



Fruit quality, phenolic and mineral profiles of new late-season European plum (*Prunus domestica* L.) cultivars from Serbia

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ABSTRACT

This study evaluated the primary metabolites, individual phenolic compounds, and fruit macro- and micro-nutrient content of two newly released late-season European plum cultivars, 'Divna' and 'Petra', compared with the standard cultivar 'Stanley'. Fruits were analysed over three years to assess the influence of genotype, seasonal variation, and their interaction on biochemical and mineral profiles. Clear cultivar-dependent differences were observed: 'Petra' accumulated more sugars and phenolics, reflecting greater sweetness and functional potential, while 'Divna' showed higher total acids, vitamin C, and mineral concentrations; importantly, both cultivars extend the plum harvest season, providing nutritionally rich fruits into late autumn. Significant cultivar × year interactions confirmed that the accumulation of metabolites and nutrients is not constant but shaped by both genetic background and environmental conditions. Correlation and principal component analyses revealed strong associations among sugars, acids, phenolics and mineral nutrients, highlighting cultivar-driven patterns of nutritional variability.

These findings emphasize the value of 'Divna' and 'Petra' as novel genetic resources for plum breeding and sustainable production, while also demonstrating their potential relevance as functional foods. By combining superior fruit quality with enhanced levels of health-promoting compounds and essential minerals, these cultivars may contribute to dietary diversity, consumer health and the development of nutritionally enriched plum-based products.

1. Introduction

Plums are the second most produced stone fruit worldwide after peaches and nectarines, with global production exceeding 12.4 million tons in 2023 (FAOSTAT, 2025). The most widely cultivated species are the diploid Japanese plum (*Prunus salicina* L.) and the hexaploid European plum (*Prunus domestica* L.). Among them, the European plum is one of the most important temperate-zone and deciduous fruit species with Serbia ranking among the leading global producers. In Serbia, plum growing has strong economic and cultural significance and represents the most important and widespread fruit species in the country.

Nevertheless, average yields often remain modest due to traditional production systems and the presence of Sharka disease caused by Plum pox virus (PPV). Similar challenges related to yield stability and disease pressure are also reported in other major plum growing regions worldwide (Sottile et al., 2022; Liu et al., 2023), highlighting the importance of breeding programs in ensuring both sustainable production and high-quality fruit.

Over 6000 plum cultivars have been identified worldwide and continuous breeding efforts have been directed toward improving fruit size, quality, yield potential and resistance to PPV (Milošević and Milošević, 2018; Neumüller et al., 2021). In addition to these agronomic

Abbreviations: SSC, soluble solids content; TS, total sugars, RS, reducing sugars; N-RS, non-reducing sugars; TA, total acids; RI, ripening index; Vit C, vitamin C content; PCA, Principal component analysis.

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traits, modern breeding increasingly integrates nutritional and functional properties as important selection criteria, reflecting global consumer demand for fruits with enhanced health-promoting value. The Fruit Research Institute, Čačak, has been a leader in this area for decades, releasing 18 cultivars, including the widely cultivated 'Čačanska Lepotica', 'Čačanska Rodna' and 'Čačanska Najbolja'. Recently developed late-season cultivars such as 'Divna' ('Stanley' × 'Čačanska Rana') and 'Petra' ('Stanley' × 'Opal') are particularly relevant because they extend the availability of fresh plums into late autumn. In the conditions of western Serbia, they ripen around September 20, approximately three weeks after 'Stanley' which was also confirmed in our study, ranking them among the latest-ripening plum cultivars. Such late-ripening genotypes are increasingly valued in breeding programs as a strategy to prolong market supply and enhance dietary continuity of fresh fruits.

From a nutritional perspective, plums are valued not only for their sensory attributes but also for their contribution to human health. They are rich in primary metabolites such as sugars, organic acids and dietary fiber, which determine fruit quality and digestibility. More importantly, plums are recognized as sources of bioactive compounds, particularly phenolic acids, flavonoids and anthocyanins, which exhibit strong antioxidant activity and play a role in reducing oxidative stress and inflammation (Milala et al., 2013; Wallace, 2017; Ghalandari et al., 2022). Among them, chlorogenic acid, rutin, quercetin, *p*-coumaric acid and cyanidin-3-glucoside are among the most abundant and biologically relevant compounds in plums, with well-documented health-promoting effects (Tomić et al., 2019; Liaudanskas et al., 2020). The regular consumption of plums and dried plums has been associated with improved gastrointestinal health, bone metabolism and metabolic regulation (Arjmandi et al., 2017; Ayub et al., 2023; Dwivedi et al., 2024).

Plums possess features that qualify them as functional foods, as they provide not only energy and phytochemicals that serve as potent dietary antioxidants but also essential nutrients. In addition to phytochemicals, plums provide macro- and micronutrients, including K, Ca, Mg, Fe, Zn, Cu and B, which contribute to diverse metabolic functions such as blood pressure and cardiovascular regulation, enzymatic activity, bone health, muscle and nerve function and immune response (Dwivedi et al., 2024; Scedei et al., 2024). The combined presence of antioxidants, minerals and dietary fiber underlines the functional relevance of plums beyond their basic nutritional value, particularly in the context of chronic disease prevention.

The balance of macro- and micronutrients in fruit composition is also of particular importance. Deficiency or excess of certain nutrients may impair physiological processes in plants, influencing fruit quality traits and postharvest storability (Milošević et al., 2025b). From a nutritional perspective, mineral imbalances may also affect human health: while insufficient intake of Fe, Ca or Mg is linked to anemia, osteoporosis and metabolic dysfunctions, excessive levels of micronutrients such as Cu or B may raise concerns regarding food safety and dietary exposure (Ekholm et al., 2007; Kabata-Pendias, 2010). Therefore, cultivar-specific mineral profiling is essential for evaluating both nutritional adequacy and potential dietary risks.

The composition of bioactive compounds and minerals in plums is strongly influenced by genotype, rootstock, environmental conditions and orchard management practices (Picchi et al., 2019; Milošević et al., 2025a). Genotype remains a primary driver of compositional variability, while environmental factors modulate the expression of these traits. Characterizing new cultivars is therefore essential for identifying those with superior nutritional and functional profiles. Despite this, only a limited number of studies in the available literature have simultaneously examined the content of primary metabolites, phenolic compounds and macro- and micronutrients in European plum fruit, as well as their interrelationships, particularly in newly released late-season cultivars.

The objective of this study was to evaluate primary metabolites, phenolic profile and mineral composition in fruits of novel late-season European plum cultivars bred at the Fruit Research Institute, Čačak, and to determine the relationships among these traits. By focusing on the

newly released cultivars 'Divna' and 'Petra' this work provides insights into their nutritional and functional relevance, within a broader context of contemporary plum breeding and functional fruit development, underlining their role in extending the seasonal availability of fresh plums and supporting the development of health-oriented food products.

2. Material and methods

2.1. Plant material, experimental layout, and environmental conditions

The study was conducted in a commercial orchard located in the village of Teočin, western Serbia (44°04'29" N; 20°14'23" E) at an elevation of 662 m. Observations and sampling were carried out over three consecutive years, from 2021 to 2023. The orchard was established in 2013 with a spacing of 5.0 × 3.0 m, corresponding to a planting density of 677 trees per hectare. A randomized block design was applied, with each cultivar represented by five trees in four replications (n = 20). Trees were trained to a central leader system, and standard orchard practices were followed, with the exception of irrigation, which was not applied.

The experiment involved two newly released late-season European plum (*P. domestica* L.) cultivars developed at the Fruit Research Institute, Čačak, 'Divna' and 'Petra'. The widely grown cultivar 'Stanley' was included as a reference standard. All trees were grafted onto Myrobalan seedling rootstocks.

The experimental orchard was established on Vertisol soil (locally referred to as "Smonitza"), a heavy clay soil distinguished by its pronounced shrink-swell behaviour and limited drainage capacity. In the surface layer (0–30 cm), the soil texture comprised 17 % sand, 24 % silt, and 59 % clay. The organic matter concentration was 3.08 %, while the soil pH measured in water was 5.48, reflecting moderately acidic conditions. The cation exchange capacity (CEC) was 26.6 cmol(+) kg⁻¹, with a sum of base cations (SB) of 14.9 cmol(+) kg⁻¹ and the base saturation (BS%) at 54.2 %. Regarding nutrient status (dry matter basis), the soil contained 0.18 % total nitrogen (N), 177 µg g⁻¹ P, 256 µg g⁻¹ K, 0.26 % calcium oxide (CaO) and 3.99 µg g⁻¹ magnesium oxide (MgO). Micronutrient concentrations were as follows: Fe 73 mg kg⁻¹, Mn 36 mg kg⁻¹, Zn 1.4 mg kg⁻¹, Cu 0.5 mg kg⁻¹ and B 0.68 mg kg⁻¹.

The climate in Serbia is largely classified as moderate continental, with steppe-like influences in certain areas, creating favorable conditions for plum production (Vujadinović Mandić et al., 2022). Meteorological data for the study period (Fig. 1a–c) were provided by the Republic Hydrometeorological Service of Serbia (<http://www.hidmet.gov.rs>). During the growing season (April–October), the mean air temperature in 2021 and 2022 was below the long-term average, whereas in 2023 it was above this reference value. Nevertheless, maximum summer temperatures across all three years of the trial consistently exceeded the long-term average, reflecting the broader warming trend associated with recent climate change. Precipitation levels during the vegetative cycle were markedly lower than average in the first two years, while in 2023 the total rainfall surpassed the average, though its temporal distribution was unfavorable. Taken together, elevated maximum temperatures, reduced or irregular rainfall and the absence of irrigation likely affected the results observed in this study.

2.2. Sample preparation and analysis

For the assessment of primary metabolites, phenolic compounds and fruit macro- and micronutrients, three sets of 25 randomly selected fruits at full maturity were analyzed. All chemical determinations were performed on fully mature fruits (flesh and peel) in four replicates, with five fruits per replicate (n = 4). Results are presented as means ± SE, based on triplicate analyses for each cultivar combination across the years 2021, 2022 and 2023.

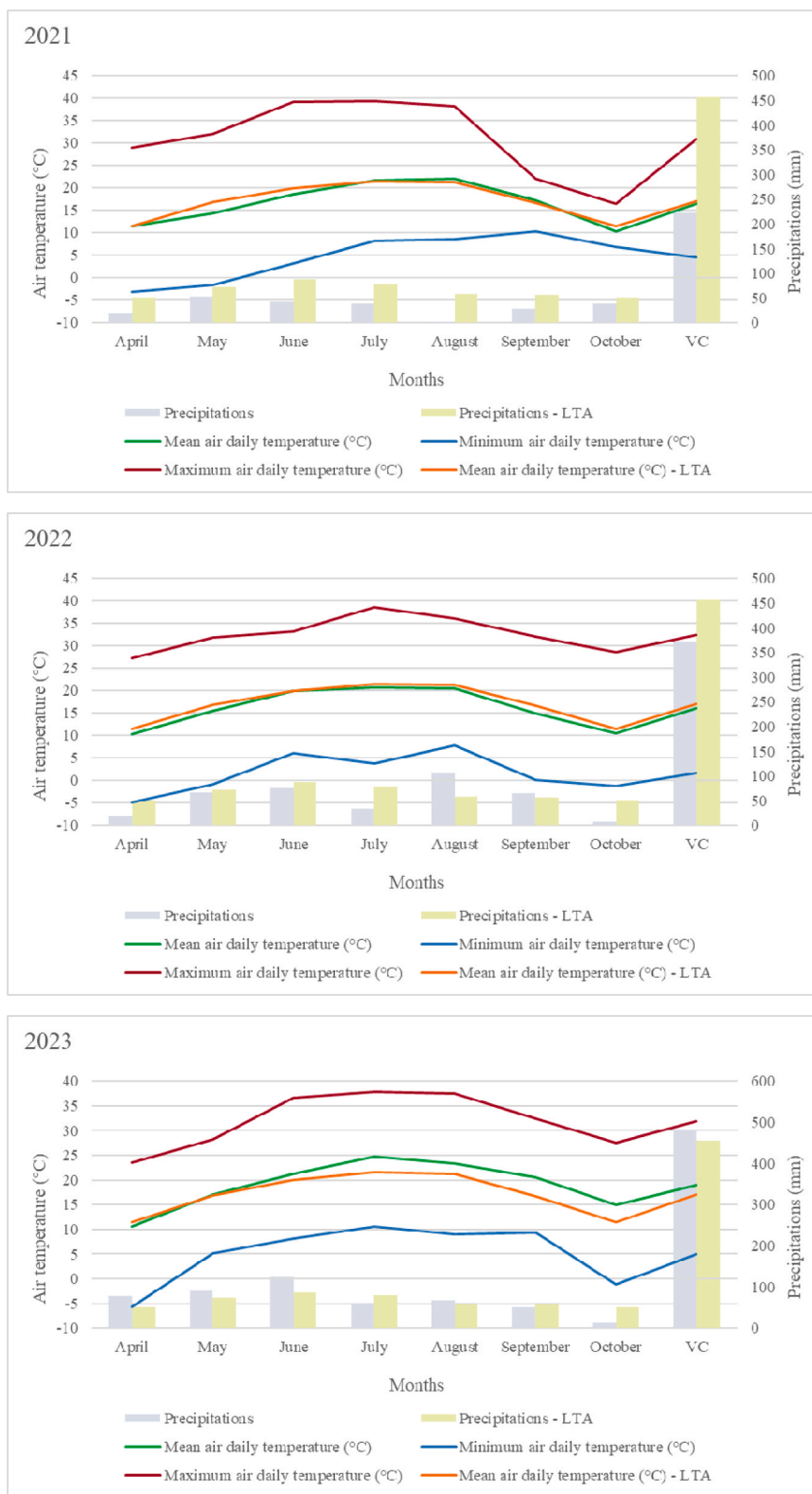


Fig. 1. a. Weather conditions (temperature and precipitation) during the vegetative cycle in the Čačak area (western Serbia) in 2021. Abbreviations: LTA = normal refers to the long-term average (45-year average, i.e. 1965–2010 period); VC = vegetative cycle. **Fig. 1b.** Weather conditions (temperature and precipitation) during the vegetative cycle in the Čačak area (western Serbia) in 2022. Abbreviations: LTA = normal refers to the long-term average (45-year average, i.e. 1965–2010 period); VC = vegetative cycle. **Fig. 1c.** Weather conditions (temperature and precipitation) during the vegetative cycle in the Čačak area (western Serbia) in 2023. Abbreviations: LTA = normal refers to the long-term average (45-year average, i.e. 1965–2010 period); VC = vegetative cycle.

2.2.1. Fruit primary metabolites

SSC ($^{\circ}$ Brix) was measured at room temperature (20 $^{\circ}$ C) using a digital refractometer (HI96801, Hanna Instruments, Vöhringen, Germany). TA, expressed as % malic acid, was determined by titration with 0.1 N NaOH in the presence of phenolphthalein as an indicator, with the endpoint marked by a persistent pink coloration. The RI was calculated as the ratio between SSC and TA. TS and RS were quantified using the Luff-Schoorl method based on iodine titration of excess copper (Alexander et al., 1985). N-RS were calculated as $(TS - RS) \times 0.95$. All sugar contents were expressed as percentage of fresh weight (fw). Vitamin C concentration ($\text{mg } 100 \text{ g}^{-1} \text{ fw}$) was determined using the 2,6-dichloroindophenol (DCIP) titration method (Arya et al., 2000).

2.2.2. Fruit phenolic composition

Phenolic compounds (chlorogenic acid, *p*-coumaric acid, rutin, quercetin and cyanidin-3-glucoside) were quantified by HPLC analysis. Fruit samples were chopped and 1 g of homogenized tissue was extracted with 5 mL of methanol containing 1 % formic acid for 1 h. Extracts were centrifuged at 10,000 rpm for 10 min at 4 $^{\circ}$ C, filtered through a 0.20 μm polyamide filter (Chromafil A-20/25, Macherey-Nagel, Düren, Germany) and transferred to vials prior to injection. Analyses were performed on a Thermo Dionex HPLC system (Thermo Scientific, San Jose, USA) equipped with a diode array detector (DAD) and a Gemini C18 column (150 \times 4.6 mm i.d., 3 μm ; Phenomenex, Torrance, USA) operated at 25 $^{\circ}$ C. The mobile phase consisted of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B), with a gradient program adapted from Celik et al. (2017). The injection volume was 20 μL , and the flow rate was 0.6 mL min^{-1} . Identification of compounds was based on retention times and UV-Vis spectra compared with authentic standards (chlorogenic acid, *p*-coumaric acid, rutin, quercetin, cyanidin-3-glucoside; Sigma-Aldrich, St. Louis, USA), following previously published protocols (Tomás-Barberán et al., 2001; Mikulic Petkovsek et al., 2012). Quantification was performed using external calibration curves, and results were expressed as milligrams per 100 g of fresh weight ($\text{mg } 100 \text{ g}^{-1} \text{ fw}$).

2.2.3. Fruit mineral composition

For the assessment of fruit macro- and micronutrient composition, sampling and sample preparation were performed following the protocols outlined by Milošević et al. (2013, 2023). Briefly, fruits were rinsed with deionized water, oven-dried at 70 $^{\circ}$ C to constant weight, weighed, and finely ground to pass through a 0.5 mm mesh sieve using a Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ, USA).

Total nitrogen (Ntot) was determined using a modified Kjeldahl method with a Tecator Kjeltac 1003 Distilling Unit (Rochester, NY, USA). P was analyzed by the phospho-vanadate colorimetric method using a Genesys 180 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). K and Mg were quantified by flame photometry (Flapho 4, Carl Zeiss, Jena, Germany), while B was assessed colorimetrically with kinalizarin on an MK 6/6 colorimeter (Carl Zeiss, Jena, Germany). The concentrations of other elements (Fe, Mn, Cu, Zn) were measured using a Perkin-Elmer PinAAcle 500 flame atomic absorption spectrometer (Waltham, MA, USA). Results were expressed on a dry weight basis: macronutrients (N, P, K, Ca, Mg) as $\text{g kg}^{-1} \text{ dw}$ and micronutrients (Fe, Mn, Cu, Zn, B) as $\text{mg kg}^{-1} \text{ dw}$.

2.3. Statistical analysis

Experimental data were analyzed using analysis of variance (ANOVA). When the *F*-test revealed significant differences, mean comparisons were carried out with the least significant difference (LSD) test at a 5 % probability level ($P \leq 0.05$). Statistical analyses and figure preparation were performed in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as mean values \pm standard error (\pm SE). Relationships among variables were evaluated through Pearson's correlation analysis using the corrplot package in R (Wei and Simko,

2017), with statistical significance set at $\alpha = 0.05$. Principal component analysis (PCA), based on 22 traits, was applied to assess overall variability and to highlight cultivar-related effects. The PCA biplot was constructed with XLSTAT software (Addinsoft, New York, USA).

3. Results

3.1. Fruit primary metabolites

Analysis of variance revealed that both cultivar and year exerted a significant effect on all examined primary metabolites. The interaction cultivar \times year was significant for SSC, TS, RS, N-RS and TA, while it was not significant for RI and Vit C (Table 1).

Across cultivars, 'Petra' exhibited the highest SSC and TS, whereas 'Divna' recorded the highest values for RS, TA and Vit C. The standard cultivar 'Stanley' was distinguished by the highest N-RS and RI and the lowest Vit C content. These results indicate clear genotype-dependent differences in primary metabolite composition.

Year also had a strong influence on fruit primary metabolites. The highest SSC was observed in 2022, while TS and N-RS peaked in 2023. RS showed a gradual increase across the three seasons, reaching a maximum in 2023. TA increased slightly from 2021 to 2023. Vit C content remained relatively stable across years, with the highest values recorded in 2021 and 2022 and a minor decline in 2023. Variation in the RI was limited, with comparable values among seasons.

The significance of the cultivar \times year interactions for most traits (except RI and Vit C) confirms that the accumulation of primary metabolite is not uniform across seasons but depends on cultivar performance under specific environmental conditions.

3.2. Fruit phenolic composition

Significant differences in the concentrations of individual phenolic compounds were observed among cultivars and across years (Table 2). The interaction cultivar \times year was significant for all examined traits, highlighting the combined influence of genetic background and seasonal on phenolic accumulation.

Among cultivars, 'Petra' consistently showed the highest concentrations of chlorogenic acid, *p*-coumaric acid, rutin, quercetin and cyanidin-3-glucoside, followed by 'Divna', whereas the standard cultivar 'Stanley' generally recorded the lowest values, except for rutin where it showed intermediate levels.

Year to year variation was also evident. The highest mean levels of all phenolic compounds were recorded in 2022, whereas the lowest were observed in 2023, with intermediate values in 2021. These results demonstrate pronounced seasonal variability in phenolic profiles.

The significance of the cultivar \times year interactions indicates that phenolic accumulation patterns varied across years and were cultivar-specific rather than stable over time.

3.3. Fruit mineral composition

The mean concentrations of macro- and micronutrients in fruits of the examined cultivars across three consecutive years are shown in Tables 3 and 4. Analysis of variance showed that cultivar significantly affected the concentrations of most macro and micronutrients, while year also exerted a significant influence on mineral composition. The interaction cultivar \times year was significant for several elements, indicating variable mineral accumulation depending on seasonal conditions.

Among macronutrients, 'Petra' contained the highest levels of N, P, K and Mg, while 'Divna' had the highest Ca concentration. The lowest N, P, K and Mg values were found in 'Stanley', whereas Ca was lowest in 'Petra'. These differences suggest distinct nutrient accumulation patterns among the studied cultivars.

Regarding micronutrients, 'Divna' recorded the highest concentrations of Fe, Mn, Cu and Zn, whereas 'Petra' had the highest B content.

Table 1

Primary metabolite content in fruits of new late-season plum cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley' over a three-year period (2021–2023).

Parameter	Soluble solids (%)	Total Sugars (%)	Reducing Sugars (%)	Non-Reducing Sugars (%)	Total acids (%)	Ripening Index	Vitamin C (mg 100 g ⁻¹ fw)
Cultivar (A)							
Divna	20.75 ± 0.31c	10.78 ± 0.19 b	7.36 ± 0.07 a	3.24 ± 0.17c	1.33 ± 0.01 a	15.58 ± 0.33c	7.02 ± 0.08 a
Petra	22.85 ± 0.22 a	11.65 ± 0.10 a	6.96 ± 0.01 b	4.46 ± 0.10 b	1.10 ± 0.01 b	20.71 ± 0.24 b	6.08 ± 0.02 b
Stanley	21.12 ± 0.31 b	11.32 ± 0.19 ab	5.97 ± 0.07c	5.08 ± 0.17 a	0.96 ± 0.01c	21.92 ± 0.33 a	5.06 ± 0.08c
Year (B)							
2021	21.16 ± 0.34 b	10.73 ± 0.15c	6.70 ± 0.12c	3.83 ± 0.11c	1.11 ± 0.02 c	19.40 ± 0.33 ab	6.08 ± 0.11 a
2022	21.95 ± 0.24 a	11.25 ± 0.18 b	6.75 ± 0.01 b	4.27 ± 0.17 b	1.13 ± 0.01 b	19.75 ± 0.27 a	6.13 ± 0.03 a
2023	21.61 ± 0.26 ab	11.77 ± 0.16 a	6.84 ± 0.01 a	4.69 ± 0.15 a	1.15 ± 0.01 a	19.05 ± 0.30 b	5.96 ± 0.03 b
ANOVA (<i>F</i> test)							
A	*	*	*	*	*	*	*
B	*	*	*	*	*	*	*
AB	*	*	*	*	*	ns	ns

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.The asterisk in column indicates a significant difference between means at $P \leq 0.05$ by LSD test.**Table 2**

Phenolic profile in fruits of new late-season plum cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley' over a three-year period (2021–2023).

Parameter	Chlorogenic acid (mg 100 g ⁻¹ fw)	<i>p</i> -coumaric acid (mg 100 g ⁻¹ fw)	Rutin (mg 100 g ⁻¹ fw)	Quercetin (mg 100 g ⁻¹ fw)	Cyanidin-3-glucoside (mg 100 g ⁻¹ fw)
Cultivar (A)					
Divna	11.55 ± 0.36 b	0.50 ± 0.03 b	7.60 ± 0.16c	0.64 ± 0.29c	2.85 ± 0.08 b
Petra	14.04 ± 0.05 a	0.72 ± 0.00 a	10.11 ± 0.03 a	0.99 ± 0.16 a	3.64 ± 0.03 a
Stanley	10.20 ± 0.36c	0.36 ± 0.03c	8.66 ± 0.16 b	0.70 ± 0.29 b	2.62 ± 0.08c
Year (B)					
2021	11.88 ± 0.43 b	0.49 ± 0.05c	8.71 ± 0.20 b	0.69 ± 0.39 b	2.94 ± 0.13 b
2022	13.03 ± 0.03 a	0.58 ± 0.00 a	9.24 ± 0.04 a	0.91 ± 0.09 a	3.23 ± 0.01 a
2023	10.88 ± 0.31c	0.51 ± 0.01 b	8.42 ± 0.11c	0.73 ± 0.25 b	2.95 ± 0.04 b
ANOVA (<i>F</i> test)					
A	*	*	*	*	*
B	*	*	*	*	*
AB	*	*	*	*	*

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.The asterisk in column indicates a significant difference between means at $P \leq 0.05$ by LSD test.**Table 3**

Macronutrient content in fruits of new late-season plum cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley' over a three-year period (2021–2023).

Parameter	Nitrogen (N) (g kg ⁻¹ dw)	Phosphorus (P) (g kg ⁻¹ dw)	Potassium (K) (g kg ⁻¹ dw)	Calcium (Ca) (g kg ⁻¹ dw)	Magnesium (Mg) (g kg ⁻¹ dw)
Cultivar (A)					
Divna	6.66 ± 0.03 ab	0.51 ± 0.00 b	6.38 ± 0.07 b	0.18 ± 0.00 a	0.15 ± 0.00c
Petra	7.20 ± 0.00 a	0.56 ± 0.00 a	7.39 ± 0.00 a	0.13 ± 0.00c	0.18 ± 0.00 a
Stanley	5.60 ± 0.03 b	0.52 ± 0.00 b	5.40 ± 0.01c	0.15 ± 0.00 b	0.17 ± 0.00 b
Year (B)					
2021	6.62 ± 0.00 a	0.53 ± 0.00 a	6.49 ± 0.01 a	0.16 ± 0.00 a	0.17 ± 0.00 a
2022	6.86 ± 0.00 a	0.53 ± 0.00 a	6.34 ± 0.00 a	0.16 ± 0.00 a	0.17 ± 0.00 a
2023	5.98 ± 0.05 a	0.52 ± 0.00 a	6.33 ± 0.00 a	0.15 ± 0.00 b	0.16 ± 0.00 b
ANOVA (<i>F</i> test)					
A	*	*	*	*	*
B	ns	ns	ns	*	*
AB	ns	*	ns	ns	ns

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.The asterisk in column indicates a significant difference between means at $P \leq 0.05$ by LSD test.

The standard cultivar 'Stanley' generally exhibited lower levels of Fe, Mn and Cu compared with the new cultivars, but intermediate values for Zn and B.

Year-to-year variation was most evident for Ca and Mg among macronutrients, with slightly higher values in 2021 and 2022 compared to 2023. For micronutrients, Fe content declined progressively from 2021 to 2023, while Mn increased slightly over the years. Cu showed a peak in 2021, but lower values in subsequent years. B content was highest in 2022, whereas Zn remained relatively stable across seasons.

3.4. Correlation among examined parameters

The Pearson's correlation matrix revealed numerous significant relationships among fruit primary metabolites, phenolic compounds and macro- and micronutrients in plum fruits (Fig. 2) indicating coordinated variation among biochemical and mineral traits.

Sugars (SSC, TS and RS) showed strong to very strong positive associations with individual phenolic compounds and antioxidant capacity, suggesting that cultivars characterized by higher sweetness also tended to accumulate elevated levels of phenolics. TA were negatively

Table 4

Micronutrient concentrations in fruits of new late-season plum cultivars 'Divna' and 'Petra' and standard cultivar 'Stanley' over a three-year period (2021–2023).

Parameter	Iron (Fe) (mg kg ⁻¹ dw)	Manganese (Mn) (mg kg ⁻¹ dw)	Copper (Cu) (mg kg ⁻¹ dw)	Zinc (Zn) (mg kg ⁻¹ dw)	Boron (B) (mg kg ⁻¹ dw)
Cultivar (A)					
Divna	39.64 ± 0.02 a	2.82 ± 0.01 a	0.10 ± 0.00 a	3.72 ± 0.03 a	2.46 ± 0.02 a
Petra	25.53 ± 0.01 b	2.19 ± 0.01 b	0.08 ± 0.00 b	1.82 ± 0.02c	2.53 ± 0.01 a
Stanley	24.86 ± 0.02 b	1.39 ± 0.01c	0.07 ± 0.00c	3.07 ± 0.03 b	2.28 ± 0.02 b
Year (B)					
2021	30.79 ± 0.02 a	20.89 ± 0.01 b	0.09 ± 0.00 a	2.93 ± 0.06 a	2.35 ± 0.03 b
2022	30.31 ± 0.02 a	21.05 ± 0.01 b	0.08 ± 0.00 b	2.85 ± 0.01 a	2.51 ± 0.01 a
2023	28.92 ± 0.02 b	22.01 ± 0.01 a	0.08 ± 0.00 b	2.82 ± 0.00 a	2.28 ± 0.01 b
ANOVA (<i>F</i> test)					
A	*	*	*	*	*
B	*	*	*	ns	*
AB	ns	*	*	ns	ns

Differences between data marked with different letters in columns are significant at $P \leq 0.05$ level by LSD test.

The asterisk in column indicates a significant difference between means at $P \leq 0.05$ by LSD test.

associated with SSC and TS, whereas the ripening index (RI) exhibited positive relationships with sugars and negative relationships with acidity, reflecting expected interdependencies among primary quality parameters.

Several mineral nutrients displayed notable associations with both primary and secondary metabolites. In particular, micronutrients such as Mn and Cu showed strong positive relationships with sugars and phenolic compounds, whereas macronutrients such as K were more closely associated with primary metabolite profiles. In contrast, Ca exhibited pronounced negative associations with sugar-related parameters and Mg, indicating divergent accumulation patterns among mineral elements.

Overall, the correlation structure highlights close interrelationships among fruit quality traits, bioactive compounds and mineral nutrients. However, because correlations were calculated using cultivar- and year-averaged data and a limited number of experimental units, the strength of individual coefficients should be interpreted with caution, with emphasis placed on general patterns rather than on single correlation values.

3.5. Principal component analysis

PCA was applied to summarize multivariate relationships among the examined traits and to visualize cultivar differentiation based on fruit biochemical and mineral composition. The first two principal components (PC1 and PC2) substantial proportion of the total variance, confirming that a limited number of components captured most of the variability present in the dataset (Fig. 3).

PC1 was primarily associated with sugars (SSC, TS and RS), individual phenolic compounds and antioxidant capacity, indicating that this axis reflects variation related to sweetness and bioactive potential. In contrast, PC2 showed stronger loadings for organic acids, vitamin C and several macro- and micronutrients, suggesting that this component is linked to acidity and mineral-related characteristics.

The PCA biplot revealed clear cultivar separation. 'Petra' was positioned along the positive side of PC1, reflecting its higher sugar content, phenolic concentrations and antioxidant capacity. 'Divna' was more strongly associated with PC2, driven by elevated acidity, Vit C and mineral concentrations, whereas the standard cultivar 'Stanley' occupied an intermediate position between these two groups. This cultivar grouping is consistent with the results of univariate analyses and correlation patterns, confirming distinct compositional profiles among the examined cultivars.

4. Discussion

4.1. Fruit primary metabolites

The present results confirm that the composition of primary metabolites in European plum fruits is shaped by the combined effects of genetic background and seasonal conditions, as consistently reported for plums and other stone fruits (Liverani et al., 2008; Serradilla et al., 2017; Popski et al., 2021). The significant cultivar × year interactions observed for most parameters indicate that genotypic differences in sugar and acid metabolism are modulated, rather than overridden, by environmental variability during fruit development and ripening.

Differences among cultivars reflect distinct metabolic strategies related to carbon allocation and organic acid balance, which are fundamental determinants of fruit sensory quality and nutritional value. Variation in sugar composition primarily influences sweetness perception, whereas differences in organic acid content contribute to flavor complexity and freshness. Vit C, although present at relatively low concentrations in plums compared with other fruit species (Gil et al., 2002), represents an additional nutritional attribute that may further differentiate cultivars in terms of antioxidant contribution (Kim et al., 2003; Tomić et al., 2019). Such cultivar dependent variation underscores the strong genetic control of primary metabolism in plums, as previously demonstrated for sugar-acid balance and related quality traits (Jiang et al., 2019; Ceccarelli et al., 2021).

Seasonal effects further influenced the accumulation of primary metabolites, particularly sugars, which are known to be highly responsive to temperature regime and water availability during the ripening period. Similar year-to-year fluctuations in sugar content have been documented in plums and apricots, emphasizing the sensitivity of carbohydrate accumulation to environmental conditions in temperate climates (Vangdal et al., 2007; Ciccioritti et al., 2021). In contrast, titratable acidity and vitamin C content exhibited comparatively lower seasonal variability, suggesting that these traits may be under tighter physiological regulation and less responsive to short-term environmental fluctuations (Fenech et al., 2006; Cervantes et al., 2020).

From a sensory and consumer-oriented perspective, fruit quality is determined not by individual parameters but by the balance between sugars and acids (Crisosto et al., 2004). The RI provides an integrative measure of this balance and has been widely used as an indicator of eating quality in plums (Crisosto et al., 2004; Drogoudi and Pantelidis, 2022). The values observed in this study fall within the optimal range previously reported for European plum cultivars, supporting the high sensory potential of the examined genotypes (Drogoudi and Pantelidis,

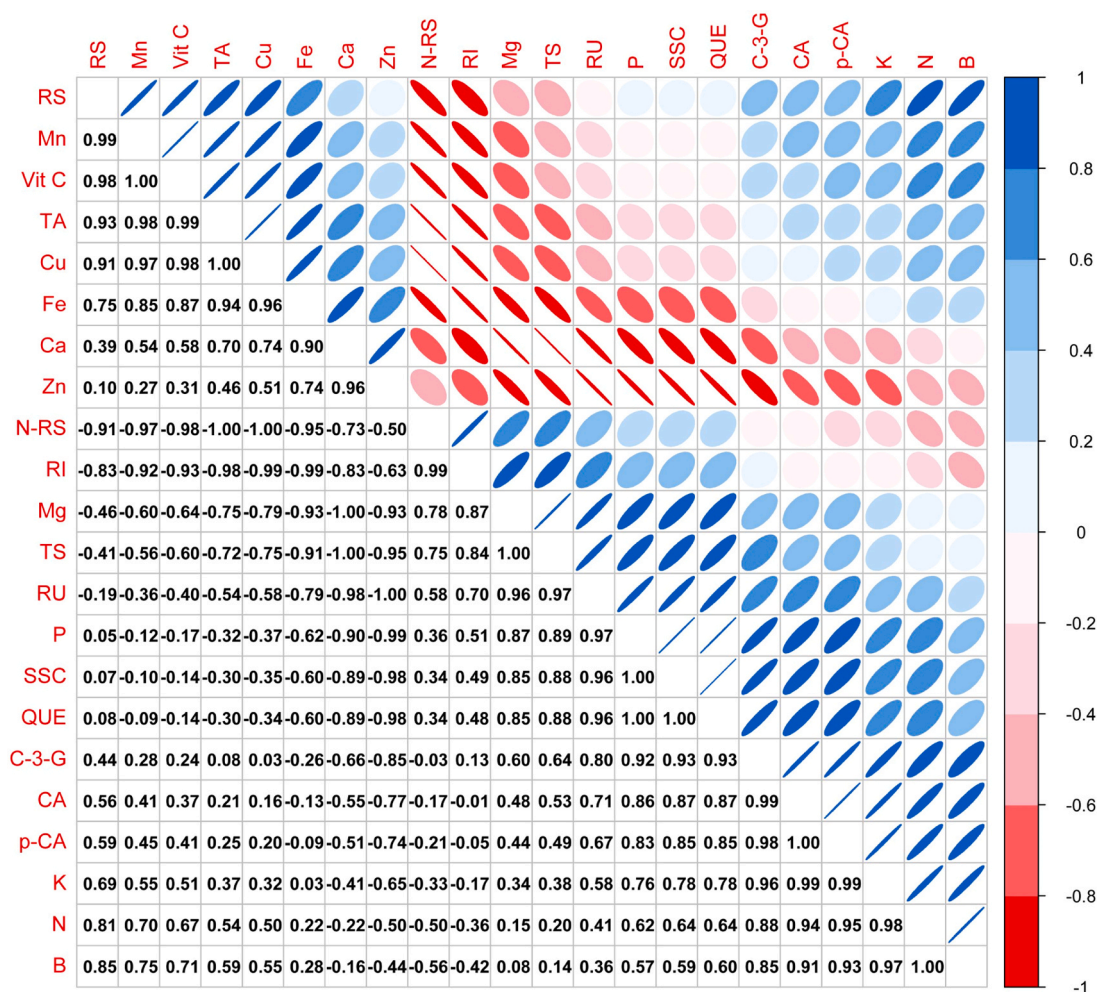


Fig. 2. Correlogram illustrating the relationships among primary metabolites, phenolic compounds and macro and micronutrient contents in fruits of two newly released late-season European plum cultivars ‘Divna’ and ‘Petra’ and standard cultivar ‘Stanley’, over a three-year period. The angle and color intensity of the ellipses are proportional to the Pearson’s correlation coefficient at $\alpha = 0.05$. Shades ranging from light blue to dark blue indicate positive correlations, while light red to dark red denote negative correlations. The scale on the right side of the figure represents the correlation strength, ranging from -1 to +1, with the corresponding Pearson’s *r*-values. Abbreviations: SSC, soluble solids content; TS, total sugars; RS, reducing sugars; N-RS, non-reducing sugars; TA, total acids; RI, ripening index; CA, chlorogenic acid; p-CA, *p*-coumaric acid; RU, rutin, QUE, quercetin, C-3-G, cyanidin-3-glucoside.

2022; Milošević et al., 2025a). Obtained results indicate that newly developed late-season cultivars, such as ‘Petra’ and ‘Divna’, combine favorable sugar–acid balance with stable Vit C levels across years, highlighting their potential to deliver consistent fruit quality under variable environmental conditions. These characteristics are particularly relevant for extending the market supply of high-quality plums into late autumn, without compromising sensory attributes or basic nutritional value.

4.2. Fruit phenolic composition

Phenolic compounds represent one of the most important groups of bioactive substances in plums, playing essential roles in plant physiology and human nutrition. They contribute to growth and development, defense against biotic and abiotic stress, and fruit sensory attributes such as colour, flavour and taste (Kim et al., 2003; Milošević et al., 2025a). They include phenolic acids, flavonoids and anthocyanins, each with distinct physiological and health-promoting roles. In particular, chlorogenic acid, rutin, quercetin, *p*-coumaric acid and cyanidin-3-glucoside are recognized as major representatives of these groups in plums and related stone fruits (Usenik et al., 2009; Liaudanskas et al., 2020; Xiao et al., 2024). These compounds exhibit strong antioxidant, anti-inflammatory and metabolic-regulating properties,

linking plum consumption to improved human health outcomes (Nile and Park, 2014; Milala et al., 2013). Their bioactivity is linked to different mechanisms, such as free radical scavenging, metal chelation and enzyme modulation (Aron and Kennedy, 2008).

Our results confirmed that both cultivar and year significantly influenced the concentrations of all examined phenolic compounds, with cultivar \times year interactions also being significant. This indicates that phenolic accumulation is shaped by the combined effects of genetic background and environmental conditions, which is consistent with findings from previous studies in plums (Drkenda et al., 2019; Tomić et al., 2019; Zezulová et al., 2022).

The observed cultivar-dependent differences in phenolic profiles reflect inherent genetic variability in phenylpropanoid metabolism, which governs the biosynthesis of hydroxycinnamic acids, flavonoids and anthocyanins in fruit tissues. Chlorogenic acid, commonly reported as the dominant hydroxycinnamic acid in plums, plays a central role in antioxidant capacity and represents a key intermediate linking primary metabolism with secondary phenolic pathways (Kim et al., 2003; Liaudanskas et al., 2020). Similarly, flavonols such as rutin and quercetin contribute substantially to the antioxidant profile of plums and are considered reliable indicators of cultivar-specific phenolic potential (Tomić et al., 2019). Anthocyanins, particularly cyanidin-3-glucoside, are primarily responsible for fruit skin coloration and represent

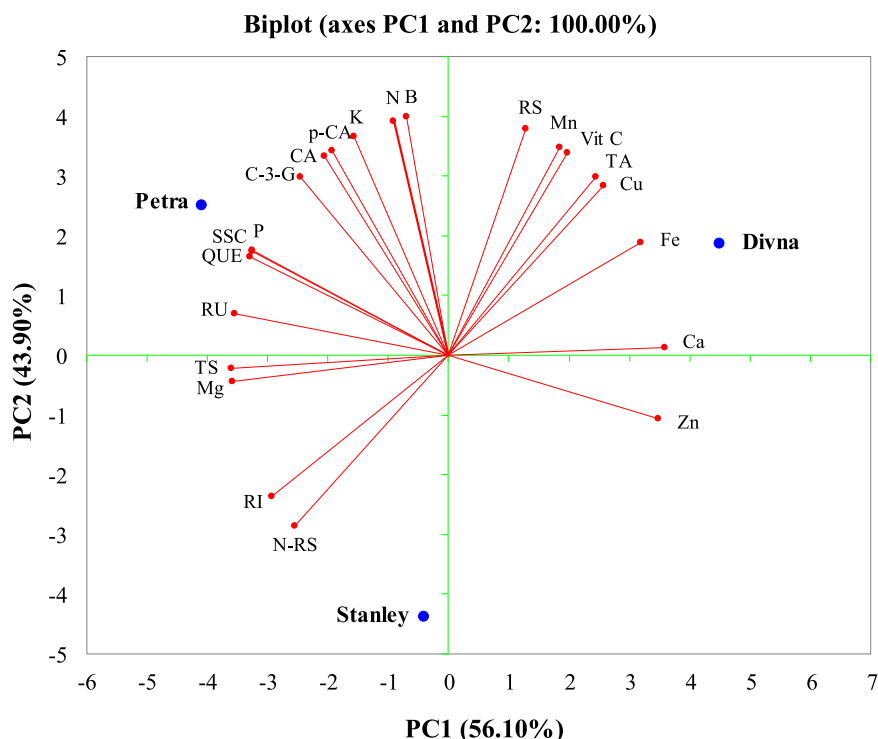


Fig. 3. Principal component analysis (PCA) biplot of late-season European plum cultivars ‘Divna’ and ‘Petra’ and standard cultivar ‘Stanley’, based on primary metabolite and phenolic profiles, as well as macro- and micronutrient contents in fruits. Abbreviations: SSC, soluble solids content; TS, total sugars; RS, reducing sugars; N-RS, non-reducing sugars; TA, total acids; RI, ripening index; CA, chlorogenic acid; p-CA, *p*-coumaric acid; RU, rutin; QUE, quercetin; C-3-G, cyanidin-3-glucoside.

important dietary antioxidants. Their accumulation is tightly regulated by genetic factors but remains sensitive to environmental modulation during late fruit development, as previously documented in plums and other stone fruits (Usenik et al., 2009; Fotirić Akšić et al., 2023). Although *p*-coumaric acid is generally present at lower concentrations than other compounds, its role as a precursor within the phenylpropanoid pathway underlines its relevance for understanding inter-cultivar differences in overall phenolic metabolism (Stalikas, 2007; Zhang et al., 2023). In particular, the higher values of phenolic compounds observed in ‘Petra’ align with reports that late-season plum cultivars often accumulate greater amounts of phenolics due to prolonged ripening under favourable climatic conditions (Tomić et al., 2019; Milošević et al., 2025a). On the other hand, the relatively lower values in ‘Stanley’ are in accordance with literature that generally describes this cultivar as nutritionally less rich compared to newly developed genotypes (Korićanac et al., 2025).

Environmental effects were also evident, with marked year-to-year variability in phenolic accumulation. Such variability reflects the strong influence of climatic factors, including temperature regime, water availability and solar radiation, on the regulation of phenolic biosynthesis (Miletic et al., 2012; Drkenda et al., 2019). Phenolic metabolism is particularly responsive to moderate temperature conditions and mild environmental stress, which may stimulate the activity of key enzymes in the phenylpropanoid pathway, whereas excessive heat or drought can constrain phenolic accumulation (Xu et al., 2011; Hamdani et al., 2024).

The ranges of individual phenolic compounds observed in this study are generally comparable with those reported in previous investigations on European plums (Usenik et al., 2009; Liaudanskas et al., 2020; Trendafilova et al., 2022), while differences among studies likely reflect the combined effects of genotype, harvest maturity, environmental conditions and analytical methodology. Such variability underscores the importance of evaluating phenolic profiles within a defined genetic and environmental context, rather than relying on absolute concentration

values alone.

Overall, these findings confirm that phenolic composition in plums is not uniform but is strongly shaped by both genotype and seasonal environment. The consistent expression of higher phenolic potential in newly developed late-season cultivars ‘Petra’ and ‘Divna’ highlights their relevance not only as valuable genetic resources for breeding, but also for the production of fruits with enhanced functional value. By extending the availability of phenolic-rich plums into late autumn, such cultivars offer added nutritional and commercial value for consumers and the food industry.

4.3. Fruit mineral composition

The mineral composition of plum fruit is strongly influenced by genotype, as confirmed by the significant differences observed among cultivars in all analyzed macro- and micronutrients. This pattern underscores the dominant role of genetic background in regulating nutrient uptake, translocation and accumulation in fruit tissues, as previously reported for plums and other *Prunus* species (Milošević and Milošević, 2012; Botelho and Müller, 2020). In addition to genetic control, year-to-year variability affected the concentrations of Ca, Mg, Fe, Mn, Cu and B, reflecting the sensitivity of mineral composition to environmental conditions such as rainfall distribution, temperature fluctuations and soil nutrient availability. Similar findings of strong genotype dependence modulated by environmental variability have been documented in various stone fruits, including plums (Stojanovic et al., 2024; Milošević et al., 2025b).

Among macronutrients, K was the most abundant element, which is consistent with earlier reports identifying plums as an important dietary source of potassium (Ekholm et al., 2007; Wallace, 2017). This nutrient plays a central role in osmotic regulation, enzyme activation and carbohydrate transport, thereby indirectly influencing overall fruit quality, particularly sweetness and juiciness. In contrast, Ca is primarily associated with cell wall stability and membrane integrity, contributing to

fruit firmness and postharvest quality (Sinha et al., 2019), while Mg is essential for chlorophyll synthesis and carbohydrate metabolism (Marschner, 2012). The observed seasonal variability in Ca and Mg concentrations highlights their susceptibility to environmental modulation, although previous studies have reported more stable patterns in specific cultivars or growing conditions (Kalcsits et al., 2020).

P and N contents also varied among cultivars, suggesting differences in nutrient acquisition efficiency and internal redistribution. Such variability may reflect genotype-specific transport mechanisms and nutrient interactions, particularly the well-documented antagonistic effects between K, Ca and Mg uptake (Maathuis and Podar, 2011). This emphasizes that fruit nutritional quality depends not only on absolute mineral concentrations but also on their relative balance, with appropriate K:Ca:Mg ratios being critical for both plant physiological performance and dietary value (Fallahi and Simons, 1996; Casero et al., 2004).

Micronutrients displayed pronounced genotypic and environmental variability, highlighting their dynamic regulation in fruit tissues. Fe and Mn, which act as essential cofactors in redox reactions and enzymatic processes related to respiration and secondary metabolism (Broadley and White, 2012), showed sensitivity to seasonal conditions. Zn and Cu, involved in enzyme activation, reproductive development and oxidative stress regulation (Xie et al., 2021; Xu et al., 2024), also exhibited significant genotype \times year interactions, indicating complex control of their accumulation. Boron a micronutrient particularly abundant in plums, plays a crucial role in cell wall structure and carbohydrate transport in plants and has been recognized as relevant for human nutrition as well (Stacewicz-Sapuntzakis et al., 2001; Thakur et al., 2023). The consistently elevated B levels observed across cultivars further support the nutritional relevance of plums as a valuable dietary source of this element.

In general, our results demonstrate that mineral composition in plum fruits arises from a complex interplay between genetic potential and environmental modulation. The newly released late-season cultivars, 'Divna' and 'Petra', exhibited mineral concentrations within the ranges reported for European plums (Cosmulescu et al., 2017; Scedei et al., 2024; Milošević et al., 2025b), while maintaining cultivar-specific mineral profiles that may be relevant for breeding strategies and for nutritional evaluation. Given the established importance of minerals such as K, Ca, Mg, Fe and Zn in human health, the identification of genotypes with balanced mineral composition supports their potential use in the development of nutritionally oriented and functional-based products (Granato et al., 2020).

4.4. Correlation among examined parameters

The correlation analysis revealed complex relationships among primary metabolites, phenolic compounds, and macro- and micronutrients in plum fruits, highlighting the integrated nature of fruit biochemical composition. Rather than isolated associations, the observed correlation structure reflects coordinated variation among groups of traits related to carbohydrate metabolism, phenolic biosynthesis and mineral nutrition.

Sugars and phenolics were closely interconnected. SSC and TS showed strong positive correlations with quercetin and P, as well as strong associations with rutin, chlorogenic acid and *p*-coumaric acid, indicating that higher sugar levels tend to coincide with enhanced phenolic accumulation. This pattern is consistent with the metabolic linkage between carbohydrate availability and the phenylpropanoid pathway, in which sugars provide both substrates and signaling cues for secondary metabolite biosynthesis (Durán Soria et al., 2020; Xiao et al., 2024). RS was also positively associated with selected micronutrients, particularly Mn and Cu, supporting the role of these elements in carbohydrate metabolism and transport, as previously reported for plums and other fruit crops (Fotirić Akšić et al., 2023; Meland et al., 2024). In contrast, Ca displayed strong negative associations with sugar-related traits, which is in agreement with reports indicating that elevated Ca availability may limit carbohydrate translocation to fruit tissues under

certain conditions (Fallahi and Simons, 1996; Shui et al., 2022).

Acidity-related traits also exhibited clear metabolic linkages. TA showed positive relationships with Vit C and selected micronutrients, particularly Mn and Cu, suggesting that higher acidity may be accompanied by enhanced ascorbic acid accumulation. Such associations are biologically plausible given the involvement of Cu and Mn as cofactors in oxidative metabolism and antioxidant systems (Mo et al., 2022; Etienne et al., 2025). Conversely, N-RS showed negative associations with TA and Cu, reflecting the well-documented complementary partitioning between sugars and organic acids during fruit ripening in stone fruits (Durán Soria et al., 2020).

Phenolic compounds themselves exhibited strong interrelationships. Hydroxycinnamic acids, flavonols and anthocyanins showed coherent association patterns, reflecting their interconnected roles within the phenylpropanoid pathway. In particular, the close linkage between chlorogenic acid and *p*-coumaric acid highlights the precursor-product relationships within this pathway, while the association between rutin and quercetin is consistent with their shared biosynthetic origin, with rutin representing a glycosylated form of quercetin (Stalikas, 2007; Magar and Sohng, 2020; Zhang et al., 2023). Similar coordinated regulation of phenolic subclasses has been reported in plums and other fruits (Michalska et al., 2016; Drkenda et al., 2022).

Mineral nutrients further contributed to the observed correlation structure. Mn showed positive associations with RS and Vit C, supporting its role in carbohydrate-related enzymatic processes and antioxidant metabolism (Li and Yang, 2018). B was associated with N and K, which is consistent with its involvement in sugar transport, cell wall integrity and metabolic regulation in plants (Marschner, 2012; Vera-Maldonado et al., 2024).

Overall, the correlation patterns observed in this study emphasize that fruit quality traits, phenolic composition and mineral nutrition are regulated in a coordinated manner rather than independently. Given that correlations were derived from cultivar- and year-averaged data, the strength of individual associations should be interpreted with caution, with greater emphasis placed on general metabolic patterns than on single pairwise relationships. Nevertheless, these patterns provide valuable insight into the integrated regulation of nutritional and functional attributes in late-season European plum cultivars.

4.5. Principal component analysis

The PCA results revealed a clear separation of cultivars according to their dominant biochemical and mineral traits, providing an integrated overview of multivariate relationships among fruit quality parameters. PC1 was associated with contrasting groups of traits, reflecting a trade-off between mineral-related characteristics and sugar- and flavonoid-related attributes. Such opposing trends between mineral nutrition and carbohydrate- or phenolic-rich profiles have been reported in fruit species and are commonly attributed to competing metabolic demands during fruit development and ripening (Usenik et al., 2008; Dias et al., 2024). The association of 'Divna' with positive PC1 values highlights its tendency toward higher mineral and Vit C content, suggesting a nutritional profile characterized by enhanced mineral density rather than elevated sweetness or phenolic concentration. This positioning supports its relevance for dietary mineral intake without implying exclusivity of functional benefits (Dabbou et al., 2017; Granato et al., 2020).

PC2 was characterized by traits related to organic acids, phenolic compounds and associated macronutrients, indicating a compositional pattern linked to bioactive potential rather than basic mineral nutrition. The positioning of 'Petra' on the positive side of PC2 reflects its tendency toward higher accumulation of phenolic acids, flavonoids and anthocyanins, which is consistent with previous reports describing enhanced phenolic development in late-ripening plum cultivars (Tomić et al., 2019; Liaudanskas et al., 2020; Milošević et al., 2025a). Within this context, compounds such as chlorogenic acid and cyanidin-3-glucoside emerge as key contributors to cultivar differentiation, rather than as

isolated markers of quality (Kim et al., 2003; Liaudanskas et al., 2020). By contrast, ‘Stanley’ clustered toward negative PC2 values, reflecting its comparatively lower phenolic content and reduced bioactive potential, which aligns with earlier characterization of this cultivar as nutritionally less rich relative to recently developed genotypes (Korićanac et al., 2025).

The clear separation of cultivars across the first two principal components demonstrates complementary nutritional profiles rather than absolute superiority of a single genotype. ‘Divna’ is associated with mineral- and vitamin-related traits, whereas ‘Petra’ is characterized by attributes linked with sugars, phenolic composition and anthocyanin content. Such genotype-specific multivariate patterns have been consistently reported in plums and related *Prunus* species, reinforcing the value of PCA as a tool for integrated cultivar evaluation (Drkenda et al., 2019; Fotirić Akšić et al., 2023; Milošević et al., 2025a).

From a practical perspective, the PCA analysis underlines the importance of multivariate approaches in cultivar assessment. By revealing distinct but complementary nutritional strengths, such as the mineral- and vitamin-dense profile of ‘Divna’ and the phenolic- and anthocyanin-rich profile of ‘Petra’, this approach supports informed decisions in breeding, orchard management and cultivar positioning particularly in the context of functional food development where nutritional attributes complement traditional quality traits (Granato et al., 2020; Yuan et al., 2024).

5. Conclusion

This study provides a comprehensive evaluation of two newly released late-season European plum cultivars, ‘Divna’ and ‘Petra’ in comparison with the standard cultivar ‘Stanley’, with emphasis on fruit quality, primary metabolites, phenolic composition and mineral profile. The results demonstrate clear genotypic differentiation indicating complementary nutritional profiles rather than superiority of a single cultivar, while seasonal effects and significant cultivar × year interactions highlight the combined influence of genetic background and environmental conditions on fruit biochemical composition.

Multivariate and correlation analyses revealed coordinated relationships among sugars, organic acids, phenolic compounds and macro- and micronutrients, emphasizing that plum fruit quality and nutritional value arise from integrated metabolic networks rather than isolated traits. The observed associations between phenolics and key mineral elements further underline the importance of balanced mineral nutrition in shaping the functional attributes of plum fruits.

From a breeding perspective, the biochemical profiles of ‘Divna’ and ‘Petra’ confirm their value as promising genetic material for programs aimed at improving nutritional quality and adaptability. From a horticultural standpoint, their late ripening extends the availability of fresh plums into late autumn, supporting orchard diversification and market differentiation.

From a consumer and nutritional perspective, these cultivars represent valuable sources of sugars, Vit C, bioactive compounds and essential minerals, reinforcing their relevance in the context of functional foods and health-oriented diets. The promotion of such cultivars may therefore contribute to both dietary diversification and the sustainability of plum production systems.

Future research should focus on multi-year and multi-location assessments to further validate the stability of these traits under diverse agroecological conditions, as well as on integrating biochemical and agronomic evaluations to support breeding and orchard management strategies aimed at long-term sustainability.

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CRediT authorship contribution statement

Nebojša Milošević: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing - review & editing. **Ivana Glišić:** Methodology, Formal analysis, Investigation. **Milena Đorđević:** Methodology, Investigation. **Sanja Radičević:** Methodology, Investigation. **Slađana Marić:** Validation, Methodology, Project administration. **Mira Milinković:** Methodology, Investigation. **Jelena Tomić:** Methodology, Investigation. **Jelena Mladenović:** Methodology, Investigation. **Tomo Milošević:** Investigation, Data curation, Formal analysis, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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