. A significant location-by-cultivar interaction for fruit weight, thickness, width and peduncle length shows that the size of the location effect was not exactly the same in all four cultivars, although its direction was consistent.

Table 5. Fruit size and peduncle length of four sweet cherry cultivars at two locations in Eskişehir province, mean $\pm$ SD (2016 to 2017, pooled across years).

Trait	Fruit Weight (g)	Fruit Thickness (mm)	Fruit Width (mm)	Fruit Length (mm)	Peduncle Length (mm)
Location					
Eskişehir (792 m)	8.51 $\pm$ 0.84 ^A	21.29 $\pm$ 0.95 ^A	24.86 $\pm$ 1.13 ^A	23.47 $\pm$ 1.37 ^A	43.16 $\pm$ 9.18 ^A
Sarıcakaya (220 m)	7.84 $\pm$ 0.94 ^B	20.58 $\pm$ 1.71 ^B	24.62 $\pm$ 1.75 ^A	23.55 $\pm$ 1.89 ^A	37.51 $\pm$ 4.13 ^B
Cultivar					
Regina	8.30 $\pm$ 0.46 ^A	21.47 $\pm$ 0.45 ^A	25.08 $\pm$ 0.77 ^A	23.95 $\pm$ 0.93 ^A	40.13 $\pm$ 5.35 ^A
Sweetheart	7.10 $\pm$ 0.88 ^B	19.42 $\pm$ 1.92 ^B	23.30 $\pm$ 1.90 ^B	21.78 $\pm$ 1.77 ^B	33.18 $\pm$ 3.44 ^B
Kordia	8.59 $\pm$ 0.57 ^A	21.45 $\pm$ 0.69 ^A	25.08 $\pm$ 0.69 ^A	24.57 $\pm$ 0.72 ^A	44.72 $\pm$ 7.99 ^A
0900 Ziraat	8.71 $\pm$ 0.83 ^A	21.41 $\pm$ 0.89 ^A	25.49 $\pm$ 1.12 ^A	23.76 $\pm$ 1.45 ^A	43.31 $\pm$ 7.38 ^A
Analysis of variance (F values)					
Location (L)	19.7 ***	7.8 **	0.7 NS	0.1 NS	17.8 ***
Cultivar (C)	24.3 ***	15.8 ***	11.6 ***	14.5 ***	14.8 ***
L $\times$ C	3.0 *	3.5 *	3.7 *	2.1 NS	3.1 *

Values in a column followed by the same letter are not significantly different (Tukey's HSD, $p < 0.05$), compared separately within the Location block and within the Cultivar block. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = not significant.

Skin and flesh colour results are given in Table 6. As with fruit size (Table 5), location had the larger effect: fruit from Eskişehir were more intensely coloured, with higher skin a^* (redness) and flesh b^* , though flesh a^* did not differ between sites. Cultivar differences were smaller and less consistent, and no location-by-cultivar interaction was significant for any colour trait.

Table 6. Skin and flesh colour (CIE L^* , a^* , b^*) of four sweet cherry cultivars at two locations in Eskişehir province, mean $\pm$ SD (2016 to 2017, pooled across years).

Trait	Skin L^*	Skin a^*	Skin b^*	Flesh L^*	Flesh a^*	Flesh b^*
Location						
Eskişehir (792 m)	32.72 $\pm$ 3.43 ^A	32.15 $\pm$ 6.70 ^A	8.23 $\pm$ 4.55 ^A	35.25 $\pm$ 5.01 ^A	27.36 $\pm$ 7.74 ^A	13.80 $\pm$ 2.25 ^A
Sarıcakaya (220 m)	29.97 $\pm$ 2.57 ^B	25.00 $\pm$ 6.22 ^B	4.91 $\pm$ 3.28 ^B	32.56 $\pm$ 6.97 ^A	27.33 $\pm$ 4.65 ^A	10.65 $\pm$ 1.71 ^B
Cultivar						
Regina	33.15 $\pm$ 4.04 ^A	31.60 $\pm$ 7.29 ^A	8.62 $\pm$ 5.29 ^A	33.37 $\pm$ 4.72 ^A	26.10 $\pm$ 7.43 ^A	13.07 $\pm$ 2.97 ^A
Sweetheart	30.77 $\pm$ 3.44 ^A	27.04 $\pm$ 8.45 ^A	5.59 $\pm$ 4.47 ^A	32.04 $\pm$ 8.46 ^A	27.37 $\pm$ 4.86 ^A	11.27 $\pm$ 3.24 ^A
Kordia	29.84 $\pm$ 2.23 ^B	26.45 $\pm$ 6.79 ^A	4.98 $\pm$ 3.07 ^A	33.06 $\pm$ 4.31 ^A	30.57 $\pm$ 5.90 ^A	12.14 $\pm$ 1.50 ^A
0900 Ziraat	31.61 $\pm$ 2.57 ^A	29.21 $\pm$ 6.24 ^A	7.09 $\pm$ 3.30 ^A	37.15 $\pm$ 5.61 ^A	25.34 $\pm$ 6.15 ^A	12.43 $\pm$ 1.95 ^A
Analysis of variance (F values)						
Location (L)	15.6 ***	20.6 ***	12.0 **	3.3 NS	0.0 NS	41.2 ***
Cultivar (C)	4.1 *	2.2 NS	2.9 *	2.2 NS	2.3 NS	2.3 NS
L $\times$ C	1.8 NS	0.9 NS	0.6 NS	0.4 NS	1.4 NS	0.4 NS

Within each column, means that share a letter do not differ significantly (Tukey's HSD, $p < 0.05$); comparisons were made separately for the Location and Cultivar blocks. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = not significant.

Stone traits are given in Table 7. As above, both location and cultivar were significant for all four traits, with larger, heavier stones from Eskişehir fruit, and no location-by-cultivar interaction.

Table 7. Stone weight and dimensions of four sweet cherry cultivars at two locations in Eskişehir province, mean $\pm$ SD (2016 to 2017, pooled across years).

Trait	Stone Weight (g)	Stone Thickness (mm)	Stone Width (mm)	Stone Length (mm)
Location				
Eskişehir (792 m)	0.40 $\pm$ 0.07 ^A	7.37 $\pm$ 0.53 ^A	9.35 $\pm$ 0.75 ^A	11.68 $\pm$ 0.84 ^A
Sarıcakaya (220 m)	0.32 $\pm$ 0.05 ^B	6.54 $\pm$ 0.40 ^B	8.24 $\pm$ 0.45 ^B	10.71 $\pm$ 1.05 ^B
Cultivar				
Regina	0.41 $\pm$ 0.06 ^A	7.24 $\pm$ 0.57 ^A	9.07 $\pm$ 0.81 ^A	11.28 $\pm$ 0.66 ^A
Sweetheart	0.32 $\pm$ 0.06 ^B	6.68 $\pm$ 0.72 ^B	8.43 $\pm$ 1.01 ^A	10.75 $\pm$ 1.53 ^B
Kordia	0.35 $\pm$ 0.05 ^A	6.76 $\pm$ 0.64 ^A	8.63 $\pm$ 0.78 ^A	11.71 $\pm$ 1.00 ^A
0900 Ziraat	0.38 $\pm$ 0.08 ^A	7.15 $\pm$ 0.38 ^A	9.04 $\pm$ 0.52 ^A	11.02 $\pm$ 0.65 ^A
Analysis of variance (F values)				
Location (L)	39.4 ***	64.5 ***	59.9 ***	21.7 ***
Cultivar (C)	8.4 ***	7.1 ***	4.9 **	3.9 *
L $\times$ C	0.3 NS	0.7 NS	0.3 NS	4.5 **

Means in a column sharing the same letter are statistically indistinguishable (Tukey's HSD, $p < 0.05$), with Location and Cultivar blocks tested separately. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = not significant.

Taste-related traits are given in Table 8. Here the direction reversed: fruit from the warmer Sarıcakaya site were sweeter and more acidic, with higher SSC and titratable acidity, though juice pH did not differ by location or cultivar. Both SSC and titratable acidity showed a significant location-by-cultivar interaction. Table 8 additionally reports the SSC/titratable-acidity ratio, a simple index of eating-quality maturity: it was significantly higher at Eskişehir than at Sarıcakaya ($F = 47.1$, $p < 0.001$), consistent with SSC itself, did not differ significantly among cultivars overall ($F = 0.0$, $p > 0.05$), and showed a significant location-by-cultivar interaction ($F = 25.8$, $p < 0.001$), indicating that the size of the location effect on this ratio varied among cultivars.

Table 8. Taste-related traits of four sweet cherry cultivars at two locations in Eskişehir province, mean $\pm$ SD (2016 to 2017, pooled across years).

Trait	SSC (°Brix)	Acidity (% Citric Acid)	Juice pH	SSC/TA Ratio
Location				
Eskişehir (792 m)	16.28 $\pm$ 1.14 ^B	0.92 $\pm$ 0.05 ^B	3.39 $\pm$ 0.27 ^A	17.80 $\pm$ 1.59 ^A
Sarıcakaya (220 m)	18.59 $\pm$ 1.45 ^A	1.17 $\pm$ 0.10 ^A	3.36 $\pm$ 0.59 ^A	15.93 $\pm$ 1.60 ^B
Cultivar				
Regina	17.25 $\pm$ 1.18 ^A	1.06 $\pm$ 0.22 ^A	3.45 $\pm$ 0.26 ^A	16.89 $\pm$ 2.94 ^A
Sweetheart	18.00 $\pm$ 1.70 ^A	1.07 $\pm$ 0.11 ^A	3.46 $\pm$ 0.30 ^A	16.86 $\pm$ 1.76 ^A
Kordia	17.31 $\pm$ 1.78 ^A	1.03 $\pm$ 0.17 ^A	3.17 $\pm$ 0.77 ^A	16.93 $\pm$ 1.16 ^A
0900 Ziraat	17.19 $\pm$ 2.20 ^A	1.02 $\pm$ 0.08 ^A	3.42 $\pm$ 0.24 ^A	16.78 $\pm$ 1.04 ^A
Analysis of variance (F values)				
Location (L)	60.1 ***	308.0 ***	0.1 NS	47.1 ***
Cultivar (C)	1.6 NS	2.8 NS	1.6 NS	0.0 NS
L $\times$ C	4.5 **	16.7 ***	1.5 NS	25.8 ***

Within each column, means that share a letter do not differ significantly (Tukey's HSD, $p < 0.05$); comparisons were made separately for the Location and Cultivar blocks. ** $p < 0.01$; *** $p < 0.001$; NS = not significant.

3.4. Total Phenolic Content, Total Flavonoid Content and Antioxidant Capacity

Total phenolic content, total flavonoid content and DPPH radical scavenging activity, pooled across the 2016 and 2017 harvests, are given in Table 9 together with the two-way ANOVA results. Location was by far the largest source of variation for all three traits: fruit from Eskişehir had about 30% higher TPC, 47% higher TFC and more than double the DPPH activity of fruit from Sarıcakaya, a pattern that held across all four cultivars (Figure 4). Cultivar was also significant for all three traits—Sweetheart had significantly lower TPC and TFC than the other three cultivars, and Kordia had the highest DPPH activity of the four, while 0900 Ziraat and Sweetheart had the lowest values. A significant location-by-cultivar interaction for DPPH indicates that cultivar differences were much larger at Eskişehir, where mean inhibition ranged from 25.6% in 0900 Ziraat and 28.3% in Sweetheart up to 54.1% in Regina and 73.9% in Kordia, than at Sarıcakaya, where all four cultivars gave similarly low values (19.1% in 0900 Ziraat, 23.2% in Kordia, 23.7% in Regina and 24.2% in Sweetheart). Unlike DPPH, the location-by-cultivar interaction was not significant for TPC or TFC once the two years were pooled ($p = 0.095$ and $p = 0.145$, respectively), even though it had been significant for TPC within the 2017 harvest alone—indicating a more consistent location effect across cultivars once both seasons were considered together. In a three-way analysis, including year as a factor (see Materials and Methods), year was a significant source of variation for TPC and TFC but not for DPPH, and the direction of the location

effect was the same in both 2016 and 2017 for all three traits, supporting the pooling of the two years as replicates here, as was done for the pomological traits in Tables 5–8.

Table 9. Total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging activity of four sweet cherry cultivars at two locations, 2016 and 2017 harvests pooled, mean $\pm$ SD.

Trait	TPC (mg GAE kg ⁻¹ FW)	TFC (mg CE kg ⁻¹ FW)	DPPH (% Inhibition)
Location			
Eskişehir (792 m)	1071.0 $\pm$ 81.9 ^A	344.3 $\pm$ 66.7 ^A	45.5 $\pm$ 21.0 ^A
Sarıcakaya (220 m)	823.3 $\pm$ 48.0 ^B	234.6 $\pm$ 28.2 ^B	22.5 $\pm$ 7.1 ^B
Cultivar			
Regina	991.5 $\pm$ 155.6 ^A	312.1 $\pm$ 76.1 ^A	38.9 $\pm$ 17.6 ^B
Sweetheart	867.7 $\pm$ 112.0 ^B	239.9 $\pm$ 43.5 ^B	26.3 $\pm$ 7.8 ^C
Kordia	977.1 $\pm$ 146.9 ^A	312.5 $\pm$ 86.2 ^A	48.5 $\pm$ 26.8 ^A
0900 Ziraat	952.4 $\pm$ 131.8 ^A	293.3 $\pm$ 71.8 ^A	22.3 $\pm$ 5.5 ^C
Analysis of variance (F values)			
Location (L)	355.9 ***	84.2 ***	184.9 ***
Cultivar (C)	17.8 ***	8.2 ***	50.5 ***
L $\times$ C	2.3 NS	1.9 NS	42.6 ***

Means in a column sharing the same letter are statistically indistinguishable (Tukey's HSD, $p < 0.05$), with Location and Cultivar blocks tested separately. *** $p < 0.001$; NS = not significant. GAE = gallic acid equivalents; CE = catechin equivalents; FW = fresh weight.

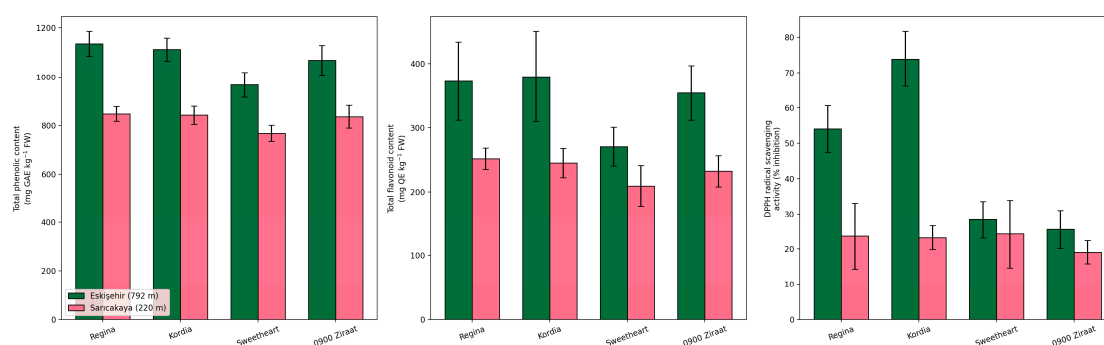


Figure 4. Total phenolic content, total flavonoid content and DPPH radical scavenging activity (mean $\pm$ SD) of four sweet cherry cultivars at two locations, 2016 and 2017 harvests pooled.

3.5. Relationships Among Phytochemical and Pomological Traits

The correlations among TPC, TFC and DPPH activity, and between these three traits and selected pomological and colour traits, are given once in Table 10 rather than repeated in the text. In summary, TPC and TFC were strongly correlated ($r = 0.93$), and both were correlated with DPPH activity, so the two colorimetric assays gave a consistent phenolic signal that matched measured radical scavenging capacity. Skin a^* , the redness value, had the strongest positive link with phytochemical content among the pomological traits tested. SSC and titratable acidity were both strongly and negatively correlated with TPC and TFC; because both traits were also linked to the warmer Sarıcakaya site, these negative correlations likely reflect the shared effect of growing location rather than a direct link between the traits. Fruit weight and skin L^* were the weakest of the pomological and colour traits considered here, but at this finer, year-resolved level both reached statistical significance for their correlation with TPC (and skin L^* also with TFC), where they had not at the coarser, two-year-pooled resolution used in our original submission. These

coefficients were calculated at the cultivar-by-location-by-year level ($n = 16$) rather than pooled over the two years as in our original submission ($n = 8$); correlations of this kind, based on group means rather than individual fruit or replicate values, tend to be stronger than the corresponding relationship at the level of individual fruit, so they are best read as a description of shared patterns among cultivar, location and year combinations rather than as proof of a direct biological link between traits.

Table 10. Pearson correlation coefficients (r) between total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity and selected pomological and colour traits (cultivar by location by year, $n = 16$).

Trait	TPC	TFC	DPPH
Fruit weight	0.56 *	0.50 NS	0.37 NS
Skin a^* (redness)	0.60 *	0.57 *	0.21 NS
Skin L^* (lightness)	0.52 *	0.50 *	0.09 NS
SSC	−0.69 **	−0.69 **	−0.52 *
Titrateable acidity	−0.86 ***	−0.75 ***	−0.67 **
TFC	0.93 ***	—	—
DPPH	0.75 ***	0.73 **	—

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = not significant.

3.6. Correlation Structure and Principal Component Analysis

The full correlation matrix of fourteen pomological, colour and phytochemical traits, at the cultivar-by-location-by-year level ($n = 16$), is shown in Figure 5. Three main blocks stand out. Fruit weight, thickness, length, peduncle length and stone weight were all strongly and positively correlated with each other, so larger fruit had larger stones and longer peduncles. Skin L^* , a^* and b^* formed a second tight block, showing that fruit colour changed together on all three axes. Total phenolic content, total flavonoid content and DPPH activity formed a third block, and this block was itself positively correlated with fruit size, stone weight and skin colour, and negatively correlated with soluble solids content and titrateable acidity. This pattern matches the location effects described above: the same fruit that were larger, more deeply coloured and more phenolic-rich also had lower sugar and acid content, and these were consistently the fruit grown at the cooler Eskişehir site.

Principal component analysis (PCA) on the same fourteen standardised traits gave a clear two-dimensional summary of the data (Figure 6). The first principal component (PC1) explained 47.6% of the total variance, and the second (PC2) a further 21.7%, together accounting for 69.3% (lower than in our original group-mean analysis, because resolving each cultivar by location combination into separate 2016 and 2017 scores introduces additional year-to-year variability that the first two components do not capture). PC1 separated the two locations for the great majority of the sixteen cultivar-by-location-by-year points: all eight Sarıcakaya points scored lower on PC1 than every Eskişehir point except one, Sweetheart in 2017, whose Eskişehir score (−2.06) fell within the range of Sarıcakaya scores, so the location separation, while strong, was not absolute at this finer, year-resolved resolution. PC1 was driven positively by fruit weight and the other fruit size traits, stone weight, skin colour and all three phytochemical traits, and negatively by soluble solids content and titrateable acidity, in agreement with the location effects reported in Tables 5–9. PC2 was driven positively by fruit length, fruit width and fruit weight and negatively by the three skin colour traits (Skin L^* , a^* and b^*), so it mainly separated cultivar by year combinations along a fruit-shape versus skin-colour gradient rather than by location; it did not give a single cultivar ranking that held across both sites and both years. This is

consistent with the significant location-by-cultivar interaction found for DPPH radical scavenging activity (Table 9): cultivar differences in phytochemical and colour traits were not uniform across sites, so a second axis, largely independent of the main location effect on PC1, was needed to capture this additional cultivar-by-site variation. Taken together, these results support the main conclusion of this study: growing location, not cultivar, was the strongest driver of variation among the pomological, colour and phytochemical traits measured in this dataset.

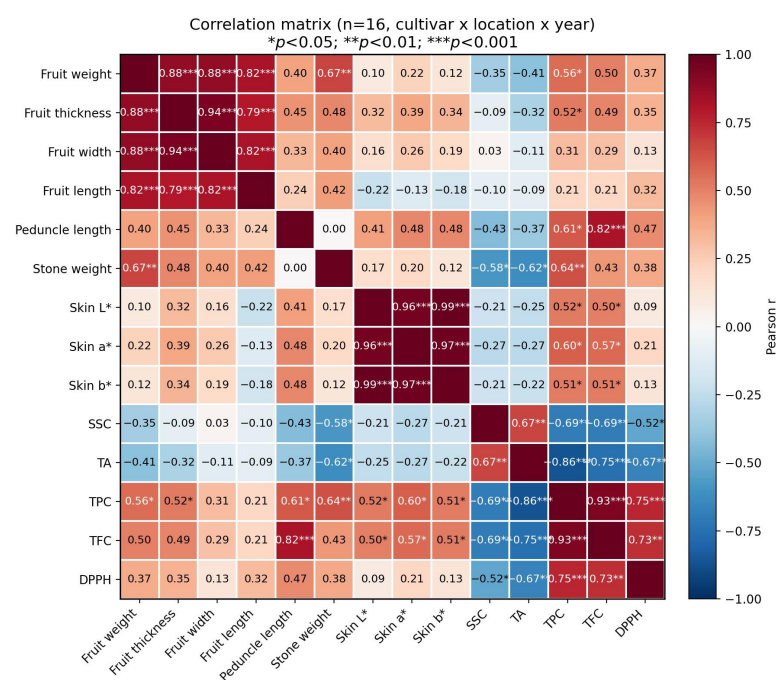


Figure 5. Correlation matrix (Pearson r) of fourteen pomological, colour and phytochemical traits, computed at the cultivar-by-location-by-year level ($n = 16$). cells without an asterisk were not statistically significant.

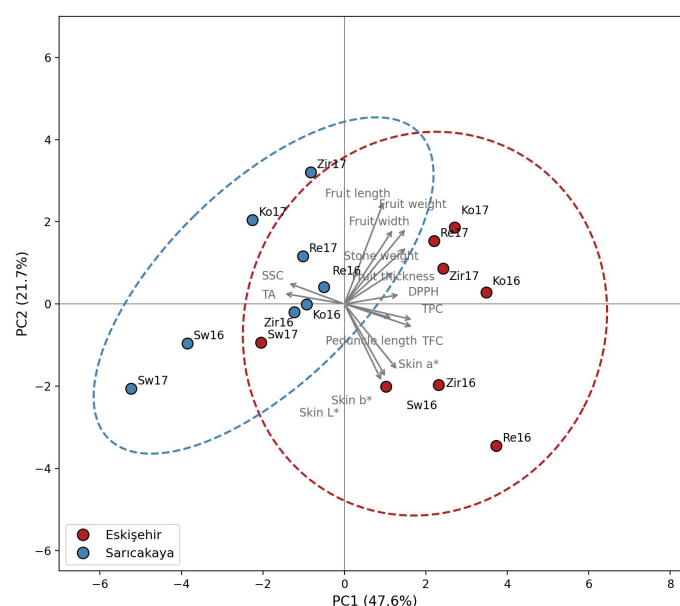


Figure 6. Principal component analysis biplot of fourteen pomological, colour and phytochemical traits, computed at the cultivar-by-location-by-year level ($n = 16$). Points are coloured by cultivar and shaped by location; shaded ellipses show the 95% confidence region for each location; grey arrows show trait loadings on the first two components.

4. Discussion

The clearest finding here is that growing location—associated with a roughly 570 m altitude difference between the two orchards, but also potentially with unmeasured differences in soil, water source and microclimate that we could not separate from altitude itself—was associated with larger phytochemical differences, across both growing seasons, than any difference found among the four cultivars themselves. Fruit from the higher, cooler Eskişehir site consistently exceeded the same cultivars grown just 60 km away at Sarıcakaya on all three phytochemical measures (Section 3.4). Cooler, higher-altitude sites have favoured phenolic accumulation in sweet cherry elsewhere too: in Lebanon [9] and, more markedly, in northern Greece, where total phenolic content and antioxidant capacity were up to 85% higher in fruit from the highest of three orchards than from the lowest, in three of the four cultivars tested [10]. Cooler temperatures, a wider day–night temperature gap and stronger solar radiation at altitude are thought to favour phenolic biosynthesis, a pattern documented in other fruit species as well [20]. These physiological mechanisms were not measured directly in the present study; however, they should be regarded as a plausible explanation drawn from the literature rather than a demonstrated causal pathway for the cultivars and sites examined here. DPPH activity was the phytochemical trait most affected by location in our data, which suggests that a roughly 3 °C difference in annual mean temperature between the two sites was enough to alter not just the amount of phenolic compounds produced but their antioxidant effectiveness. These phytochemical results rest on two harvest years, with the direction of the location effect consistent in both 2016 and 2017, though confirmation at additional sites would still be needed before generalising the size of this effect beyond a single pair of orchards. Because the sampled fruit were not retained for post hoc analysis, we were also unable to re-express phytochemical content on a dry-weight basis, a conversion increasingly used to remove the confounding effect of moisture content when comparing phenolic and flavonoid concentrations across sites and studies; this is a limitation of the present dataset that future sampling at these orchards should address. A similarly consistent site effect across harvest years, despite real year-to-year shifts in individual phytochemical traits, has been reported for altitude and harvest year effects on fruit nutraceutical content in *Rubus ellipticus* [21], and season has recently been shown to affect total flavonoid content and antioxidant capacity more consistently than DPPH activity in sour cherry, much as we found here [22]. Crop year effects on phenolic and antioxidant traits, by contrast, have not always been consistent across cultivars or fruit tissues in other *Prunus* species [23]. Phytochemical content also shifts sharply within a single season as sweet cherry fruit ripens—total phenolics, antioxidant activity and especially anthocyanins can increase several-fold between early development and full colouration [24]—so the single, defined maturity stage sampled here is a further source of variation worth bearing in mind alongside harvest year. Flesh firmness, a widely used direct indicator of ripening stage and postharvest quality, was not measured in the 2016 and 2017 seasons; the SSC/titratable-acidity ratio reported here (Table 8) offers an indirect maturity index computed from traits that were measured, but it is not a substitute for a direct firmness measurement, and its absence is a limitation we aim to address in future harvests at these orchards.

More specifically, the cooler, higher-UV-B conditions typical of higher elevations are generally understood to favour phenolic accumulation through transcriptional activation of the phenylpropanoid pathway: UV-B perception through the UVR8 photoreceptor upregulates MYB transcription factors that drive anthocyanin and flavonoid structural genes, a pathway demonstrated experimentally in blueberry peel [25]. Night-time temperature may act in the opposite direction: in sweet cherry specifically, elevated temperature has been shown to suppress anthocyanin accumulation by promoting abscisic-acid catabolism rather

than by directly repressing biosynthetic gene expression [26], a mechanism broadly consistent with the lower phenolic and antioxidant values recorded at the warmer Sarıcakaya site here. These pathways remain untested in the present material; however, the relationship between altitude and phenolic content is not universal: a recent systematic review across thirty strawberry studies found no consistent altitude effect on phenolic composition or antioxidant capacity once study heterogeneity was accounted for [27], underscoring that the pattern reported here is best treated as specific to these two orchards and cultivars rather than a generalisable altitude effect.

The cultivar rankings for phytochemical content fit well with earlier Turkish trials. Kordia had the highest DPPH activity of the four cultivars and was also among the richest in total phenolics and flavonoids, matching its ranking among five sweet cherry cultivars grown in Ordu, Türkiye [5], and in trials from Poland [28,29] and Greece [30]—suggesting its high phytochemical content is a fairly stable genetic trait rather than something tied to one growing region. The 0900 Ziraat values measured here for total phenolics and antioxidant activity were broadly similar to those reported for the same cultivar at another Turkish site of comparable altitude [31], while Sweetheart was consistently among the lowest, as it was in the genotypes tested by Ateş and Öztürk [5] and by Ockun et al. [11]. Cultivar differences in DPPH activity were far more pronounced at Eskişehir than at Sarıcakaya, where all four cultivars converged to similarly low values—pointing to a genotype-by-environment interaction in which a high-phenolic cultivar such as Kordia may need a favourable, cooler environment to reach its full antioxidant potential. A comparable interaction has been reported for cultivar by rootstock effects on phenolics by Milošević et al. [12] and for fruit size by bioactive compound effects by Aglar et al. [13]. Antioxidant-related parameters in Regina specifically have also been shown to respond strongly to management practice: regulating fruit load under plastic covers altered harvest and postharvest antioxidant capacity in a Chilean trial, underlining that phytochemical traits in this species are plastic to growing conditions well beyond site or cultivar alone [32].

The pomological differences between sites are consistent with known temperature effects on cherry fruit development. Warmer conditions at Sarıcakaya shortened the bloom-to-maturity interval by about 2.5 days relative to Eskişehir and advanced dormancy release by almost two weeks, in line with the general finding that warmer spring temperatures speed up sweet cherry phenology and shorten the interval from flowering to harvest [7,8]—although the shift seen here was more modest than the 7- to 11-day shortening of the bloom-to-maturity period reported for two cultivars in Slovenia [33], or the roughly 4-day advance in flowering and 8-day advance in harvest per degree of mean temperature increase found across Turkish cultivar trials [34]. Temperature governs the timing of dormancy release and flowering in fruit trees mainly through its effect on bud developmental rate, so the warmer autumn and winter conditions at Sarıcakaya would be expected to bring bud break and bloom forward [35]. Earlier dormancy release at the warmer site could also raise exposure to damaging spring frosts, since flower bud frost tolerance changes sharply within a few weeks around bloom and varies among cultivars, including 0900 Ziraat [36]. The higher soluble solids content and titratable acidity, but lower fruit weight and less intense skin colour, seen in Sarıcakaya fruit are consistent with a shorter, warmer development period that favours rapid sugar accumulation over the longer window for cell growth and pigment build-up available at the cooler Eskişehir site. A comparable location effect on fruit weight has been reported for two of the same cultivars in Slovenia, where a five-year rootstock trial found location, rootstock and year all significantly affecting fruit weight of Kordia and Regina, with smaller fruit at the lower, warmer site [37]. The strong negative correlation between titratable acidity and both TPC and TFC (Table 10) most likely reflects this shared dependence on site temperature rather than any direct link

between the two properties; the positive association between skin a^* and both TPC and TFC, meanwhile, is consistent with the substantial contribution of anthocyanins—a major group of cherry phenolics that also give the skin its red colour—to total phenolic content, and Aglar et al. [13] and Hayaloğlu and Demir [6] reported a similar link between fruit colour and bioactive compound content in Turkish sweet cherry. Sweetheart was also the last cultivar to flower and the slowest to ripen among six cultivars grown at a high-altitude site in Isparta, Türkiye (921 m)—the same late-flowering habit it showed at both of our sites [38]. Two further Turkish studies of 0900 Ziraat, one comparing five provinces [39] and one comparing seven orchards over a smaller altitude range near İzmir [40], likewise found growing location to significantly affect most fruit quality traits, including total phenolic content and DPPH activity in the first of the two.

The location effect measured here is larger than in some other sweet cherry studies, and this difference in magnitude is worth placing in context. The direction of the altitude effect on phenolic content has varied among cultivars in Lebanese orchards spanning 900 to 2200 m [9], and in the northwestern United States, phenolic and antioxidant levels peaked at an intermediate rather than the highest of four elevations [41]. Season can matter as much as site: in a Moroccan sweet cherry collection, total phenolic and flavonoid content varied more between a milder and a hotter year than between orchards [42], and at two other Moroccan sites differing mainly in rainfall and continentality, four cultivars showed no significant location effect on total phenolics, flavonoids or antioxidant activity—only total anthocyanins differed significantly by site [43]. In sour cherry, year, location and cultivar all significantly affected polyphenol and anthocyanin content, with cultivar the largest single source of variation [44], and a broadly similar climate-dependent pattern has been reported outside cherry, in pomegranate [45]. The two Eskişehir province sites compared here differ by 572 m—a wider gradient than in several of these studies—which may explain why the location effect came through so clearly and consistently across nearly all traits measured, in both harvest years sampled for phytochemistry.

5. Conclusions

Growing location, tied to the roughly 572 m altitude difference between Eskişehir and Sarıcakaya, was the main factor shaping fruit size, colour, taste-related traits, and flowering and ripening time across the four sweet cherry cultivars over the two years studied; it was also associated with substantially higher phenolic, flavonoid and antioxidant content in both the 2016 and 2017 harvests. In most respects, location mattered more than cultivar. Fruit from the higher, cooler Eskişehir site were larger and more deeply coloured in both years, and much richer in total phenolics, flavonoids and DPPH radical scavenging activity, while fruit from the warmer, lower-altitude Sarıcakaya site ripened earlier and had higher soluble solids content and acidity. Kordia combined large fruit with the highest DPPH activity and was consistently among the cultivars richest in phenolics and flavonoids in both years, but reached this potential fully only under the cooler Eskişehir conditions—suggesting that site selection, not cultivar choice alone, matters when the goal is antioxidant-rich sweet cherry fruit. All of these results, including the phytochemical measurements now based on two harvest years, still come from a single orchard per location, so location and altitude remain confounded with other site-specific factors such as soil, water source and orchard microclimate. Phytochemical monitoring at additional sites, and a design that separates altitude from these other factors, would help confirm and extend the pomological, phenological and phytochemical findings reported here before generalising them more broadly.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
CE	Catechin equivalents
CIE	International Commission on Illumination
DPPH	2,2-diphenyl-1-picrylhydrazyl
FW	Fresh weight
GAE	Gallic acid equivalents
HSD	Honestly significant difference
NS	Not significant
PC1	First principal component
PC2	Second principal component
PCA	Principal component analysis
SD	Standard deviation
SSC	Soluble solids content
TFC	Total flavonoid content
TPC	Total phenolic content
UV-Vis	Ultraviolet-visible

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