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Phenolic, Amino Acid, Mineral, and Vitamin Contents during Berry Development in 'Italia' and 'Bronx Seedless' Grape Cultivars

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Abstract: Understanding the variations in amino acids, phenolic compounds, elements, and vitamins between grape varieties is essential for optimizing grape production, fine-tuning dietary recommendations, and harnessing the health potential of grapes. In this regard, this comprehensive study investigated the compositional diversity of two distinct table grape cultivars, 'Bronx Seedless' and 'Italia', at various critical phenological stages (BBCH-77, -79, -81, -83, -85, and -89). The research findings demonstrated remarkable differences in the concentrations of key nutritional components. Bronx Seedless consistently exhibited higher levels of several amino acids, including glutamate, phenylalanine, and aspartate with concentrations reaching 49.6, 52.7, and 24.8 pmol μL^{-1}, respectively, in contrast to Italia. Regarding phenolic compounds, Italia emerged as the richer source, with concentrations notably higher for compounds such as vanillic acid (18.2 $\mu g\ g^{-1}$ FW) and gallic acid (37.4 $\mu g\ g^{-1}$ FW). Mineral analysis revealed variable concentrations, with Italia grapes containing higher levels of Fe (91.0 mg/kg) compared to Bronx Seedless (87.1 mg/kg); however, Bronx Seedless had slightly elevated levels of K (31,089 mg/kg) compared to Italia (28,184 mg/kg). Concidering vitamins, Italia grapes showcased superior levels of Vitamin B1 (14.1 mg/100 g FW) and Vitamin A (11.0 mg/100 g FW), while Bronx Seedless had higher concentrations of Vitamin B6 (29.5 mg/kg), C (3.9 mg/100 g FW) and Vitamin B2 (36.9 mg/100 g FW). Principal component analysis (PCA) elucidated complex relationships within these components, offering insights into potential correlations and interactions. The heatmap visualization further indicated the concentration gradients across various samples, unveiling the intricate nutritional profiles of these grape cultivars. This research can aid grape growers and consumers in making informed decisions about grape cultivars and their corresponding health advantages.

Keywords: table grape cultivars; grape berries; nutritional composition; *Vitis vinifera* L.

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1. Introduction

The aromatic profiles of grapes, particularly those containing Muscat and Foxy aromas, play a pivotal role in the world of viticulture and ecology [1]. These distinct aroma characteristics significantly contribute to the sensory complexity and overall quality of wines [2]. The term Foxy is used to describe the earthy and musky fragrance commonly found in American grape varieties, with references to grapes such as Niagara and Concord [3]. This aroma provides a unique, nostalgic quality to wines and has historical and cultural

significance, particularly in American winemaking traditions. On the other side, Muscat grape cultivars, known for their enchanting bouquet of floral and fruity notes, are often utilized in winemaking to create wines with a pronounced, alluring aroma reminiscent of orange blossoms and lychee [4]. The importance of these distinct grape aromas extends beyond mere olfactory pleasure; they can influence consumer preferences, marketability, and the overall sensory experience of the wine, highlighting their significance in the wine industry [1,5]. The mineral composition of grapes exerts a dual influence by not only impacting grapevine health but also shaping the sensory attributes of the resulting wines. This mineral content plays a crucial role in defining the unique terroir of the wine and can have significant implications for winemaking, as an excess of potassium may pose challenges related to elevated pH levels and microbial stability concerns, as documented by Bordiga et al. [6]. Key minerals like potassium and calcium are crucial for grape physiology, influencing cell wall osmoregulation, structure, and enzymatic functions [7]. Understanding and effectively managing mineral content in grapes is essential for grape growers and winemakers to optimize grape quality and produce wines with the desired characteristics. Conversely, amino acids, as fundamental constituents of proteins, exert a substantial influence on various aspects of grape development, crop productivity, and overall quality, as elucidated by Hannah et al. [8]. These compounds assume a central role in grape physiology, the winemaking process, and the sensory characteristics of the resulting wine. Amino acids contribute to wine flavor and aroma by generating volatile compounds that define sensory profiles. In winemaking, amino acids influence fermentation dynamics, serving as nitrogen sources for yeast metabolism, crucial for fermentation success and desired wine attributes [9]. Understanding amino acid composition in grapes is vital for grape growers and winemakers to enhance wine quality and flavor profiles. Phenolic compounds in grapes are crucial in winemaking and viticulture, impacting the aroma, flavor, color, and aging potential of wines. Grapes also offer nutritional benefits, supplying dietary fiber, vitamins (notably vitamin K and vitamin C), and minerals like potassium, contributing to human health [10]. These compounds influence the astringency, mouthfeel, and bitterness of wines, shaping their character [11]. Phenolic acids, flavonoids, and tannins, as natural antioxidants, preserve wine taste and are vital for wine quality, terroir understanding, and the uniqueness of different grape varieties and growing conditions [11,12]. Vitamins in grapes, particularly vitamin K and vitamin C, contribute to the nutritional value and health benefits of this berry. Grapes are notably rich in vitamin C, which acts as a potent antioxidant, playing a crucial role in protecting cells from oxidative stress and contributing to overall health [13]. Vitamin K, found in grapes in moderate amounts, is essential for blood clotting and bone metabolism, and its consumption through grape-derived products can provide valuable dietary contributions [14,15]. Incorporating grapes into one's diet can therefore be a way to harness the health-promoting properties of these vitamins, enhancing overall well-being.

In the intricate mosaic of grape berry development stages, the interplay of phenolic compounds, minerals, amino acids, and vitamins not only influences grape quality but also offers a fascinating glimpse into the complex and multifaceted journey from vine to wine. However, there is limited scholarly work that comprehensively explores the dynamic interactions of these compounds throughout grape berry development stages, especially in the case of Foxy (*V. vinifera* L. cv. 'Bronx Seedless') and Muscat (*V. vinifera* L. cv. 'Italia'). Therefore, our academic purpose is to address this knowledge gap by conducting an in-depth analysis of the roles, changes, and interdependencies of phenolic compounds, amino acids, minerals, and vitamins at different stages of grape development, providing a more profound understanding of the intricate processes that shape the grape's journey to becoming exceptional wine.

2. Materials and Methods

2.1. Plant Material

This research was conducted using grapevine cultivars, Bronx Seedless and Italia, which were 20 years old and grafted onto 5 BB rootstock. Italia is a seeded grape cultivar characterized by a slight muscat flavor of the berries, vigorous vegetative behavior, and mid-late maturity period. Bronx Seedless is a cultivar that is attractive and preferred by consumers because of its pink berries characterized by a strawberry flavor. The study took place at the Manisa Viticulture Research Institute in Turkey, situated at 27°23′57.36′′ East Longitude and 38°37′57.14′′ North Latitude. The vineyard had well-drained, calcium-rich, clay loam soils ideal for growing high-quality grapes. In the vineyard, the climate data showed a pattern with hot, dry summers and mild, rainy winters. Total annual precipitation was around 612 mm, with most rainfall occurring from October to April (430 mm) compared to the dry period of May to September (178 mm). Average temperatures ranged from around 8 °C in winter to 22 °C in summer months. Relative humidity was highest in the winter at around 81% and lowest in the summer at 74%. Evaporation peaked in July at 6.5 mm and was the lowest in December at 0.7 mm. In our study, precise fertilization schedules were diligently formulated based on the grapevine's developmental stages, soil nutrient analysis, and fertilizer composition, the goal being to provide a balanced nutrient supply during critical periods like post-harvest, bud break, and pre-bloom. Irrigation practices were adapted to the local climate, real-time weather, soil moisture levels, and vine's hydration requirements at each growth phase. The vineyard's drip system had two 2.4 L/h pressure-compensated emitters per vine, positioned on either side. Decomposed cow manure was the organic fertilizer source applied via drip fertigation throughout the season. During the entire growth cycle, 8 irrigation events occurred at 7- to 10-day intervals. The three fertilizer treatments received irrigation on the same dates and durations. The fertigation utilized urea (46% N), monoammonium phosphate (12% N, 60% P_2O_5), and potassium sulfate (50% K_2O) to supply nutrients. The standard pesticide program in the vineyard employed an integrated pest management approach to control diseases, insects, and weeds. Fungicide applications followed a calendar-based schedule, with copper compounds applied at bud break against downy mildew, followed by rotating applications of synthetic fungicides throughout the growing season to combat powdery mildew and other fungal diseases. Insecticides were applied on an as-needed basis determined by regular scouting for insect pests like leafhoppers and mealybugs, while pre-emergence herbicides kept weed pressure low in the vine rows. The vines were planted in a 2 × 3 m layout with a high trunk cordon trellis system. These spur-pruned cultivars typically had 12–15 shoots per plant. Sample collections were performed randomly from the top, middle, and bottom sections of the grape clusters We carefully inspected the grape bunches at multiple points during the growing season for signs of gray mold caused by *Botrytis cinerea*, looking for telltale fuzzy gray growth on the berries. We visually examined representative grape clusters from different sections of the vineyard, gently pulling apart the bunches to inspect the interior berries for any presence of the fuzzy gray mold growth characteristic of *Botrytis* infection. Since the standard drug trial was effective in combating *Botrytis cinerea*, there were no diseased bunches. Clusters were collected six times between 27 July (referred to as BBCH-77, the first week before *veraison*) and 28 August (BBCH-89, harvest time). In this study, BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89 corresponded to specific stages in grape development, including the beginning of berry touch, completion of berry touch, the onset of berry color change, significant berry color change, berry softening, and optimal ripeness for harvest. To guide our sampling, we referred to the protocol established by Lorenz et al. [16]. Subsequently, collected clusters were immediately stored at 4 °C in the laboratory and then preserved at −80 °C for further analysis.

2.2. Chemical and Reagents

Solutions, standards for analytical measurements, and HPLC mobile phases utilized high-purity chemicals from Sigma-Aldrich (St. Louis, MO, USA). Chemicals required for

inline pre-column derivatization reactions before HPLC injection were purchased from Agilent Technologies (Palo Alto, CA, USA). This study employed analytical grade and HPLC grade solvents and chemicals to ensure optimal analytical performance and accuracy. Also, this study employed an external standard approach for analyte identification and quantification. The authentic reference standards for all analyte compounds were procured from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Determination of Phenolic Compounds from Grape Cultivars by HPLC

Grape samples from the Bronx Seedless and Italia cultivars were collected at various developmental stages, as described in Section 2.1. Whole berry samples obtained from clusters were triturated with a conventional beater until a homogeneous berry sample was obtained for analysis. The following phenolic compounds were analyzed: gallic acid, vanillic acid, trans-caffeic acid, trans-p-coumaric acid, ferulic acid, kaftaric acid, catechin, epicatechin, quercetin, routine, myricetin, and tyrosol. Chromatography assays were carried out using an Agilent 1100 HPLC device equipped with a diode-array detector (Agilent, Santa Clara, CA, USA) and a 4-µm octadecyl-silica column (4.6 × 250 mm, Hichrom, Reading, UK). A mobile phase was prepared using two components: A—methanol:water:acetic acid (10:28:2) and B—methanol:water:acetic acid (90:8:2). For the analysis, 25 mg of pomace extract was dissolved in 1 mL of methanol, and the injection volume of the sample solution was 10 µL. A gradient program was employed for the separation of phenolic compounds. The phenolic compositions of the extracts were determined using a modified method based on Sagdic et al. [17]. Gallic acid, vanillic acid, trans-caffeic acid, trans p-coumaric acid, ferulic acid, kaftaric acid, catechin, epicatechin, quercetin, rutin, myricetin, and tyrosol were utilized as standards. Identification and quantitative analysis were conducted by comparing with these standards. For characterizing phenolic compounds, liquid chromatography coupled with mass spectrometry (LC-MS) was employed. An HPLC instrument was interfaced to a Bruker ion trap mass spectrometer model Esquire 3000+, equipped with an electrospray ionization source. The control software facilitated data acquisition and processing. Negative ion mode was used to detect phenolic acids. The mass range scanned was m/z 50–1000 at a rate of 13,000 m/z per second. The mobile phase consisted of two eluents—eluent A was 2% acetic acid in water, while eluent B comprised 0.5% acetic acid in a 50:50 mixture of water and acetonitrile. A gradient elution program was employed as follows: 10 to 15% B over 10 min, holding at 15% B for 3 min, then increasing from 15 to 25% B over 7 min, then further raising from 25 to 55% B across 30 min, ramping up to 100% B within 1 min and maintaining 100% B for 5 min before rapidly decreasing to 10% B in 0.1 min. The total run time was 60 min. Injection volumes ranged from 5 to 10 µL for all samples. Simultaneous monitoring was carried out at 280 nm to detect hydroxybenzoic acids and at 320 nm for hydroxycinnamic acids, with a flow rate of 1.0 mL/min. The nebulizer temperature was maintained at 365 °C. Helium acted as the collision gas for collision-induced dissociation, held at 4.0×10^{-6} mbar pressure. Identifications were performed by spiking samples with pure compounds and comparing retention times (tR) with those of standards.

2.4. Determination of Amino Acid Profiling in Grape Cultivars by HPLC

Grape samples from the Bronx Seedless and Italia cultivars were collected at various developmental stages, as outlined in Section 2.1. Amino acids were determined by slightly modifying the method developed by Barrado et al. [18]. The collected grape berries were homogenized, and the resulting pulp was freeze-dried to obtain a fine powder. Amino acids were extracted from 1 g of the freeze-dried grape powder by suspending it in 10 mL of 0.1 M HCl. The suspension was thoroughly mixed and sonicated for 15 min to ensure efficient extraction. Subsequently, the supernatant was obtained following centrifugation. Amino acids in the supernatant were derivatized using o-phthaldialdehyde (OPA) reagent. To achieve this, 20 µL of the extracted sample was mixed with 80 µL of OPA reagent (10 mg OPA dissolved in 1 mL methanol) and incubated for 2 min at room temperature. This derivatization process enhances the detectability of amino acids during HPLC analysis. The

derivatized amino acids were separated and quantified using a high-performance liquid chromatograph (HPLC) equipped with a C18 column. The mobile phase typically consisted of two components: buffer A (0.1 M sodium acetate, pH 7.2) and buffer B (acetonitrile). An isocratic elution at a suitable flow rate was employed for the separation of amino acids. A fluorescence detector set at specific excitation and emission wavelengths for OPA-derivatized amino acids was used for detection. The flow rate was 0.6 mL/min. The elution profile had the following proportions (v/v) of phase B: 6% held for 3 min; 4–14% over 7 min, then held for 3.5 min; 14–19.5% over 2 min; 19.5–20% over 2.5 min, then held for 1 min; 20–26% over 1 min; 26–30% over 3 min; 30–50% over 1 min; 50–63% over 1 min, held for 1.5 min; and 63–100% over 1 min, held for 1 min. The photodiode array (PDA) detector was utilized for detecting and monitoring the analytes eluting from the HPLC column, with each amino acid exhibiting a characteristic retention time. Data acquisition and processing were facilitated by the MassLynx V4.1 2011 software (Waters, Milford, MA, USA). Calibration curves were constructed using the peak areas and retention times, enabling subsequent quantification of amino acid concentrations in the samples. Identifications were performed by spiking samples with pure compounds and comparing retention times (tR) with those of standards. All analyses were performed in triplicate to ensure reproducibility and reliability of the analytical data.

2.5. Determination of Vitamin Profiling in Grape Cultivars by HPLC

During the analysis, samples were first weighed, and then they were combined with 2.5 mL of an extraction solution, which differed based on the specific analysis: 8% acetic acid for MPA–acetic acid extraction, 0.1% oxalic acid for oxalic acid extraction, and 3% for MPA. This mixture underwent titration with an indophenol solution (comprising 25% DCIP and 21% $NaHCO_3$ in water) until a distinct rose-pink color appeared. For vitamin A analysis, 0.5 g samples were immersed in 20 mL of ethanol and subjected to a 30-min water bath at 85 °C. The cooled solution was then filtered through a separator funnel. Subsequently, heptane (10 mL) was added to the solution, followed by a 5-min shaking. To allow for layering, 20 mL of a 1.25% sodium sulfate solution was introduced into the tubes, and a 2-min shaking was conducted. The total tocopherols in the samples were determined through their reaction with cupric ions and complexation with 2,20-biquinoline (cuproine), following the procedure outlined by Kumar et al. [19]. The solution was then poured into a conical flask, to which 25 mL of the extraction solution was added. A shaking water bath at 70 °C for 40 min was employed to sonicate the solution. Subsequently, after cooling, the samples were filtered with the extraction solution to reach a final volume of 50 mL. The solution for berries underwent further filtration using 0.45 µm filter tips, and 20 µL aliquots of the solution were injected into the HPLC using an autosampler. An analytical reversed-phase C-18 column (STR ODS-M, 150 mm × 4.6 mm I.D., 5 µm, Shimadzu Corporation, Tokyo, Japan) was utilized for the separation of B complex vitamins in the berry samples. The mobile phase consisted of a mixture of 100-mM sodium phosphate buffer (pH 2.2) containing 0.8-mM sodium-1-octane sulfonate and acetonitrile at a 9:1 (v/v) ratio at 40 °C. The flow rate was maintained at a constant 0.8 mL min^{-1}, and a PDA detector was employed with an absorption wavelength of 270 nm. The detection and quantification of B vitamins were performed according to the methodology described by Mozumder et al. [20]. Identifications were performed by spiking samples with pure compounds and comparing retention times (tR) with those of standards.

2.6. Determination of Mineral Element Profiling in Grape Cultivars

Grape samples from the Bronx Seedless and Italia cultivars were collected at various developmental stages, as outlined in Section 2.1. Samples from Bronx Seedless and Italia cultivars were dried in an oven at 68 °C for 48 h. After drying, the samples were ground into a fine powder. The total nitrogen content in the berry samples was determined using the Kjeldahl method. A Vapodest Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany) was employed for the distillation process. The method followed was in

accordance with AOAC guidelines. Macroelements, including potassium (K), magnesium (Mg), phosphorus (P), sodium (Na), and calcium (Ca), as well as microelements, such as iron (Fe), zinc (Zn), sulfur (S), chlorine (Cl), copper (Cu), manganese (Mn), and boron (B), were detected using an inductively coupled plasma spectrophotometer (Optima 2100 DV, Perkin-Elmer, Shelton, CT, USA). The analytical procedure was performed in accordance with the guidelines specified by AOAC [21].

2.7. Statistical Analysis

In this study, all descriptive analyses were conducted using the R statistical programmer. To assess the impact of cultivar (two levels), phenological stage (six levels), and their interactions on phenolic compounds, amino acids, minerals, and vitamins, an analysis of variance (ANOVA) was performed using the stats package in R Studio. The model incorporated all main effects and interaction effects and was subjected to tests for normality assumptions. Four separate models were developed to evaluate the main effects of cultivar and phenological stage on phenolic compounds, amino acids, minerals, and vitamins. In cases where significance was observed in the ANOVA, Tukey's test was applied for post hoc analysis using the stats package in R Studio [22]. Principal Component Analyses (PCAs) were carried out on phenolic compounds, amino acids, minerals, and vitamins using ggbiplot2 within R Studio [22]. The heatmap was made by using the package pheatmap in R Studio [22].

3. Results

3.1. Physico-Chemical Characteristics of Grape

In our study, we delved into the intriguing journey of grape berry development in the Italia and Bronx Seedless cultivars, with a specific focus on their progression through various phenological stages. Through meticulous data collection and analysis, we uncovered a clear and significant trend: as these grapes advanced from the early stages (BBCH-77) to full maturity (BBCH-89), there was a remarkable increase in berry weight, size, and sugar contents (Total Soluble Solids-^{o}Brix). Simultaneously, titratable acidity (TA) showed a corresponding shift. The Maturity Index, a key indicator of grape ripeness, demonstrated a continuous ascent, reaching its pinnacle at BBCH-89 (Table 1).

Table 1. Parameters of Table Grapes (Italia and Bronx Seedless) at Various Phenological Stages (BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89).

Berry Development Stages	Berry Weight (g/Berry)		Berry Width (mm)		Berry Length (mm)		Total Soluble Solid (^{o}Brix)		Titratable Acidity (g/L as Tartaric Acid)-TA		Maturity Index (MI-^{o}Brix)	
	Italia	Bronx Seedless	Italia	Bronx Seedless	Italia	Bronx Seedless	Italia	Bronx Seedless	Italia	Bronx Seedless	Italia	Bronx Seedless
BBCH-77	0.68	0.41	12.35	8.89	16.15	11.65	2.87	2.70	34.98	29.78	0.88	0.95
BBCH-79	2.67	1.34	16.65	12.54	20.34	14.78	4.54	4.67	29.76	24.56	1.48	1.65
BBCH-81	4.43	1.72	18.45	13.23	22.43	16.98	9.83	10.45	18.56	15.43	5.34	6.88
BBCH-83	6.41	2.58	19.34	14.15	23.45	17.76	14.34	14.67	10.45	8.34	13.34	16.67
BBCH-85	7.86	3.21	20.87	15.85	25.42	18.45	15.21	15,54	9.58	7.45	16.23	20.76
BBCH-89	8.13	3.59	21.76	16.49	26.37	19.67	16.85	17.56	6.56	5.67	25.12	32.87

3.2. Amino Acid Content

Additionally, we found that amino acids like aspartate, glutamate, and valine, among others, exhibited highly significant differences between the two cultivars ($p < 0.001$). Considering cultivar variation in amino acid profiles, overall, when comparing the two cultivars, Bronx Seedless consistently exhibited higher concentrations of amino acids, such as aspartate, glutamate, asparagine, cystine, valine, methionine, tryptophan, phenylalanine, lisin, and proline, among others. However, some amino acids like glutamine and arginine in certain phenological stages showed similar concentrations across the two cultivars. Regarding phenological stage differences, differences across phenological stages were also highly significant for many amino acids, such as aspartate, glutamate, asparagine, cystine, and proline, with *p*-values mostly less than 0.001. Aspartate for BBCH-89 in Bronx Seedless exhibited the highest concentration, while in Italia, the concentration remained relatively

consistent across stages. Glutamate and asparagine amino acids peaked in concentration at the BBCH-77 stage. Tryptophan and phenylalanine amino acids were most concentrated in BBCH-89. Tryptophan and phenylalanine concentrations were highest at the BBCH-89 stage (Table 2).

3.3. Phenolic Compounds

We also analyzed the concentration of various phenolic compounds in the Italia and Bronx Seedless table grape cultivars harvested at different BBCH phenological stages. The differences in phenolic compounds between the Italia and Bronx Seedless grape cultivars were highly significant ($p < 0.001$) for nearly all compounds, emphasizing a distinct phenolic profile for each cultivar. Italia generally exhibited higher phenolic compound concentrations across all compounds except ferulic acid. The changes in phenolic compound concentration across the BBCH stages were also statistically significant ($p < 0.001$), reinforcing the influence of grape development stages on its phenolic composition. The concentration of phenolic compounds varied across the BBCH stages. BBCH-89 stage exhibited some of the highest concentrations for several compounds like gallic acid, vanillic acid, and kaftaric acid in both grape cultivars. In contrast, the BBCH-77 stage showed lower concentrations of phenolic compounds in both grape cultivars, especially evident in compounds like gallic acid, vanillic acid, and trans-caffeic acid. Stages like BBCH-81, BBCH-83, and BBCH-85 demonstrated intermediate levels of phenolic compounds (Table 3).

3.4. Minerals

The study also analyzed the elemental composition of table grapes, particularly two cultivars: Italia and Bronx Seedless, harvested at various phenological stages namely BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89. The effect of the cultivar on the elemental composition was statistically significant for elements like N, K, Mg, S, Mn, Cu, Fe, Zn, and B, as indicated by their respective *p*-values. For instance, the *p*-value for N was significantly low at 0.0001. The cultivar Italia exhibited higher concentrations of N at 3.87 mg/kg compared to Bronx Seedless, which had 3.55 mg/kg. On the contrary, Bronx Seedless exhibited marginally higher K levels (31,089 mg/kg) than the Italia cultivar (28,184 mg/kg). The elemental composition of other elements, such as Ca, Mg, Na, P, S, Mn, Cu, Fe, Zn, and B, also varied between the two cultivars. Similarly, the influence of phenological stages on elemental content was also significant for many elements, with *p*-values like <0.0001 for K. The Italia grapes showed lower Fe (81.9 mg/kg) compared to Bronx Seedless (87.1 mg/kg). As grapes progressed through phenological stages, variations in the elemental composition were observed. The Ca concentration increased from 5147 mg/kg at BBCH-77 to 8752 mg/kg at BBCH-89. Similarly, K values also showed an increment from 22,359 mg/kg during BBCH-77 to 38,017 mg/kg by BBCH-89. Other elements such as Mg, Na, P, S, Mn, Cu, Fe, Zn, and B displayed varied concentrations across different phenological stages (Table 4).

3.5. Vitamins

The type of cultivar (C) and the phenological stage (PS) had significant impacts on the vitamin content, as demonstrated by the very low *p*-values. For Vitamin A, 'Italia' grapes had an average of 11.0 mg/100 g FW while 'Bronx Seedless' grapes showed slightly less at 10.4 mg/100 g FW. However, the interaction between the cultivar and the phenological stage (C × PS) was not significant for any of the vitamins, suggesting that the pattern of vitamin changes across the stages was similar for both 'Italia' and 'Bronx Seedless'. This difference was statistically significant ($p = 0.0001$). For Vitamin B1, 'Italia' grapes had 14.1 mg/100 g FW, while 'Bronx Seedless' grapes contained 11.5 mg/100 g FW. For Vitamin B2, 'Italia' grapes exhibited higher levels (36.9 mg/100 g FW) compared to the 'Bronx Seedless' grapes (31.6 mg/100 g FW).

Table 2. Amino acid content (pmol μL^{-1}) of table grapes (Italia and Bronx Seedless) harvested in BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89 phenological stages.

Cultivar [X] (C)	Aspartate	Glutamate	Asparagine	Cerin	Glutamine	Histidine	Glycine	Thionine	Arginine	Alanine	Tyrosine
Italia	5200 ± 44 b	2866 ± 51 a	9803 ± 13 b	11,265 ± 29 b	8004 ± 14	2590 ± 4.4 b	1566 ± 3 b	5979 ± 11 b	10,508 ± 27 b	7974 ± 13	753 ± 13 b
Bronx Seedless	5537 ± 34 a	2500 ± 58 b	12,995 ± 15 a	14,045 ± 23 a	7804 ± 15	3044 ± 4.8 a	2325 ± 4 a	7374 ± 19 a	11,691 ± 20 a	8357 ± 12	860 ± 12 a
Phenological stage [Y] (PS)											
BBCH-77	3949 ± 74 f	2820 ± 12	8343 ± 25 e	9547 ± 504 e	5859 ± 23 e	2125 ± 81 e	2045 ± 43	5037 ± 33 d	8374 ± 48 d	5857 ± 22 e	848 ± 11
BBCH-79	4431 ± 71 e	2764 ± 10	9378 ± 27 d	10,617 ± 517 de	6556 ± 21 de	2363 ± 23 d	2004 ± 34	5601 ± 31 cd	9312 ± 49 cd	6630 ± 24 d	831 ± 23
BBCH-81	4972 ± 72 d	2709 ± 13	10,540 ± 23 c	11,806 ± 407 cd	7336 ± 25 cd	2628 ± 41 cd	1964 ± 53	6229 ± 35 bc	10,355 ± 46 bc	7505 ± 26 c	814 ± 20
BBCH-83	5578 ± 73 c	2654 ± 15	11,847 ± 24 b	13,128 ± 307 bc	8209 ± 22 bc	2922 ± 83 bc	1925 ± 55	6926 ± 11 ab	11,514 ± 68 ab	8496 ± 29 bc	798 ± 18
BBCH-85	6259 ± 71 b	2601 ± 16	13,316 ± 26 ab	14,598 ± 506 ab	9186 ± 21 ab	3249 ± 45 ab	1886 ± 34	7702 ± 33 ab	12,804 ± 45 a	9618 ± 22 ab	782 ± 19
BBCH-89	7022 ± 73 a	2549 ± 14	14,968 ± 25 a	16,233 ± 517 ab	10,279 ± 23 a	3613 ± 74 a	1849 ± 32	8565 ± 35 a	14,238 ± 46 a	10,887 ± 26 a	766 ± 12
Significance											
C	***	***	***	***	0.3490	***	***	***	**	0.0513	***
PS	***	0.4420	***	***	***	***	0.1991	***	***	***	0.1045
C × PS	0.9521	0.9999	0.8999	0.9118	1.0000	0.9047	0.9975	0.9755	0.9970	0.9991	1.0000
Cultivar [X] (C)	**Cystine**	**Valine**	**Methionine**	**Tryptophan**	**Phenylalanine**	**Isolosin**	**Losin**	**Lisin**	**Hydroxyproline**	**Sarcosine**	**Proline**
Italia	1030 ± 11 b	435 ± 22 b	1843 ± 43 b	1229 ± 18 b	2753 ± 40 a	1420 ± 31 b	2630 ± 45 b	2039 ± 34 b	1391 ± 36 b	4174 ± 103 b	257 ± 7 b
Bronx Seedless	1289 ± 16 a	969 ± 24 a	2450 ± 40 a	1769 ± 11 a	2463 ± 50 b	1864 ± 32 a	3772 ± 65 a	4509 ± 55 a	3427 ± 56 a	5730 ± 104 a	460 ± 7 a
Phenological stage [Y] (PS)											
BBCH-77	875 ± 21 e	530 ± 35 d	1620 ± 71 e	1075 ± 32 e	1967 ± 83 e	1239 ± 16 e	2296 ± 111 e	2470 ± 95 e	1728 ± 91 e	3736 ± 170 e	271 ± 14 e
BBCH-79	973 ± 18 d	589 ± 32 cd	1801 ± 60 de	1217 ± 23 d	2188 ± 87de	1377 ± 45 de	2599 ± 114 d	2747 ± 92 d	1956 ± 87 de	4154 ± 183 de	301 ± 32 de
BBCH-81	1082 ± 23 c	655 ± 31 bc	2003 ± 45 cd	1378 ± 24 c	2433 ± 37 cd	1532 ± 56 cd	2942 ± 124 cd	3054 ± 90 cd	2215 ± 96 cd	4620 ± 180 cd	335 ± 13 cd
BBCH-83	1203 ± 12 bc	729 ± 33 abc	2227 ± 65 bc	1559 ± 31 b	2705 ± 83 bc	1703 ± 53 bc	3331 ± 143 bc	3397 ± 65 bc	2507 ± 90 bc	5137 ± 185 bc	372 ± 11 bc
BBCH-85	1338 ± 23 ab	810 ± 18 ab	2476 ± 73 ab	1765 ± 33 a	3008 ± 59 ab	1894 ± 54 ab	3770 ± 134 ab	3777 ± 92 ab	2838 ± 77 ab	5712 ± 179 ab	414 ± 13 ab
BBCH-89	1487 ± 26 a	901 ± 28 a	2754 ± 71 a	1998 ± 35 a	3345 ± 46 a	2106 ± 51 a	4268 ± 115 a	4200 ± 93 a	3212 ± 59 a	6352 ± 174 a	460 ± 22 a
Significance											
C	***	***	***	***	***	***	***	***	***	***	***
PS	***	***	***	***	***	***	***	***	***	***	***
C × PS	0.5546	0.9887	0.6087	0.8765	0.9897	0.6915	0.8976	0.9596	0.8799	0.6099	0.9876

[X], Mean seperation in cultivars; [Y], Mean seperation in Phenological stages; C, Cultivar; PS, Phenological Stages; C × PS, interactions; For a given factor (different letters within a column represent significant differences (Tukey test, **, Significant at p-value < 0.01; ***, Significant at p-value < 0.001). Data are expressed as mean of the data.

Table 3. Phenolic compounds ($\mu g\ g^{-1}$ FW) of table grapes (Italia and Bronx Seedless) harvested in BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89 phenological stages.

Cultivar [X] (C)	Gallic Acid	Vanillic Acid	Trans-Caffeic Acid	Trans P-Coumaric Acid	Ferulic Acid	Kaftaric Acid	Catechin	Epicatechin	Quercetin	Rutin	Myricetin	Tyrosol
Italia	5.1 ± 0.1 a	6.3 ± 0.0 a	3.6 ± 0.1 a	5.9 ± 0.0 a	3.1 ± 0.03 a	7.54 ± 0.0 a	8.5 ± 0.1 a	3.8 ± 0.1 a	7.1 ± 0.0 a	3.2 ± 0.10 a	2.9 ± 0.0 a	9.1 ± 0.0 a
Bronx Seedless	3.7 ± 0.0 b	4.84 ± 0.2 b	2.9 ± 0.0 b	4.9 ± 0.1 b	2.4 ± 0.02 b	5.35 ± 0.1 b	5.7 ± 0.2 b	3.2 ± 0.0 b	5.7 ± 0.0 b	2.4 ± 0.0 b	1.7 ± 0.0 b	6.3 ± 0.2 b
Phenological stage [Y] (PS)												
BBCH-77	3.2 ± 0.1 f	4.2 ± 0.0 f	2.4 ± 0.0 e	4.1 ± 0.0 f	2.8 ± 0.1 a	2.6 ± 0.1 f	5.3 ± 0.1 e	2.5 ± 0.0 f	6.7 ± 0.2 a	2.1 ± 0.0 e	1.8 ± 0.1 d	5.8 ± 0.2 e
BBCH-79	3.6 ± 0.2 e	4.7 ± 0.2 e	2.7 ± 0.0 d	4.52 ± 0.2 e	2.82 ± 0.0 ab	3.9 ± 0.2 e	5.9 ± 0.1 d	2.8 ± 0.0 e	6.6 ± 0.1 ab	2.3 ± 0.2 de	1.9 ± 0.3 cd	6.4 ± 0.3 d
BBCH-81	4.1 ± 0.0 d	5.2 ± 0.0 d	3.0 ± 0.1 c	5.0 ± 0.3 d	2.8 ± 0.0 ab	4.8 ± 0.1 d	6.6 ± 0.2 cd	3.2 ± 0.1 d	6.4 ± 0.1 abc	2.6 ± 0.0 cd	2.2 ± 0.1 bcd	7.2 ± 0.1 c
BBCH-83	4.6 ± 0.0 c	5.8 ± 0.1 c	3.4 ± 0.0 bc	5.6 ± 0.0 c	2.7 ± 0.3 ab	5.1 ± 0.2 c	6.6 ± 0.2 bc	3.6 ± 0.1 c	6.3 ± 0.2 abc	2.9 ± 0.1 bc	2.4 ± 0.2 abc	7.9 ± 0.1 b

Table 3. *Cont.*

Cultivar [X] (C)	Gallic Acid	Vanillic Acid	Trans-Caffeic Acid	Trans P-Coumaric Acid	Ferulic Acid	Kaftaric Acid	Catechin	Epicatechin	Quercetin	Rutin	Myricetin	Tyrosol
BBCH-85	5.2 ± 0.3 b	6.4 ± 0.3 b	3.8 ± 0.3 ab	6.2 ± 0.0 b	2.7 ± 0.0 ab	6.6 ± 0.1 b	8.1 ± 0.1 ab	4.1 ± 0.0 b	6.2 ± 0.1 bc	3.2 ± 0.0 ab	2.7 ± 0.1 ab	8.9 ± 0.3 a
BBCH-89	5.8 ± 0.3 a	7.1 ± 0.0 a	4.2 ± 0.0 a	6.9 ± 0.0 a	2.6 ± 0.1 b	7.4 ± 0.1 a	9.1 ± 0.1 a	4.6 ± 0.0 a	6.0 ± 0.1 c	3.6 ± 0.1 a	3.0 ± 0.3 a	9.9 ± 0.1 a
Significance												
C	***	***	***	***	***	***	***	***	***	***	***	***
PS	***	***	***	***	0.0107 *	***	***	***	0.0050 **	***	***	***
C × PS	0.9401	0.8947	0.6219	0.8731	0.9957	0.9122	0.0885	0.5099	0.9978	0.8495	0.9525	0.9019

[X], Mean separation in cultivars; [Y], Mean separation in phenological stages; C, Cultivar; PS, Phenological stages; C × PS, interactions. For a given factor (different letters within a column represent significant differences (Tukey test, *, Significant at *p*-value < 0.05; **, Significant at *p*-value < 0.01; ***, Significant at *p*-value < 0.001). Data are expressed as mean of the data.

Table 4. Elements (mg/kg) of table grapes (Italia and Bronx Seedless) harvested in BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89 phenological stages.

Cultivar [X] (C)	N (%)	Ca	K	Mg	Na	P	S	Mn	Cu	Fe	Zn	B
Italia	3.9 ± 0.2 a	6833 ± 4	28,184 ± 211 b	6054 ± 46 a	480 ± 14	5861 ± 33.1	5280 ± 68 a	50.1 ± 1.1 b	46.8 ± 3.3 a	81.9 ± 0.3 b	33.5 ± 3.5 a	18.0 ± 2.0 a
Bronx Seedless	3.6 ± 0.0 b	6812 ± 5	31,089 ± 215 a	5900 ± 35 b	506 ± 11	5758 ± 38.6	4694 ± 66 b	56.0 ± 1.6 a	34.2 ± 3.8 b	87.1 ± 0.2 a	20.8 ± 3.8 b	11.7 ± 1.0 b
Phenological stage [Y] (PS)												
BBCH-77	2.8 ± 0.0 f	5147 ± 10 f	22,359 ± 37 f	4397 ± 83 f	518 ± 21	4252 ± 66 f	3762 ± 118 e	39.3 ± 2.3 d	30.6 ± 6.1	88.8 ± 0.4 a	20.5 ± 6.2	11.2 ± 3.4
BBCH-79	3.1 ± 0.1 e	5724 ± 13 e	24,863 ± 373 e	4933 ± 80 e	508 ± 22	4779 ± 64 e	4184 ± 111 d	44.0 ± 2.3 cd	34.0 ± 6.6	87.1 ± 0.5 ab	22.7 ± 5.6	12.5 ± 2.4
BBCH-81	3.5 ± 0.0 d	6365 ± 20 d	27,648 ± 323 d	5535 ± 81 d	498 ± 23	5372 ± 65 d	4652 ± 132 c	49.2 ± 2.5 bc	37.8 ± 6.0	85.3 ± 0.7 bc	25.3 ± 6.4	13.9 ± 3.3
BBCH-83	3.9 ± 0.2 c	7077 ± 12 c	30,744 ± 333 c	6210 ± 86 c	488 ± 24	6038 ± 62 c	5174 ± 121 b	55.1 ± 2.4 abc	42.0 ± 6.3	83.6 ± 0.6 cd	28.1 ± 5.6	15.4 ± 2.5
BBCH-85	4.3 ± 0.0 b	7870 ± 21 b	34,188 ± 345 b	6968 ± 70 b	478 ± 19	6787 ± 55 b	5753 ± 112 ab	61.6 ± 2.5 ab	46.7 ± 6.4	81.9 ± 0.3 d e	31.3 ± 5.6	17.1 ± 3.5
BBCH-89	4.8 ± 0.3 a	8752 ± 13 a	38,017 ± 356 a	7818 ± 82 a	469 ± 18	7628 ± 49 a	6397 ± 122 a	69.0 ± 2.8 a	52.0 ± 6.2	80.3 ± 0.5 e	34.8 ± 6.0	19.1 ± 3.1
Significance												
C	***	0.7951	***	***	0.2078	0.0708	***	0.0203 *	0.0283 *	***	0.0285 *	0.0393 *
PS	***	***	***	***	0.7081	***	***	***	0.2414	***	0.6661	0.6296
C × PS	0.7235	1.000	0.7046	0.9989	1.000	0.9996	0.9413	0.9986	0.9992	0.9999	0.9992	0.9995

[X], Mean seperation in cultivars; [Y], Mean seperation in phenological stages; C, Cultivar; PS, Phenological stages; C × PS, interactions. For a given factor (different letters within a column represent significant differences (Tukey test, *, Significant at *p*-value < 0.05; ***, Significant at *p*-value < 0.001). Data are expressed as mean of the data.

For Vitamin B6: 'Italia' grapes contained 29.5 mg/100 g FW, and 'Bronx Seedless' grapes had slightly lower levels at 24.7 mg/100 g FW. For Vitamin C: 'Italia' grapes had 3.97 mg/100 g FW, whereas 'Bronx Seedless' grapes displayed 3.48 mg/100 g FW. Considering vitamin content across phenological stages, for Vitamin A, the content was lowest at BBCH-77 with 8.06 mg/100 g FW and peaked at BBCH-89 with 13.71 mg/100 g FW. For Vitamin B1, the grapes harvested at BBCH-77 contained the least amount at 10.5 mg/100 g FW, whereas it was the highest at BBCH-89 with 15.4 mg/100 g FW. For Vitamin B2, the concentration ranged from a minimum of 25.6 mg/100 g FW at BBCH-77 to a maximum of 44.3 mg/100 g FW at BBCH-89. For Vitamin B6, levels were lowest at BBCH-77 with 20.6 mg/100 g FW and reached their highest at BBCH-89 with 34.6 mg/100 g FW. For Vitamin C, the content was at its lowest in grapes harvested at BBCH-77 (2.74 mg/100 g FW) and reached its zenith at BBCH-89 (4.89 mg/100 g FW) (Table 5).

Table 5. Vitamins (mg/100 g FW) of table grapes ('Italia' and 'Bronx Seedless') harvested in BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89 phenological stages.

Cultivar [X] (C)	Vitamin A	Vitamin B_1	Vitamin B_2	Vitamin B_6	Vitamin C
Italia	11.0 ± 0.2 a	14.1 ± 0.2 a	36.9 ± 0.6 a	29.5 ± 0.3 a	3.9± 0.0 a
Bronx Seedless	10.4 ± 0.0 b	11.5 ± 0.1 b	31.6 ± 0.5 b	24.7 ± 0.2 b	3.5± 0.1 b
Phenological stage [Y] (PS)					
BBCH-77	8.1 ± 0.2 f	10.5 ± 0.4 d	25.6 ± 1.1 e	20.6 ± 0.3 e	2.7 ± 0.0 f
BBCH-79	8.9 ± 0.1 e	11.3 ± 0.3 cd	28.6 ± 1.3 de	22.8 ± 0.5 d	3.1 ± 0.1 e
BBCH-81	9.9 ± 0.2 d	12.2 ± 0.4 bc	31.9 ± 1.0 cd	25.3 ± 0.4 c	3.5 ± 0.0 d
BBCH-83	11.1 ± 0.1 c	13.2 ± 0.2 ab	35.6 ± 1.4 bc	28.1 ± 0.5 b	3.9 ± 0.1 c
BBCH-85	12.3 ± 0.2 b	14.3 ± 0.1 a	39.7 ± 1.1 ab	31.2 ± 0.2 ab	4.4 ± 0.0 b
BBCH-89	13.7 ± 0.1 a	15.4 ± 0.4 a	44.3 ± 1.0 a	34.6 ± 0.3 a	4.9 ± 0.0 a
Significance					
C	***	***	***	***	***
PS	***	***	***	***	***
C × PS	0.9267	0.9564	0.9372	0.8664	0.9873

[X], Mean separation in cultivars; [Y], Mean separation in phenological stages; C, Cultivar; PS, Phenological stages; C × PS, interactions. For a given factor (different letters within a column represent significant differences (Tukey test, ***, Significant at p-value < 0.001). Data are expressed as mean of the data.

The image contains four separate PCA biplots for different groups of compounds in berries: amino acids, phenolic compounds, elements, and vitamins (Figure 1). In our recent investigation into the amino acid composition of various berry cultivars, we employed PCA to dissect and visualize the intricate relationships between different amino acids present. The analysis yielded two principal components, which together account for a significant portion of the dataset's variance. The first principal component (Dim1) proved to be particularly influential, explaining a remarkable 72.3% of the total variance. In contrast, the second component (Dim2) accounted for a further 18.4% (Figure 1A,B). An interesting divergence from this trend is the position of glycine. This amino acid finds itself anchored towards the negative end of the Dim1 axis. This placement implies an inverse relationship with the amino acids. In simpler terms, berries that are rich in glutamine, aspartate, and phenylalanine might tend to have lower levels of glycine. One of the standout observations from the biplot was the strong representation of certain amino acids, notably glutamine, aspartate, and phenylalanine, along the positive side of the Dim1 axis. Their pronounced positioning indicates a consistent and high concentration of these amino acids across the studied berry samples. Their co-location also hints at a potential correlation, suggesting that when one of these amino acids is present in high amounts, the others likely are as well. Turning our attention to the Dim2 axis, it is apparent that there is not a significant distinction among the amino acids in this direction. This suggests that, in the context of the variance captured by Dim2, the amino acids' concentrations remain relatively consistent across the berry samples. This PCA-based exploration into amino acid composition provides a compelling snapshot of the intricate relationships between different

amino acids in berries. As researchers continue to unravel the dietary significance and health implications of these amino acids, such analyses become instrumental in guiding dietary recommendations and understanding the nutritional nuances of different berry cultivars (Figure 1B). The x-axis (Dim1) explains 81.1% of the variance, and the y-axis (Dim2) explains 15.8%. Compounds like quercetin, ferulic acid, and myricetin are positioned towards the negative x-axis. Trans-caffeic acid and gallic acid are towards the positive x-axis. The y-axis does not looked to differentiate these compounds much. Quercetin, ferulic acid, and myricetin are distinctly clustered towards the negative side of Dim1. This co-location suggests a potential shared distribution pattern across the berry samples, indicating berries rich in one of these compounds might similarly exhibit high concentrations of the others. On the opposite spectrum, trans-caffeic acid and gallic acid find prominence on the positive x-axis, potentially indicating an inverse relationship with the previously mentioned compounds. The Dim2 axis, however, remains relatively non-distinctive for phenolic compounds, implying consistent concentrations in this dimension across the berry samples (Figure 1D). On the other hand, the x-axis (Dim1) explains 64.1% of the variance, and the y-axis (Dim2) explains 26.1%. Elements like Na, Mn, and K are more prevalent on the positive x-axis. Zn is distinct on the negative x-axis. Mg, K, and Ca have a pronounced representation on the positive y-axis. Also, Fe stands apart on the negative x-axis, suggesting berries rich in Fe might have contrasting elemental profiles from those rich in Na, Mn, or S; Mg, K, and Ca have a distinct representation on the positive y-axis, signaling potential correlations amongst these elements in the studied samples (Figure 1F). Regarding vitamins (bottom-right plot), the x-axis (Dim1) explains a significant 92.5% of the variance, and the y-axis (Dim2) only explains 4.9%. Vitamin B6 and B2 are along the positive x-axis. Vitamins B6 and B2 feature prominently on the positive end of Dim1, hinting at a consistent and potentially correlated presence across the berry samples. Conversely, Vitamins A, C, and B2 gravitate slightly towards the negative side of the x-axis, which may suggest a different distribution pattern when compared to the other vitamins. Much like with phenolic compounds, the y-axis remains relatively unvaried for vitamins, suggesting consistent concentrations in this regard (Figure 1H).

Heatmap analysis scrutinizes a plethora of components ranging from phenolic compounds and essential elements to critical vitamins. This vivid visual representation provides a deep dive into the concentration gradients of these compounds across berry samples, facilitating a comparative understanding. The heatmap presents a gradient from deep blue to intense red, with blue signifying lower concentrations and red representing higher concentrations of the respective compounds in the berry samples. This gradient is complemented by a numeric scale running from -6 to 6, reinforcing the intensity of these concentrations. Myricetin, ferulic acid, and N compounds exhibit high concentrations (deep blue) across most berry samples. Trans-caffeic acid and gallic acid compounds manifest moderate to high concentrations in the Bronx Seedless cultivar, especially within the BBCH 77 and BBCH 79 samples. Regarding minerals, Fe, Na, and Zn elements display a moderate concentration (lighter blue to white) across most samples. Italia samples under the BBCH 89 tag lean towards higher levels of these elements. K exhibits consistently high levels across almost all berry samples. On the other hand, Vitamins B6 and B2 exhibit consistently high concentrations (intense red) in the Bronx Seedless berry samples, especially within BBCH 77 and BBCH 79. Also, considering Vitamins A and B1, the heatmap indicates varying concentrations, with some Bronx Seedless samples showing high levels, while others, especially within the Italia cultivar, reflecting lower concentrations. Glutamate, aspartate, and phenylalanine amino acids present high concentrations across most Bronx Seedless samples. In contrast, most Italia samples, particularly under BBCH 89 and BBCH 85, exhibit slightly lower concentrations. A consistent high concentration pattern of glycine and alanine can be observed across both Bronx Seedless and Italia cultivars. The Bronx Seedless samples, especially BBCH 77 and BBCH 79, tend to exhibit higher concentrations for many of the studied compounds. In contrast, the Italia cultivar under BBCH 85 and BBCH 89 tags shows a diverse concentration gradient, pointing to a heterogeneous composition (Figure 2).

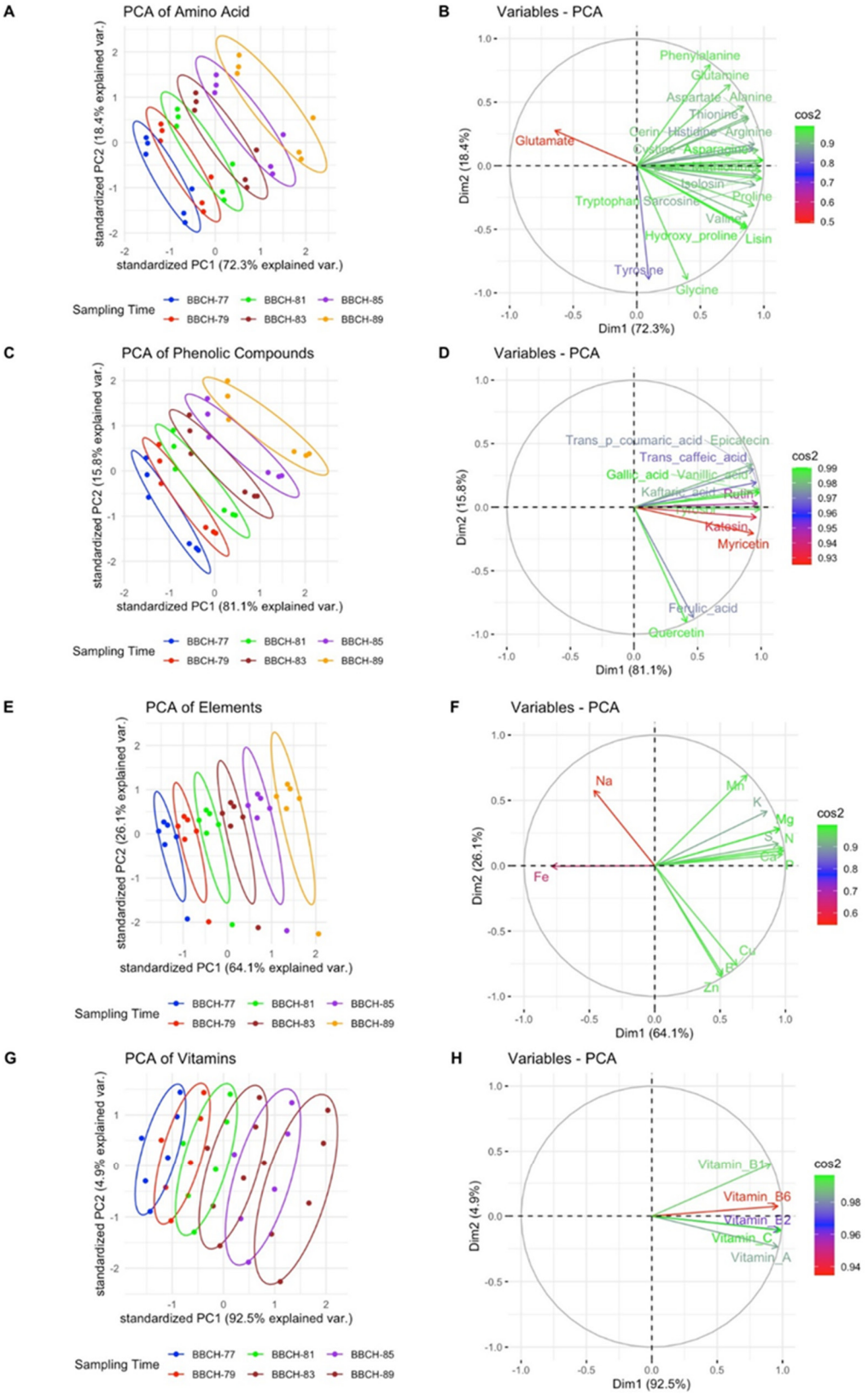

Figure 1. PCAs biplot of berries colored by cultivars. All amino acids (**A**,**B**), phenolic compounds (**C**,**D**), minerals (**E**,**F**) and vitamins (**G**,**H**) are demonstrated. Each point is the average of quaternary plicate of each organic acid.

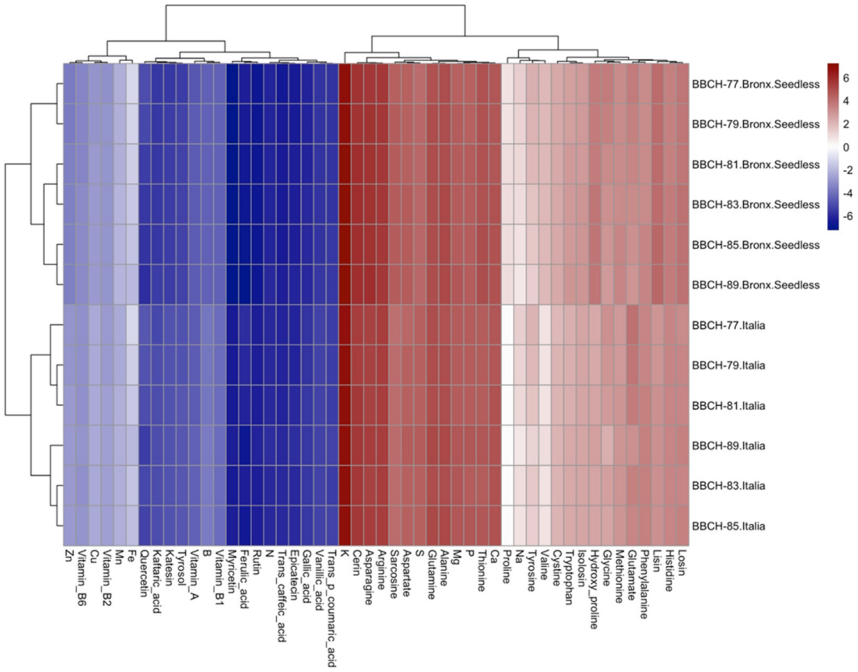

Figure 2. A heatmap analysis of various components, including amino acids, phenolic compounds, minerals, and vitamins, in table grapes (Italia and Bronx Seedless) harvested at BBCH-77, BBCH-79, BBCH-81, BBCH-83, BBCH-85, and BBCH-89 phenological stages.

4. Discussion

The findings of this study shed light on the dynamic nature of amino acid composition in grape berries across different phenological stages and grape cultivars, contributing to our understanding of grape quality and potential applications in winemaking and viticulture. The observed variations in amino acid levels between phenological stages and cultivars align with the complex biochemical processes occurring during grape development. This is consistent with research by Kaya et al. [1], which emphasizes the influence of phenological stages on the grape's chemical composition, impacting wine quality and flavor profiles. The higher concentrations of amino acids in Bronx Seedless, including aspartate, glutamate, and valine, suggest its potential for producing wines with distinctive sensory attributes. This is in line with prior studies highlighting the influence of grape variety on amino acid composition and its implications for wine flavor, as noted by Murillo-Peña et al. [23] and Moukarzel et al. [24]. Moreover, the observed changes in amino acid content emphasize the necessity of precise harvesting decisions to ensure the desired flavor profiles in wines, as discussed in previous research by Ma et al. [25]. The study also corroborates the intricate interplay between genetics, metabolism, and environmental conditions in shaping amino acid profiles in grapes. Similar findings on the genetic basis for amino acid composition differences among grape cultivars have been reported in the work of Shiraishi et al. [26]. The findings from the analysis of phenolic compounds in Italia and Bronx Seedless grape cultivars at different BBCH phenological stages provide valuable insights into the varietal and developmental aspects of grape phenolic profiles, with implications for wine production and grape breeding. The highly significant differences observed between the two cultivars for nearly all phenolic compounds underline the distinct phenolic fingerprints characterizing each cultivar, which is consistent with previous studies emphasizing the impact of grape variety on phenolic composition, as documented by Esparza et al. [27] and Van Leeuw et al. [28]. Italia's generally higher phenolic compound concentrations reflect its potential for contributing to wines with enhanced antioxidant and sensory properties. Conversely, Bronx Seedless, while exhibiting lower concentrations overall, still maintains significant levels, particularly for compounds like tartaric acid, catechin, and

epicatechin, suggesting its unique phenolic qualities that may be harnessed in winemaking. The statistically significant variations in phenolic compound concentrations across BBCH stages indicate the influence of grape development on the phenolic composition. These findings are in line with research by Gómez-Plaza et al. [29], which emphasizes the evolution of phenolic compounds during grape ripening and its impact on wine quality. The BBCH-89 stage displayed some of the highest concentrations for several compounds in both grape cultivars, suggesting its suitability for harvesting grapes rich in certain phenolic compounds. Conversely, the BBCH-77 stage exhibited lower concentrations, indicating the grapes' earlier development stage with less ripering phenolic profiles. The intermediate levels observed at stages BBCH-81, BBCH-83, and BBCH-85 indicate the complexity of phenolic changes during grape development. Furthermore, the significant interaction effects between grape cultivar and phenological stage for specific compounds like gallic acid and vanillic acid highlight the nuanced relationship between cultivar and stage. This finding aligns with research by Pérez-Magariño et al. [30], which discusses the intricate interactions between grape cultivar and ripening stages influencing phenolic compounds.

The mineral composition analysis of table grapes, with a focus on the Italia and Bronx Seedless cultivars at different phenological stages, reveals intriguing insights into how both the grape cultivar and developmental stages impact the grape's elemental content, with implications for viticulture and agriculture. The statistically significant differences between the two cultivars in terms of elemental composition, including N, K, and other elements, underline the distinct chemical signatures associated with each grape cultivar. This aligns with previous research highlighting the importance of grape cultivars in determining nutrient content and overall grape quality, as emphasized by Askari-Khorasgani and Pessarakli [31]. The variations in elemental content across different phenological stages emphasize the dynamic nature of grape development. The substantial increase in Ca and K concentrations as grapes progress through phenological stages exemplifies the impact of grape maturation on mineral content. These findings resonate with the work of Sweetman et al. [32] and Conde et al. [33], which discusses the changing mineral content in grapes during ripening and its influence on wine quality. Additionally, the fluctuations in other elements like Zn highlight the intricate relationship between grape ripering and elemental composition, which may be critical for grape breeders and growers in optimizing grape production. The limited significance of the interaction between grape cultivar and phenological stage (C $\times$ PS) in affecting elemental composition implies that the elemental content variations are primarily driven by the individual effects of cultivar and phenological stage. This observation is in line with research by Rolle et al. [34], which discusses the independent influences of grape cultivar and ripening stage on grape composition. On the other hand, the study's findings regarding the impact of grape cultivar and phenological stage on vitamin content provide valuable insights into the multifaceted nature of grape chemistry and the factors contributing to the nutritional profile of grapes. These results align with previous research emphasizing the significance of grape ripening stages in shaping the phenolic and nutritional composition of grapes [35,36]. The observed higher vitamin content in Italia grapes for Vitamins A, B1, B2, B6, and C concurs with the notion that grape cultivar plays a pivotal role in determining the nutritional composition of grapes. Additionally, the fact that Vitamins A, B1, B2, B6, and C exhibited peak concentrations at BBCH-89 indicates the importance of timing in grape harvesting to optimize their nutritional content [37,38]. The study also examines vitamin variations across different berry development stages, notably BBCH-77 and BBCH-89. The interaction between grape cultivar and phenological stage (C $\times$ PS) did not significantly affect vitamin content, indicating similar vitamin changes across phenological stages for both Italia and Bronx Seedless grapes, as supported by low *p*-values. The trend continues for Vitamins B1, B2, B6, and C, with Italia grapes consistently exhibiting higher levels than Bronx Seedless, aligning with prior research on grape composition [39,40]. It becomes evident that the choice of harvest time significantly impacts the nutritional profile of grapes. For instance, Vitamin A content was at its lowest at BBCH-77 and reached its zenith at BBCH-89. This pattern was consistent for

Vitamins B1, B2, B6, and C, highlighting the importance of considering the phenological stage in grape cultivation and harvesting practices.Upon conducting a heatmap analysis, which visually represents the similarity or difference between samples, alongside PCA variables employed to condense multidimensional datasets, our results were consistent with previous research findings [41]. Indeed, the multifaceted relationship between the intricate composition of various compounds in berries, including amino acids, phenolic compounds, elements, and vitamins, has been dissected and visualized through PCA in this study (Figure 1). In the context of amino acids, the PCA analysis yielded two principal components, Dim1 and Dim2, which collectively accounted for a substantial portion of the dataset's variance. Dim1 emerged as a dominant influence, explaining an impressive 72.3% of the total variance, while Dim2 contributed an additional 18.4%. This analysis highlighted the distinctive positioning of glutamate towards the negative end of the Dim1 axis, signifying a potential inverse relationship with other amino acids, particularly glutamine, aspartate, and phenylalanine, which were prominently clustered on the positive side of Dim1, suggesting a potential correlation. This assertion resonates with the overarching comprehension regarding the influence of developmental stages on the accrual of minerals in berries [42,43]. Therefore, our results indicate the significance of considering various growth phases in comprehending the intricate dynamics of these compounds' accumulation within berry structures. The Dim2 axis showed consistent concentrations of amino acids without significant differentiation, offering insight into their interplay in berries. This has potential implications for dietary recommendations and understanding nutritional variations in cultivars. For elements, PCA analysis highlighted Dim1 (explaining 64.1% of variance) and Dim2 (explaining 26.1%). N, Mn, and Cu were on the positive side of Dim1, while Fe and Na were on the negative side. Mg, K, and Ca showed a strong representation on the positive y-axis, suggesting potential correlations among them in the samples. In the vitamin analysis, Dim1 (explaining 92.5% of variance) and Dim2 (explaining 4.9%) showed Vitamins B6, B2, and C consistently on the positive side of Dim1, while Vitamins A and B1 had a different distribution. This aligns with the understanding of temporal variations in vitamin content during fruit development [42,43], emphasizing the documented interest in ascorbate metabolism and strategies for increasing vitamin C concentrations in various fruits, although grape berries are not among those with high vitamin C content [44]. These findings provide insights into the distribution and potential relationships among elements in berries, aiding our understanding of mineral content and elemental profiles in different berry cultivars. Regarding phenolic compounds, PCA analysis revealed distinct patterns with Dim1 (explaining 81.1% of variance) and Dim2 (explaining 15.8%). Compounds like quercetin, ferulic acid, and myricetin clustered together on the negative side of Dim1, while trans-caffeic acid and gallic acid were prominent on the positive side. Dim2 did not differentiate much among phenolic compounds. Much like the phenolic compounds, the Dim2 axis did not reveal significant differentiation among vitamins, indicating relatively consistent concentrations in this regard. These findings offer insights into the distribution and relationships among vitamins in berries, which are valuable for understanding their nutritional composition and dietary implications. The comprehensive heatmap analysis presented in this study offers a profound exploration of the concentration profiles of diverse compounds in berries, encompassing phenolic compounds, essential elements, and vitamins. This visually striking representation employs a color gradient ranging from deep blue to intense red, where blue signifies lower compound concentrations and red signifies higher concentrations within the berry samples. Augmenting this color gradient is a numerical scale that spans from −6 to 6, effectively conveying the intensity of these compound concentrations. Notable findings within the heatmap analysis include the prevalence of high concentrations of K, cerin, and aspargine, denoted by deep red coloration, across most berry samples. Conversely, trans-caffeic acid and gallic acid exhibit moderate to high concentrations, especially in Bronx Seedless berries, prominently within the BBCH 77 and BBCH 79 samples. In the realm of essential elements, Fe, Zn, and Cu manifest moderate concentrations across most samples, indicated by lighter blue to white coloration. In contrast,

Mn consistently exhibits low concentrations across almost all berry samples, representing a commonality in the elemental profile of these berries. Turning to vitamins, Vitamins B6 and B2 emerge as having consistently moderate concentrations, represented by white-blue coloration, particularly in Bronx Seedless berry samples, notably within the BBCH 77 and BBCH 79 categories. However, for Vitamins A and B1, the heatmap depicts varying concentrations. Amino acids such as glutamate and aspartate consistently present high concentrations across most Bronx Seedless and Italia samples. A distinct pattern emerges for Alanine, which exhibit consistently high concentrations across both Bronx Seedless and Italia cultivars. Bronx Seedless and Italia samples, particularly those from BBCH 77 and BBCH 79, tend to exhibit higher concentrations for many of the studied compounds. The presented heatmap offers an in-depth comparative understanding of the concentration gradients of these compounds in berries, shedding light on the diversity and uniqueness of various berry samples. These findings are in line with established biochemical pathways and prior studies that indicate the complexity of metabolic interactions [45–49]. It has also demonstrated that the ripening stage of grape berries exerts a significant influence on the composition and concentrations of phenolic compounds, including flavonoids and phenolic acids, with substantial variations observed across the various developmental phases [50].

5. Conclusions

This comprehensive study provided insights into the phenolic compounds, amino acids, minerals, and vitamins at different berry development stages of two table grape cultivars, Bronx Seedless and Italia. Based on our result, Italia showcased more abundant phenolic profiles with elevated levels of vanillic acid and gallic acid, whereas Bronx Seedless consistently displayed higher levels of specific amino acids, including glutamate, aspartate, and phenylalanine. Mineral composition varied notably between the grape cultivars, impacting levels of nitrogen, phosphorus, and iron. Vitamin content also showed distinctions, with Italia having higher levels of Vitamins A and B1 and Bronx Seedless showing higher levels of Vitamins B2, B6, and C. Additionally, the heatmap demonstrated concentration gradients across berry development stages, and the PCA analysis revealed intriguing relationships between these compounds. This comprehensive investigation of grape composition offers a valuable resource for researchers and grape growers. Looking to the future, further research could explore the potential health benefits associated with these nutrient variations in table grapes and investigate strategies to optimize mineral content for consumer health and preferences.

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