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Characterization of Betalain Content and Antioxidant Activity Variation Dynamics in Table Beets (*Beta vulgaris* L.) with Differently Colored Roots

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Abstract: Antioxidant properties, betalain profiles and biochemical composition were studied in table beets with maroon and yellow root colors. Features of dynamic changes during the growing season were described. Significant differences in antioxidant activity were found in table beet accessions with differently colored roots. Negative dynamics of antioxidant activity were observed in all accessions. Statistically significant differences were registered among the accessions in the total amounts of phenolic compounds, chlorophylls, and betalains. The group of maroon accessions demonstrated strong positive correlations between their antioxidant activity and total phenolics (r = 0.91), antioxidant activity and betacyanins (r = 0.80), and between betacyanins and phenolics (r = 0.90). The antioxidant activity in the accessions with yellow roots was associated with chlorophyll b (r = 0.85), ascorbic acid (r = 0.83), and total phenolics (r = 0.83). The data are presented on the structure of betalains in two table beet groups contrasting in their root color. The results of the study made it possible to identify key components in the biochemical profile of differently colored beetroots, associated with their high antioxidant activity. Dynamic changes were shown for the antioxidant activity and fractional composition of betalains in table beet during its growing season, and a conclusion was made concerning the higher nutritional value of maroon cultivars.

Keywords: *Beta vulgaris* L.; betalains; betacyanins; betaxanthins; antioxidants; total phenolic content

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1. Introduction

Recently, interest in food ingredients and bioactive additives that can prevent the development of pathological processes in the human body has increased throughout the world [1,2]. A high demand is observed for natural food colors that combine low toxicity, environmental safety, and high antioxidant activity [3].

Compounds that inhibit the formation and free radicals in the human organism are referred to as antioxidants [4]. Free radicals are reactive molecules with unpaired electrons; they are formed in the human body during natural metabolic processes as well as under a negative impact of external factors. Their accumulation leads to redox imbalance and oxidative stress. Free radicals can react with many types of biomolecules (lipids, proteins, carbohydrates or DNA), which can become a prerequisite for the occurrence of oncological processes [5,6]. Enriching human diets with natural antioxidants is an effective way to neutralize free radicals and prevent diseases. Since plant products make up a significant part of the diet in human nutrition, natural antioxidants, in particular plant pigments, become of great importance.

The classes of plant pigments with distinct antioxidant properties include betalains, carotenoids, and some flavonoids (anthocyanins, chalcones, aurones and some flavonols). Flavonoids and betalains are water-soluble pigments. Flavonoids are a large group of the

most thoroughly studied secondary metabolites with a wide range of coloring. This class of pigments includes anthocyanins, which provide various plant organs with a color from orange to dark blue. Betalains, yellow-red tyrosine-derived nitrogen-containing pigments, are inherent in representatives of only one order, Caryophyllales, and never occur in plants together with anthocyanins. Despite this fact, a functional analogy is visible between both groups of pigments. Anthocyanins and betalains both attract insect pollinators and act as protectors against biotic and abiotic stressors [7,8].

Betalains are primarily present in food plants such as beet and amaranth and in prickly pear fruits. Their positive effect on human health has been confirmed by the results of numerous medical studies: noticeable anti-inflammatory, anticarcinogenic and antioxidant properties have been reported [9–12]. Moreover, betalain-containing plant extracts have already been patented in formulations for cancer therapy [13,14]. An increased antioxidant activity of betalains is associated with their high electron-donating ability [15].

Table beet (*Beta vulgaris* L. ssp. *vulgaris* var. conditiva Alef.) is the most popular source of betalains. Published data on its exceptionally high antioxidant activity arouse interest in the crop's bioactive compounds [12,16,17]. Betalains are divided into two subgroups: red-violet betacyanins (BCs) are conjugates of betalamic acid with cyclo-DOPA, while yellow-orange betaxanthins (BXs) are conjugates of betalamic acid with various amines and amino acids. BCs are represented mainly by betanin and isobetanin, while vulgaxanthins (I and II) predominate among BXs [18]. The betalain group consists for 70–75% of betanin (betanidin 5-O-β-glucoside), used in the food, pharmaceutical, and cosmetic industries as the E 162 dye [19].

The objective of this study was to examine the antioxidant activity, betalains and biochemical parameters in table beets differing in their root color and analyze their dynamic changes during the growing season.

2. Materials and Methods

Five table beet cultivars from the collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) with differently colored roots served as the research material (Table 1, Figure 1). The accessions were grown in the experimental fields of Pushkin and Pavlovsk Laboratories of VIR (59°7111275′ N, 30°43032647′ E; Pushkin, St. Petersburg, Russian Federation) in 2021. The soils on the experimental field are predominantly soddy–podzolic and sandy loam. A randomized block planting pattern was used, with three repetitions for each block. The plantings were arranged in six-meter rows according to a 70 × 8 cm scheme. Seeds were sown manually on May 21 into open ground to a depth of 2.5–3 cm. Fertilizers or plant protection products against pests or diseases were not applied during the growing season. The first harvesting of beetroots for research purposes took place on 27 July 2021, with the next two undertaken in three-week intervals.

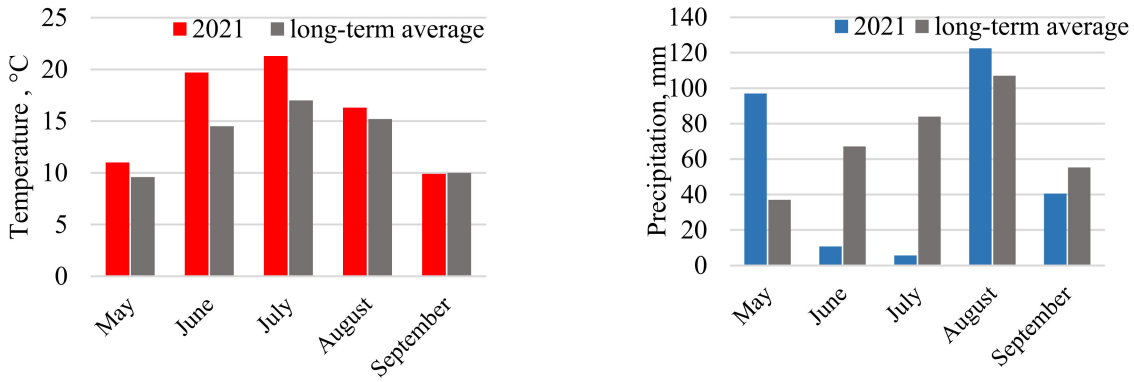
Table 1. Table beet accessions from the VIR collection selected for the experiment.

VIR Catalogue No.	Cultivar Name	Origin	Beetroot Color
k-3151	Bordo Odnosemyannaya	Russia	Maroon
k-3677	Detroit Rubinovy–5	Russia	Maroon
k-3698	Russkaya Odnosemyannaya	Russia	Maroon
k-3880	Boldor	Netherlands	Yellow
k-4017	Serdolik	Russia	Yellow

Figure 1. Table beet accessions with differently colored roots.

2.1. Weather Characteristics

The weather in 2021 was characterized by a long dry season, with virtually no rainfall in June or July (16 and 7% of annual averages, respectively) (Figure 2A). The lack of moisture (84–93% below the norm) coupled with high air temperature (25–36% above the norm) in those months produced a negative effect on plant development, restraining the growth of leaf biomass. Profuse rainfall in August during the root growth phase compensated for the deficit of precipitation, so the beetroot harvest reached the long-term average level.



(A)

Figure 2. *Cont.*

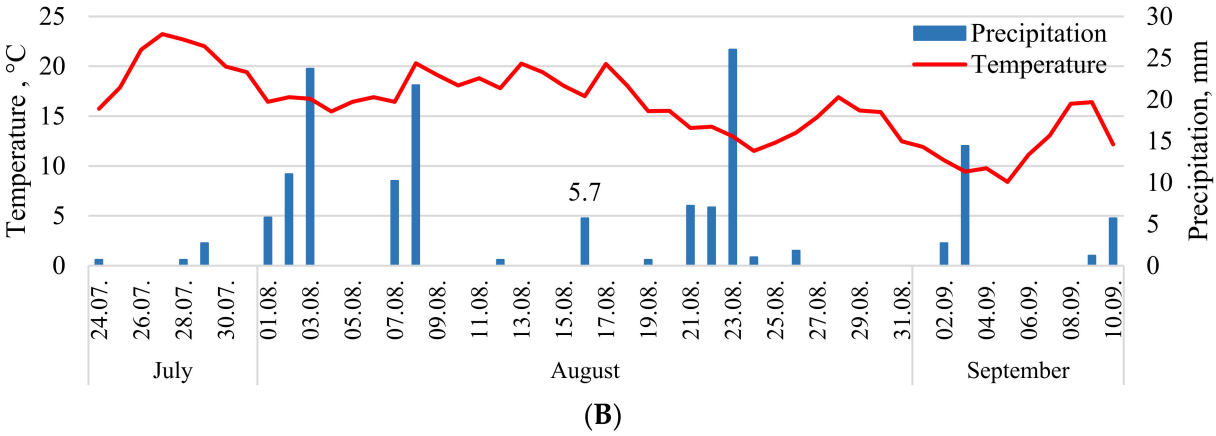


Figure 2. Weather characteristics during the growing season in 2021 (Pushkin and Pavlovsk Laboratories of VIR, Town of Pushkin, St. Petersburg, Russia). (**A**) Average monthly indicators; (**B**) dynamics of the indicators during the period of analysis. Source: Department of Automated Plant Genetic Resources Information Systems, Hydrometeorological Station of VIR.

2.2. Sample Preparation

Samples for the study were analyzed within three hours after harvesting. All samples were thoroughly cleaned using deionized water to remove adherent contaminants. An average beet sample of 5 roots per each accession was analyzed. For grinding, 1/4 of each beetroot was taken and cut lengthwise, and an average sample was formed from these parts. This average sample was ground using a food processor to a thickness of 2 mm for 1 min at room temperature. Then each sample was mixed and samples were taken from fresh material for analysis. The plant material was tested according to the methods developed at VIR [20].

2.3. Quantification of Dry Matter Content

Dry matter content was measured by %, using the gravimetric method. A sample of fresh matter (50 g) was dried in a thermostat at 80 °C for 12 h, then at 105 °C for 4 h down to a constant weight.

2.4. Quantification of Ascorbic Acid Content

Vitamin C was determined on the same day after harvesting. Vitamin C was determined using the procedure outlined in the Food Analysis Laboratory Manual (Chapter 11: Vitamin Analysis—Vitamin C: 2,6-Dichloroindophenol Titrimetric Method) [21] and AOAC International Methods of Analysis [22]. A 10 g quantity of each experimental sample was exactly weighed and ground in a mortar and pestle with the addition of 20 mL of a 1% HCl solution with oxalic acid. The mixture was then ground and filtered through muslin, and the volume of the extract was adjusted to 100 mL using oxalic acid. A 5 mL quantity of 1% HCl-oxalic acid solution was pipetted into a 50 mL Erlenmeyer flask, and then 2 mL of sample extract was added. Samples were titrated separately with indophenol dye solution until the light pink color persisted for 5 s. The amount of dye used in titration was determined and used in calculating the vitamin C content.

2.5. Quantification of Sugar Content

To measure the sugar content of beet roots, a standard protocol was used, the Munson–Walker method [22], a popular method for estimating total saccharides by titration with potassium permanganate [21,22]. The alkaline copper solution was reduced to cuprous oxide whose precipitate was collected. Roots of each experimental beet sample (500 g) were crushed down, and a fragment of the crushed pulp (25 g) was put into a 250 cm^3 volumetric flask. Water was heated to 80 °C, and 225 mL was poured into the flask. The sample was then ground in a blender and left in the flask for 12 h. Filter paper was thereafter used

to filter the sample into a clean and dry flask. Then the sample (20 mL), a 15% NaOH solution (20 mL), and a 4% $CuSO_4$ solution (20 mL) were thoroughly mixed in the flask with glycerin (1 mL).

Sucrose hydrolysis required placing 50 mL of the filtrate into a 100 mL volumetric flask, where 20% hydrochloric acid (5 mL) was added. The sample in the flask was kept for 30 min at 100 °C, then quickly cooled to an ambient temperature of 20 ± 1 °C, and then brought up to 100 mL by adding distilled water. The next step was mixing 5 mL of the sample with a 15% NaOH solution (20 mL), a 4% $CuSO_4$ solution (20 mL), and glycerin (1 mL) in a conical flask, which was stirred well and afterwards heated to the boiling point. Boiling continued for 3 min exactly from the moment when first bubbles appeared. A glass filter was then used to filter the obtained liquid. The precipitate left both in the flask and on the filter was cleansed several times with hot water. The Cu_2O precipitate was then dissolved in an ammonium ferrous sulfate (III) solution (20 mL). The obtained liquid underwent suction filtering, and the resulting precipitate was washed. Potassium permanganate was used to titrate the green-colored solution in the suction flask down to a thin pink hue. The percentage of total saccharides and reducing sugar (monosaccharides) content was calculated according to the following formula:

$$X\ (\%) = (a \cdot V \cdot 100)/(V1 \cdot m)$$

where a—the amount of saccharides in a given volume (V1), mg; V—the volume of the extract obtained from the tested sample, mL; V1—the volume of the extract's fragment taken for the analysis, mL; and m—the weight of the sample (g).

$$\text{Disaccharides (\%)} = \text{Total saccharides (\%)} - \text{Reducing sugar (\%)}.$$

2.6. Quantification of Organic Acid Content

Titratable acidity is determined by neutralizing the acid present in a known quantity (weight or volume) of food sample using a standard base as outlined by the Food Analysis Laboratory Manual in Chapter 13 [21]. The end-point for titration is the color change of a pH-sensitive dye, typically phenolphthalein. The volume of titrant used, along with the normality of the base and the volume (or weight) of sample, is used to calculate the titratable acidity, expressed in terms of the predominant organic acid. To measure the total acidity, a sample of fresh matter (25 g) was homogenized in 250 mL of hot distilled water and then filtered, and 10 mL was titrated with 0.1 N alkali in the presence of an indicator. The results are expressed as percentage of malic acid.

2.7. Quantification of Chlorophyll and Carotenoid Content

Chlorophylls and carotenoids of beet roots were extracted with 100% acetone [20]. A sample (1 g) was homogenized in 10 mL of 100% acetone, then the plant mass was ground and filtered. The amount of acetone used was taken into account. Then the extracts were centrifuged at room temperature for 3–5 min at 300–500 g. The absorbance of the filtrate was measured on an Ultrospec II spectrophotometer (Cambridge, UK) at different wavelengths (nm): 645, 662 for chlorophylls *a* and *b*, 470 for carotenoids.

$$\text{Chl } a\ (\text{mg}/100\ \text{g}) = 9.784\ D662 - 0.99\ D645$$

$$\text{Chl } b\ (\text{mg}/100\ \text{g}) = 21.426\ D645 - 4.65\ D662$$

$$\text{Total carotenoids (mg}/100\ \text{g}) = 4.695\ D470 - 0.268\ (\text{Chl } a + \text{Chl } b);$$

where Dx—optical density at x nm.

The amount of pigment was calculated using the following formula:

$$X = (C \cdot V \cdot V2 \cdot 100)/(m \cdot V1);$$

where C—pigment concentration (mg/1000 g); V—total volume of extract (ml); V1—volume of extract taken for titration (mL); V2—volume of diluted extract (mL); m—mass of the analyzed material (g).

2.8. Quantification of Protein Content

The Kjeldahl method was applied to assess protein content. Three to four samples (300 mg each) underwent mineralization with concentrated sulfuric acid (5 mL) at 420 °C for 1.5 h. A Kjeltec 2200 semi-automatic analyzer (FOSS, Hoganas, Sweden) with an automatic distillation unit was used to calculate nitrogen. Then, the sample was titrated with a 0.1 N sulfuric acid solution. Factor 6.25 was employed to deduce total proteins from the nitrogen content.

2.9. Quantification of Total Phenolics

Phenolic compound determination was based on the reduction of Mo^{6+} to Mo^{5+}, as a result of which the analyzed solution acquires a blue color and becomes optically measurable. The phenolic compounds of beetroots were extracted with 80% EtOH [23]. A fragment of the crushed pulp (10 g) was homogenized in 50 mL of 80% EtOH in a blender and left in the flask for 12 h. Then the extracts were centrifuged at room temperature for 25 min at 300 to 500× *g*.

The extract (0.5 mL) was mixed with Folin–Ciocalteu reagent and saturated sodium carbonate solution at a 1:1:2 ratio and left in the dark for 2 h. The absorbance in the final mixture was measured at 765 nm. The obtained values are expressed in mg/100 g of the initial material in terms of gallic acid (mg GA/100 g). Total polyphenols in a sample are usually expressed as gallic acid equivalents, which are used as quantitative standards.

$$\text{Total phenolics (mg GA/100 g)} = (C \cdot V)/m \cdot 1000$$

where C—concentration of phenolic compounds (mg/1000 g); V—the total volume of extract (mL); m—the mass of the analyzed material (g).

2.10. Quantification of Total Antioxidant and Antiradical Activity

Antioxidant activity (DPPH radical scavenging assay): The DPPH method (a method for determining antioxidant activity by quenching free radicals using the 2,2-diphenyl-1-picrylhydrazyl reagent) is based on the reaction of a stable synthetic DPPH (2,2-diphenyl-1-picrylhydrazyl) radical dissolved in ethanol with an antioxidant sample presumably contained in the extract [24]. The antioxidant activity (AOA) of beetroots was extracted with 80% EtOH. A fragment of the crushed pulp (10 g) was homogenized in 50 mL of 80% EtOH in a blender and left in the flask for 12 h. Then the extracts were centrifuged at room temperature for 25 min at 300 to 500× *g*. The extract (0.1 mL) was mixed with the 3.9 mL of 0.06 mM DPPH solution (2,2-diphenyl-1-picrylhydrazyl dissolved in 96% EtOH) and left in the dark for 30 min. When the DPPH free radical is reduced with antioxidant substances in the studied extracts, the saturated blue color of DPPH gradually changes to yellow: the free radical of 2,2-diphenyl-1-picrylhydrazyl (with a saturated blue color) transforms into a stable molecule of yellow-colored 2,2-diphenyl-1-picrylhydrazyl. Free radicals of DPPH were determined at a wavelength of 515 nm. The data are expressed in mg eq.·ascorbic acid/100 g (mg eq. AA/g):

$$\text{AOA (mg eq. AA/g)} = (C \times 176 \times V)/(1000 \times m),$$

where C—concentration of ascorbic acid (mg/1000 g); V—the total volume of extract (mL); m—the mass of the analyzed material (g); 176—molecular weight of ascorbic acid.

The following formula was applied to calculate the percentage of free radical inhibition (DPPH):

$$\text{Inhibition \% (DPPH)} = (\text{A blank} - \text{A sample/A blank}) \times 100,$$

where A blank—absorbance of the control reaction (containing all reagents except the test compound), A sample—absorbance of the test compound.

2.11. Quantification of Betalains

The content of betalains was measured spectrophotometrically. A 1 g sample of ground fresh beets was exhaustively (until the starting material became discolored) extracted with a 2% formic acid solution. Then it was filtered through a paper filter and collected in a volumetric flask, the resulting volume (V, mL) was recorded. The absorbance of the obtained and diluted (1:50) extracts was read (Ultrospec II, Cambridge, UK) at λ = 542 nm for betacyanins and at λ = 480 nm for betaxanthins. The betalain, betacyanin, and betaxanthin levels were calculated from optical density for each sample using the following formula:

$$\text{BC or BX (mg/100 g)} = \text{A542/480} \times \text{DF} \times \text{MW} \times \text{V}/\varepsilon \times \text{L} \times \text{Wd} \times 100,$$

where BC or BX is the content of betacyanins (BCs) or betaxanthins (BXs) (mg/100 g); A542/480 is the absorbance at an absorption maximum for BCs (λ = 542 nm) or BXs (λ = 480 nm); DF is the dilution factor; MW is the molecular weight: 550 g/mol for BCs and 308 g/mol for BXs; V is the volume of sample solution (mL); ε is the molar extinction coefficient 60,000 L/(M × cm) for BCs and 48,000 L/(M × cm) for BXs; L is the path length of the cuvette (1 cm); and Wd is the weight of the sample (g).

The betalain complex was separated into betacyanins and betaxanthins using the solid-phase extraction method on DIAPAK C18 cartridges (Grace, Deerfield, MA, USA). The extracts were passed through a cartridge prepared by activation with acetone and conditioning with an extractant. The first eluate portions were yellow-colored and contained BXs, practically nonsorbable under the conditions applied. The passage of the extract was stopped before the breakthrough of BCs started. The cartridge was washed with a small portion of the extractant (5–15 mL) to remove BXs until the yellow color of the eluate disappeared. BCs were desorbed with a solution containing 50% of acetonitrile and 2% of formic acid (*v*/*v*) in water.

2.12. HPLC-Assisted Quantification of Individual Betalains

Separation and measuring of individual betalains by means of HPLC were performed using the Agilent 1200 Infinity chromatographic system (Agilent Technologies, Santa Clara, CA, USA) under the following conditions: Zorbax SB-C18 column (Agilent Technologies, Santa Clara, CA, USA) and MWD detector; effluent CH_2O_2:H_2O = 10:90, at 0.3 mL/min; column temperature of 25 °C. BCs and BXs were detected at 540 nm and 480 nm, respectively [25], and betanin was used as the reference. The following HPLC separation conditions were applied: the mobile phase was 10% formic acid (A), and 50% acetonitrile plus 10% formic acid (B). The following multi-step linear gradient was applied: from 100% of A for 30 min, then 80% of B from 30 up to 35 min, then 80% of B from 35 up to 40 min, and then 100% of A from 40 up to 50 min. The total time for the analysis was 50 min. Qualitative identification of individual pigments in the separated fractions (BXs and BCs) was performed by comparing retention times and analyses of the UV spectra of the separated compounds with reference and published data.

All data presented are based on wet weight, except for protein content, which is expressed on an absolutely dry weight basis.

2.13. Statistical Analysis

Statistical data processing was performed using the MS Excel 2007 and Statistica 10.0 software, and in the R environment (metan packages). Descriptive statistics (mean, standard error of the mean, and coefficient of variation) were calculated for all parameters. Factor analysis was applied to assess the variability in the structure of relationships among the characters. Factor loadings were calculated using the principal components method. Tukey's HSD test was used to compare the means. The values of the Pearson correlation

coefficient at r < 0.3 were considered weak, 0.3 > r > 0.5 moderate, 0.5 > r > 0.7 conspicuous, 0.7 > r > 0.9 strong, and r > 0.9 very strong.

3. Results and Discussion

Antioxidant activity (AOA) in the studied accessions during the growing season varied widely, from 6.18 to 119.96 mg*eq AA/100 g FW (Table 2). The group of maroon genotypes had significant ($p < 0.05$) differences from the yellow-colored group (the indicator was higher by 64–67% than that of the yellow biotypes), which was confirmed by the factor analysis results (Figure 3).

Table 2. Dynamics of antioxidant activity and betalains in table beets.

VIR Catalogue No.	Beetroot Color	AOA, mg*eq AA/100 g			Betalains, mg/100 g FW		
		Dates of Analysis					
		27 July	17 August	10 September	27 July	17 August	10 September
k-3151		99.11	89.21	78.12	87.96	218.80	160.14
k-3677		116.66	94.75	92.77	56.80	179.37	168.05
k-3698		119.96	81.81	92.11	64.47	180.50	160.61
	Mean	111.91	88.59	87.67	69.74	192.89	162.93
	SD	11.21	6.49	8.27	16.23	22.46	4.44
k-3880		49.47	33.50	18.59	130.94	3.86	2.40
k-4017		49.21	30.33	6.18	56.50	4.99	3.55
	Mean	49.34	31.91	12.38	93.72	4.42	2.97
	SD	0.18	2.24	8.77	52.64	0.80	0.81

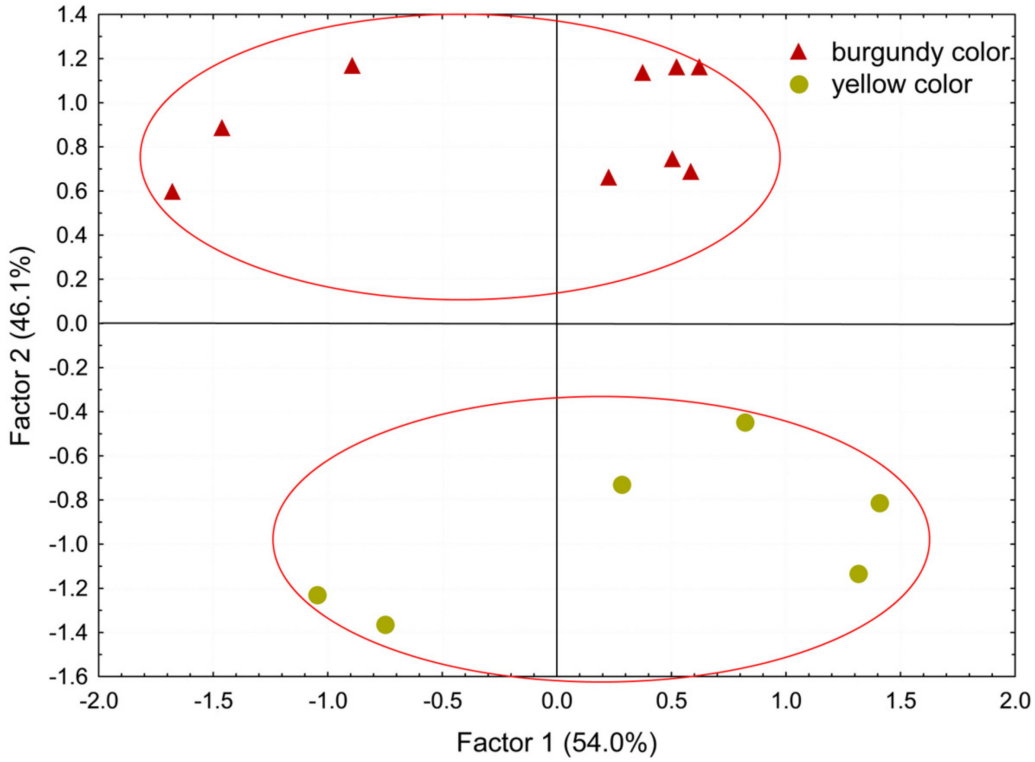


Figure 3. Contributions of the principal components of the factor analysis to the total variance. Interpretation of the factors: Factor 1—taste characteristics; Factor 2—AOA.

A decrease in AOA by an average of 28% was recorded in all tested accessions towards the end of the growing season. The only exception was cv. 'Russkaya Odnosemyannaya' (k-3698): it showed a slight increase in AOA by the end of its growing season. Yellow-colored biotypes demonstrated a more significant loss of AOA (by 30–43%).

The content of betalains in the group of maroon accessions during the growing season ranged from 64.47 to 218.8 mg/100 g and averaged 141.85 mg/100 g. Betalains in the yellow cultivars averaged 23.8% of the content in the maroon ones. By the date when beetroots were collected for analysis for the last time, the total content of betalains had increased in the maroon biotypes and decreased in the yellow ones. The highest value was recorded for cv. 'Bordo Odnosemyannaya' (218.80 mg/100 g). The absence of linear dynamics was observed in the variation of betalain content, which was noticed earlier [26]: when beetroots were collected on 8 August 2021, an increase was observed in this indicator in the maroon-colored group.

The dry matter and protein content levels in the tested table beet accessions were the closest and most stable indicators, with little variation over the growing season: the coefficient of variation (% CV) for all accessions did not exceed 16.2% (Table 3). There were no significant differences between the groups in the levels of ascorbic acid, saccharides, carotenoids or organic acids. Phenolic components and pigment composition (chlorophylls and betalains) demonstrated statistically significant differences. The lowest chlorophyll content values were recorded for cv. 'Russkaya Odnosemyannaya' (k-3698). On the basis of the total phenolic content, the accessions divided into two significantly different groups according to the color of their roots: maroon (96.43–118.97 mg of GAE/100 g) and yellow (21.71–28.47 mg of GAE/100 g).

In terms of BC content, two significantly different groups with contrasting root coloring were identified. The highest values were shown by the maroon accessions (k-3151, k-3677, and k-3698), and the lowest by the yellow ones (k-3880, and k-4017). A similar pattern, but with the yellow-colored group at the lead, was observed for BXs: these compounds in the maroon group accounted for 11.3% of the level recorded for the yellow accessions.

The correlation analysis of the tested table beet groups revealed significant differences in the relationships of AOA with biochemical parameters. For example, the maroon group demonstrated strong positive correlations between AOA and the amount of phenolic compounds ($r = 0.91$, $p < 0.001$), AOA and BCs ($r = 0.80$, $p < 0.01$), and between BCs and phenolics ($r = 0.90$, $p < 0.001$) (Figure 4). Phenolic compounds are known to have a synergistic effect with betalains, enhancing their antioxidant properties [27]. The decrease in AOA, marked in Table 2, was associated mainly with a decrease in the level of phenolics, and secondarily, with BCs. This conclusion was supported by Chavez-Santoscoy et al. [28] who reported that in the juice of betalain-synthesizing prickly pear fruits (*Opuntia* spp.) with the same high BC levels the highest AOA was observed only in the species with high phenolic content. The BX content in the maroon group had no significant relationships with other biochemical parameters. Saccharides were observed to have positive correlations with dry matter, organic acids, and carotenoids.

AOA in the yellow beetroot group was associated with chlorophyll *b* ($r = 0.85$, $p < 0.01$), ascorbic acid ($r = 0.83$, $p < 0.01$), and total phenolics ($r = 0.83$, $p < 0.05$). No relationship between betalains and AOA was found (Figure 5).

Table 3. Biochemical parameters of table beets contrasting in their root color. Average of three measurements. Standard distribution difference—Tukey's test for $p = 0.05$; abnormal distribution difference—Kruskal–Wallis test for $p = 0.05$.

Parameters	M ± SE * (Cv ***, %), Median (Min ÷ Max) **				
	k-3151	**k-3677**	**k-3698**	**k-3880**	**k-4017**
Dry matter, %	16.63 ± 1.10 (11.5) [a]	15.83 ± 1.33 (14.6) [a]	15.79 ± 1.34 (14.7) [a]	13.28 ± 0.93 (12.2) [a]	15.52 ± 1.24 (13.8) [a]
Protein, %	6.40 ± 0.44 (11.8) [a]	5.90 ± 0.55 (16.2) [a]	5.87 ± 0.33 (9.7) [a]	6.83 ± 0.34 (8.6) [a]	7.10 ± 0.29 (7.0) [a]
Ascorbic acid, mg/100 g	42.63 ± 13.92 (56.6) [a]	39.50 ± 10.19 (44.7) [a]	37.93 ± 2.74 (12.5) [a]	35.58 ± 2.38 (11.6) [a]	34.80 ± 3.85 (19.2) [a]
Monosaccharides, %	1.25 ± 0.54 (75.4) [a]	0.99 ± 0.17 (28.9) [a]	1.02 ± 0.11 (18.6) [a]	0.68 ± 0.18 (45.6) [a]	0.92 ± 0.28 (51.8) [a]
Disaccharides, %	8.71 ± 1.69 (33.5) [a]	8.67 ± 1.55 (30.9) [a]	8.67 ± 1.25 (25.0) [a]	6.94 ± 1.40 (34.9) [a]	7.62 ± 1.25 (28.5) [a]
Total sugars, %	9.96 ± 1.41 (24.5) [a]	9.66 ± 1.54 (27.6) [a]	9.68 ± 1.28 (22.9) [a]	7.62 ± 1.38 (31.4) [a]	8.54 ± 1.01 (20.4) [a]
Organic acids, %	0.51 ± 0.11 (38.0) [a]	0.37 ± 0.09 (40.3) [a]	0.44 ± 0.12 (47.6) [a]	0.59 ± 0.08 (23.0) [a]	0.53 ± 0.08 (26.6) [a]
Total phenolics, mg of GAE/100 g	118.97 (103.3 ÷ 133.6) [b]	115.32 (113.9 ÷ 116.3) [b]	96.43 (74.4 ÷ 112.3) [b]	28.47 (22.5 ÷ 37.5) [a]	21.71 (17.5 ÷ 27.1) [a]
Chlorophyll *a*, mg/100 g	0.15 (0.1 ÷ 0.3) [a]	0.15 (0.1 ÷ 0.2) [a]	0.11 (0.08 ÷ 0.1) [b]	0.41 (0.2 ÷ 0.5) [a]	0.28 (0.1 ÷ 0.4) [a]
Chlorophyll *b*, mg/100 g	0.25 (0.1 ÷ 0.3) [a]	0.25 (0.2 ÷ 0.4) [a]	0.13 (0.04 ÷ 0.2) [b]	0.47 (0.1 ÷ 0.8) [a]	0.25 (0.1 ÷ 0.4) [a]
Chlorophylls, mg/100 g	0.40 (0.1 ÷ 0.6) [a]	0.40 (0.3 ÷ 0.6) [a]	0.24 (0.1 ÷ 0.3) [b]	0.88 (0.6 ÷ 1.3) [a]	0.52 (0.4 ÷ 0.7) [a]
Carotenoids, mg/100 g	0.42 (0.25 ÷ 0.7) [a]	0.37 (0.1 ÷ 0.7) [a]	0.34 (0.2 ÷ 0.4) [a]	0.23 (0.1 ÷ 0.6) [a]	0.18 (0.1 ÷ 0.35) [b]
Betacyanins, mg/100 g FW	151.49 (87.3 ÷ 214.6) [b]	131.82 (56.2 ÷ 176.1) [b]	131.01 (62.4 ÷ 175.7) [b]	0.58 (0.2 ÷ 0.8) [a]	0.35 (0.0 ÷ 0.8) [a]
Betaxanthins, mg/100 g FW	4.14 (0.6 ÷ 7.6) [b]	2.92 (0.6 ÷ 4.9) [b]	4.18 (2.1 ÷ 5.7) [b]	45.15 (2.2 ÷ 130.1) [a]	21.3 (3.3 ÷ 56.5) [a]
AOA, mg*eq AA/100 g	88.81 (78.1 ÷ 99.1) [b]	101.39 (92.8 ÷ 116.7) [b]	97.96 (81.8 ÷ 119.9) [b]	33.85 (18.6 ÷ 49.5) [a]	28.57 (6.2 ÷ 49.2) [a]
DPPH, %	42.34 (39.3 ÷ 47.5) [b]	49.83 (43.7 ÷ 58.2) [b]	47.69 (35.6 ÷ 60.2) [b]	9.43 (5.5 ÷ 17.2) [a]	6.92 (0.15 ÷ 17.1) [a]

* M ± SE—mean ± standard error for standard data distribution; ** Median (min÷ max)—for abnormal data distribution; *** Cv—coefficient of variation; [a,b]—identical letters in the same row mean that the data do not differ significantly.

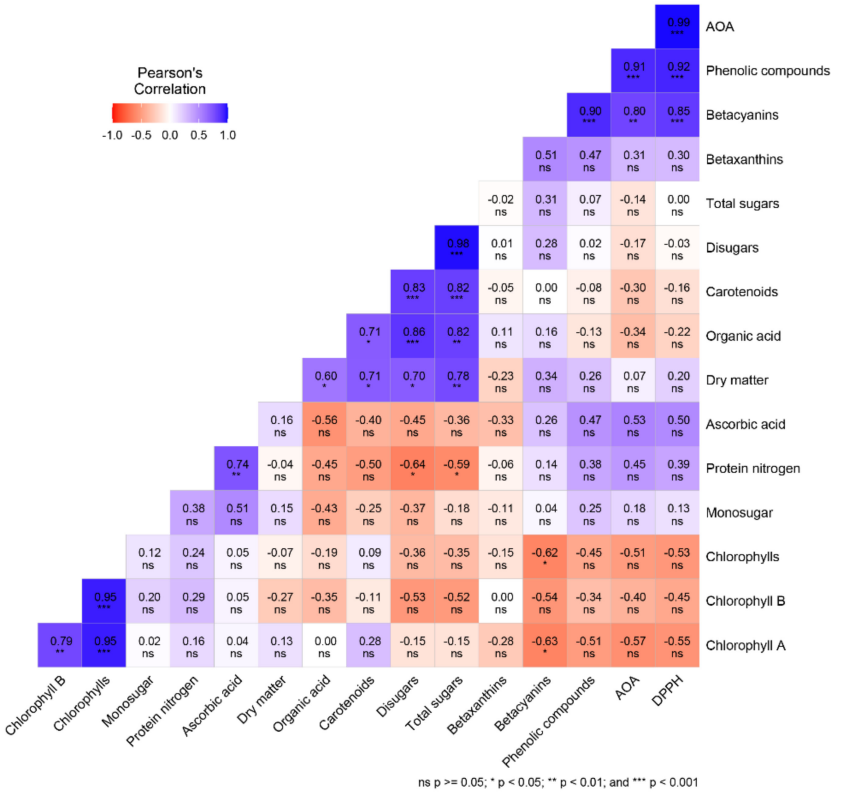


Figure 4. Correlation matrix of biochemical indicators and AOA for the maroon table beet group. The numbers inside each square show the Pearson *R* correlation values.

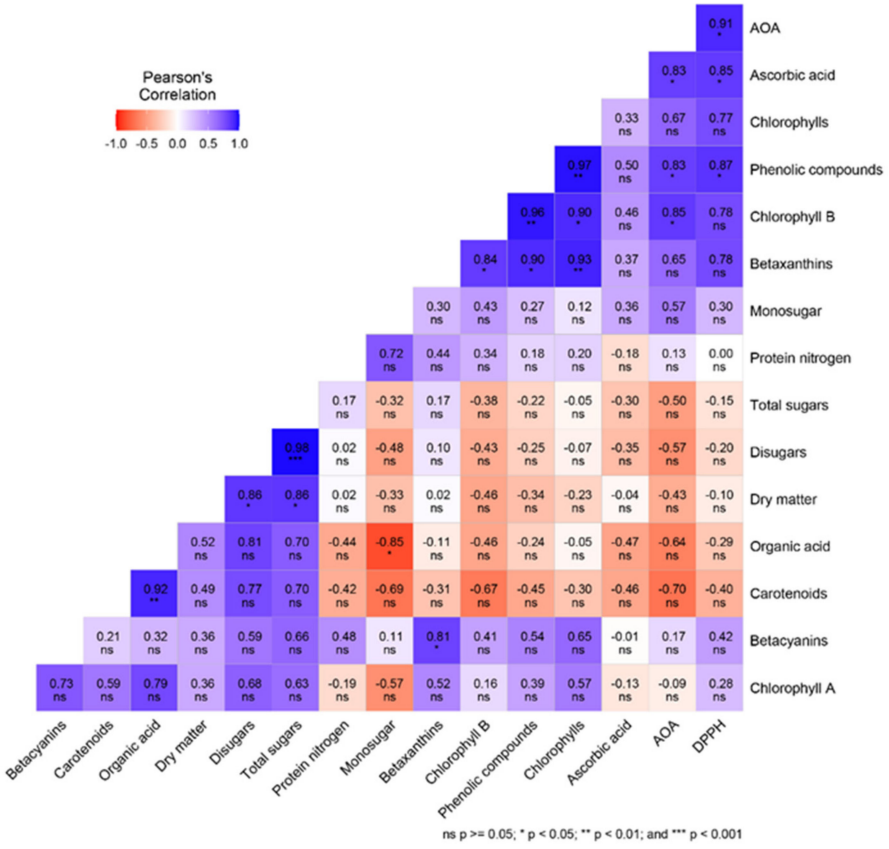


Figure 5. Correlation matrix of biochemical indicators and AOA for the yellow table beet group. The numbers inside each square show the Pearson *R* correlation values.

Deciphering the fractional composition of betalains in the accessions resulted in identification of 16 BCs and 10 BXs (Table 4, Supplementary Materials). The BCs common in all accessions were prebetanin, isoprebetanin, betanin, and isobetanin (a stereoisomer of betanin). The group of maroon biotypes was characterized by the presence of the full range of the identified BCs. The yellow-colored accessions contained 4 BCs and 10 BXs. BCs were present in small amounts, represented (in descending order) by isobetanin (0.64–0.56 mg/100 g), betanin (0.80–0.01 mg/100 g), isoprebetanin (0.19–0.01 mg/100 g), and prebetanin (0.08–0.02 mg/100 g).

Table 4. Betalain fractions in table beets contrasting in their root color.

Betalains	Trivial Name/Abbreviation	[a] λ_{max} [nm]		
Betacyanins				
6′-O-Sulfate-betanin	Prebetanin	538	+	+
Betanidin 5-O-β-glucoside	Betanin	537	+	+
Isoprebetanin		538	+	+
Betanidin	Betanidin	539	+	-
Isobetanin	IBt	537	+	+
17-Decarboxy-neobetanin	17-dNBt	485	+	-
17-Decarboxy-betanidin	17-dBt	433	+	-
15-Decarboxy-betanidin	15-dBt	433	+	-
17-Decarboxy-betanin		507	+	-
17-Decarboxy-isobetanidin	17-IdBt	510	+	-
2,17-Bidecarboxy-betanin	2,17-dBt	507	+	-
2,17-Bidecarboxy-neobetanin	2,17-dNBt	485	+	-
6′-O-Feruloyl-betanin		532	+	-
6′-O-Feruloyl-isobetanin		532	+	-
2-Decarboxy-isobetanin	2-IdBt	535	+	-
2-Decarboxy-betanin	2-dBt	535	+	-
Betaxanthin				
Glutamine-betaxanthin	Vulgaxanthin I	475	+	+
Valine-betaxanthin		470	+	+
Leucine-betaxanthin	Vulgaxanthin IV	471	+	+
Tryptophan-betaxanthin		470	-	+
Tyramine-betaxanthin	Miraxanthin III	452	-	+
Phenylalanine-betaxanthin		472	-	+
3-Methoxytyramine-betaxanthin		456	-	+
Threonine-betaxanthin		471	-	+
Aspartic acid-betaxanthin	Miraxanthin II	470	-	+
γ-Aminobutyric-acid-betaxanthin	GABA-betaxanthin	455	+	+

[a] absorption maxima of betalains; + detected; - not detected.

The similarly identified BX components for all accessions were vulgaxanthin IV (leucine-betaxanthin), vulgaxanthin I (glutamine-BX), valine-BX, and GABA-betaxanthin (γ-aminobutyric acid-BX).

F.C. Stintzing et al. [29] also found 10 BXs in yellow-colored beets, but the fractional composition differed in four components: serine-BC, indicaxanthin, miraxanthin V, and isoleucine-BC. Differently from the mentioned authors, we identified miraxanthin III, 3-methoxytyramine-betaxanthin, threonine-betaxanthin, and miraxanthin II. It is interesting that vulgaxanthin IV (leucine-betaxanthin) dominated in the yellow accessions, while vulgaxanthin I (glutamine-betaxanthin) dominated in the maroon ones. A number of authors have observed a predominance of vulgaxanthin I in the BX profile [30,31]. However, W. Schliemann et al. [32] identified the main BX as dopamine-betaxanthin. The authors' attempts to find an enzyme that catalyzed the last crucial step in the BX biosynthesis—the formation of aldimine—proved unsuccessful. It was shown that there was a competition

among amino acids for betalamic acid, and its condensation with amino acids was a spontaneous reaction, not catalyzed by enzymes. This fact explains some differences in the component composition of BXs.

The percentage of the betalain profile components in the accessions is presented in Figures 6 and 7. It was found that the largest share among BCs in the maroon biotypes belonged to betanin (betanidin 5-O-*β*-glucoside) (59%) and its stereoisomer isobetanin (23%). Betanin is characterized by high AOA, associated with the presence of the phenolic and cyclic amino group—good donors of electrons. It should be noted that the free radical scavenging activity of betanin is 3–7.5 times higher than that of vitamin C, the standard for high AOA [15].

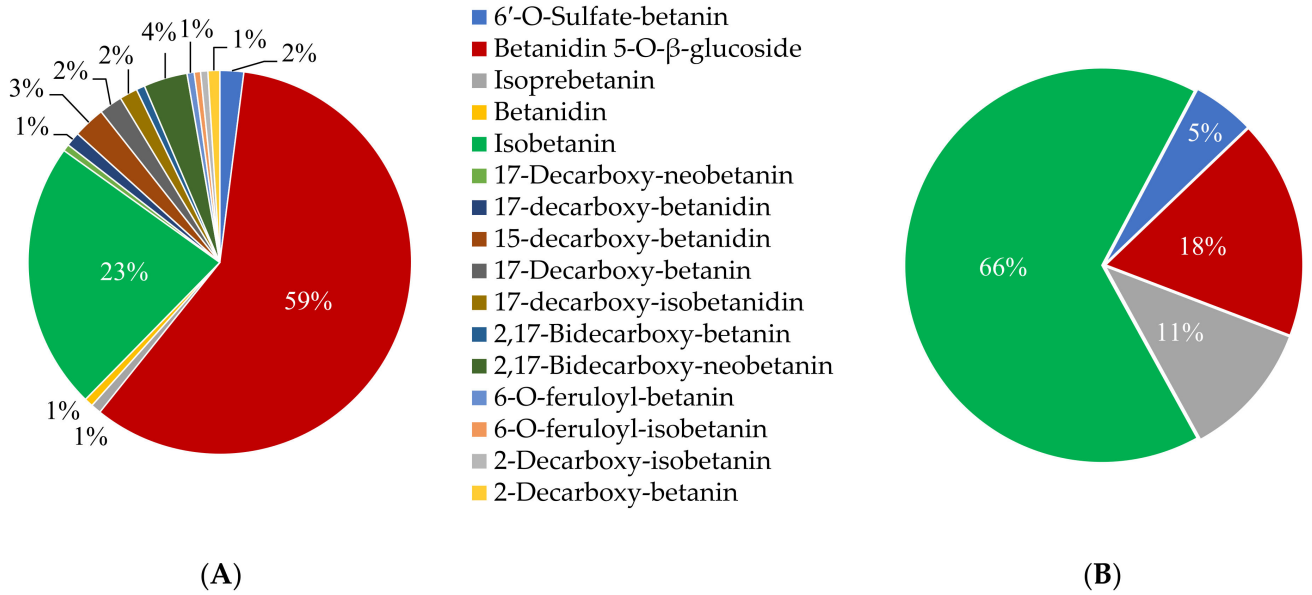


Figure 6. BC fractional composition ratios in table beets contrasting in their root color: (**A**) maroon, (**B**) yellow.

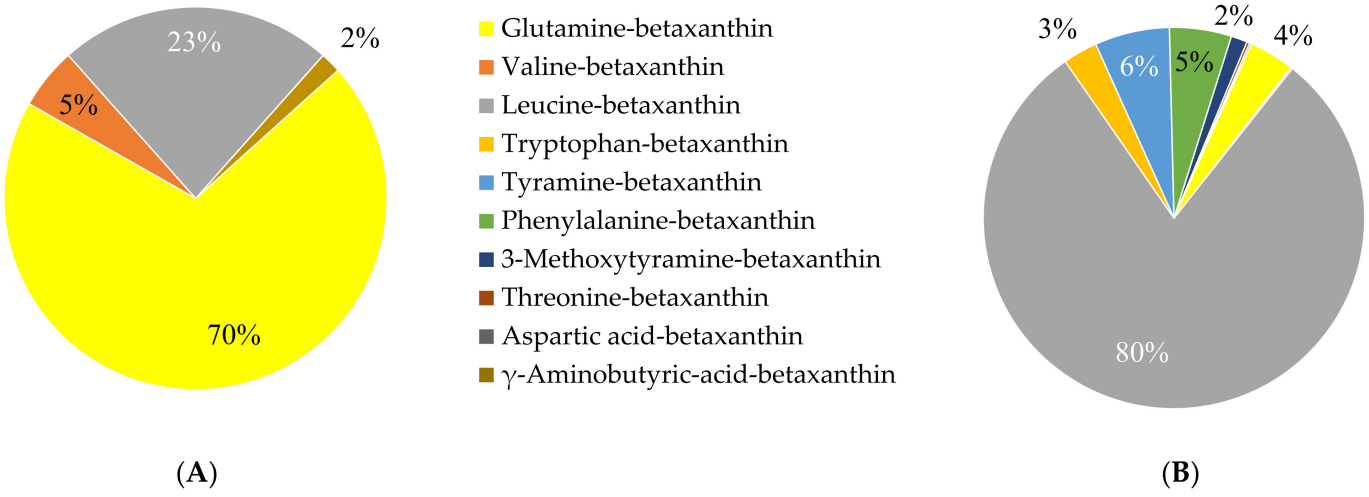


Figure 7. BX fractional composition ratios in table beets contrasting in their root color: (**A**) maroon, (**B**) yellow.

A positive correlation between AOA and BCs has been observed by a number of researchers [33–35]. Earlier studies into the effect of betanin on AOA in table beet identified higher AOA compared to vulgaxanthin of the BX group [32]. Meanwhile, a number of researchers found that betanidin (betanin aglycone which could be derived from betanin in

the human digestive tract) had more effective antioxidant properties than betanin [36,37]. However, it is present in beetroots in insignificant amounts, has a short life span, and is not always identifiable [38]. Among the accessions tested in this study, betanidin was identified only in maroon beetroots.

The dominant BCs in the yellow accessions were also betanin (18%) and isobetanin, and the latter predominated (66%) (Figure 6B).

The information about BXs within the Amaranthaceae family is limited. It was earlier noted that vulgaxanthins (I and II) predominated in maroon beetroots and yellow chard petioles [31], while miraxanthin V (dopamine-betaxanthin) was the dominant BX in the inflorescences of *Celosia argentea* var. *plumosa* [39]. In this study, the highest content and diversity of BXs was observed in the yellow-colored accessions (Figure 7B). The largest share (80%) in this group belonged to vulgaxanthin IV (leucine-betaxanthin). The component levels of 3-methoxytyramine-betaxanthin, threonine-betaxanthin, aspartic acid-betaxanthin, and γ-aminobutyric acid-betaxanthin were less than 1% (not shown in the diagram). As for the maroon biotypes, 70% of BXs was represented by vulgaxanthin I (glutamine-betaxanthin) (Figure 7A).

The highest AOA level for all accessions was recorded at the first harvesting of beetroots for analysis on 27 July 2021. By the last phase of analysis, there was a decrease in AOA (Figure 8A). A similar decrease in antioxidant activity during the growing season has been previously noted in the works of a number of authors [40,41]. The negative AOA dynamics could be associated both with a slowdown in metabolic processes of table beet, as its leaves died and roots ripened, and with unfavorable weather conditions in the first half of the growing season. It is known that one of the consequences of stress impacts on plants is an increase in the amount of reactive oxygen species (ROS) in plant cells, which leads to oxidative stress. The control over the amount of ROS is induced by the antioxidant defense system which delays or inhibits oxidation [42,43]. We assumed that the combination of a moisture deficit and high air temperature, preceding the first phase of analysis, caused oxidative stress which provoked the activation of the antioxidant defense system. Meanwhile, the dynamics of phenolics and AOA practically coincided, indirectly confirming the main function of phenolic compounds: protection of plant cells from the negative effect of environmental stressors (Figure 8A,C). The results indicate the greater usefulness of young beet roots.

The level and stability of betalain pigments in beetroots depends on many factors, such as genotype, root size, developmental phase, weather conditions, and edaphic features [44]. Betalains are very sensitive to the effect of environmental temperature. This negative impact was previously described by a number of authors [45–47]. In our earlier studies, we identified limiting abiotic factors that affect the changes in the level of betalains during the growing season [26,48]. A negative effect of an air temperature increase on betalains in the peel of maroon-colored beet genotypes was shown: it manifested itself on the second or third day. The pigment composition of the root flesh turned out to be less susceptible to the negative effects of high temperatures. The BC variations observed in this work are consistent with the previously reached conclusions (Figures 2B and 8B). The analysis of BCs in the group of maroon beetroots collected on 27 July showed low values, which may be explained by the temperature characteristics during the previous period. In the second phase of analysis (August 17), the combination of favorable weather characteristics (temperature and precipitation) during the previous days activated the process of BC biosynthesis, resulting in the abrupt growth of the BC content.

There was a significant decrease in BCs in the yellow accessions at the second and third phases of analysis (Figure 8D). It should be clarified that the main amount of betalains is concentrated in the beetroot peel [48]. Visually, the colors of the peel and flesh in the second group of cultivars differed significantly: the skin was orange, and the flesh was light yellow (Figure 1, k-3880). The ratio of BXs in the peel to those in the flesh was 7.76, in contrast to the maroon genotypes with a ratio of 1.59. At the time of the first phase of analysis, the average weight of yellow beetroots did not exceed 5.5 g. Therefore, the

peel accounted for a significant share of the total beetroot weight, which explains the high BX values. Subsequent phases of analysis were performed at the stage of active beetroot growth—up to 54.4 g (17 August) and 113 g (10 September), when the proportion of the flesh abruptly increased, leading to a decrease in the BX levels within this group.

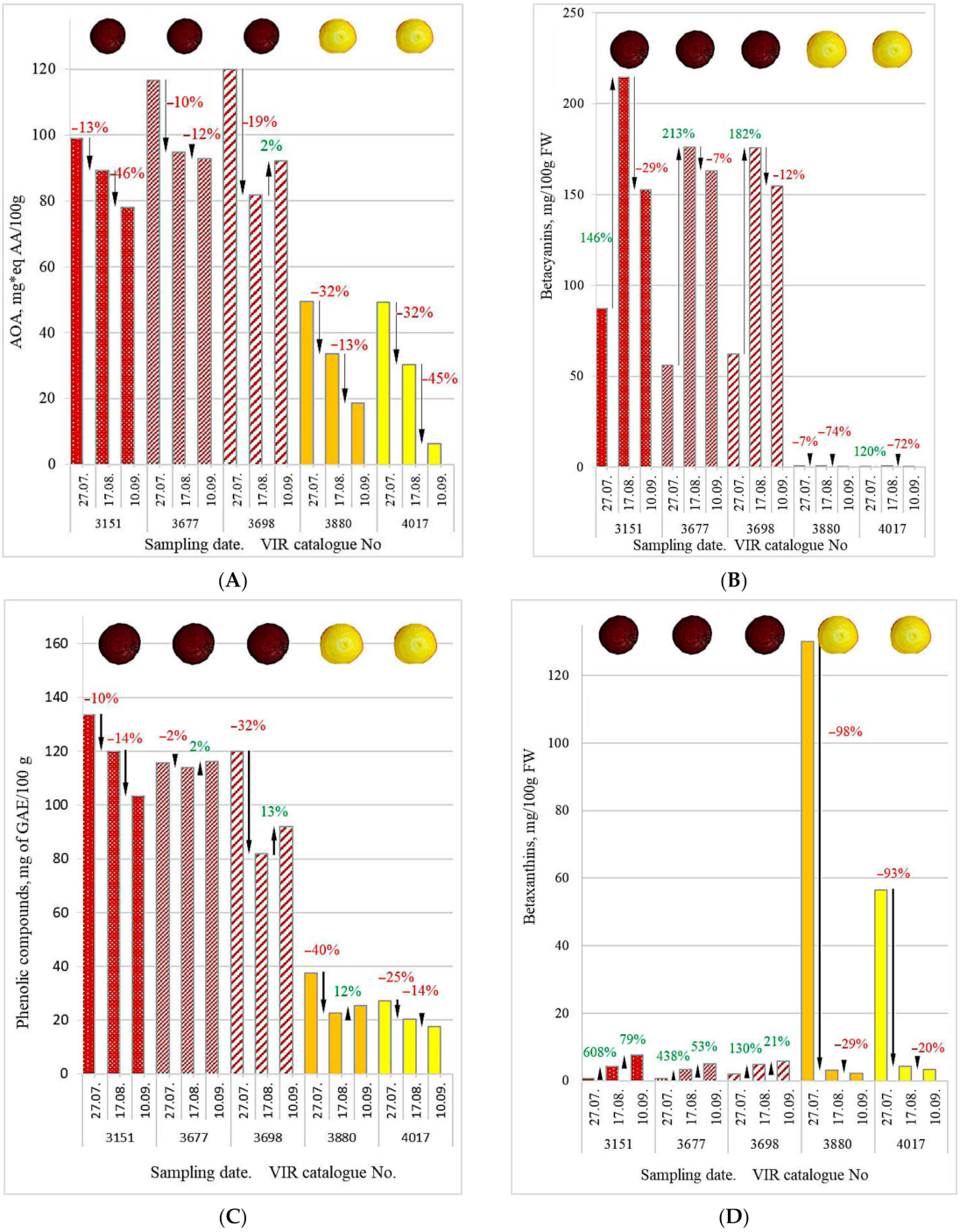


Figure 8. Dynamics of antioxidant activity (**A**) and the content of betacyanins (**B**), phenolic elements, (**C**) and betaxanthins (**D**) in beetroots.

Dynamic changes in the dominant components of the betalain fractional composition were identical to the total content of both BCs and BXs. No correlations with the pigment's dynamic variations were found for minor components (less than 5%) of the betalain biosynthesis.

4. Conclusions

Significant differences in AOA were identified in table beet accessions contrasting in their root color. The AOA in the group with maroon coloring was higher by 64–67% than in the yellow biotypes. Negative AOA dynamics were observed in all accessions, which can be presumably explained by a slowdown in active plant metabolism towards the end of the growing season and a response to stressful weather factors.

The content of betalains in the maroon group averaged 141.85 mg/100 g, which was higher by 77.2% than that in the yellow cultivars. The maroon accessions increased their betalains by the end of the growing season, while no linear dynamics was recorded, which confirmed their pronounced sensitivity to changing weather conditions. The amount of betalains in the yellow accessions decreased by 96.8% close to the end of the growing season.

Statistically significant differences were revealed between the groups in the amount of phenolic compounds, chlorophylls, and betalains. Strong positive correlations were found in the group of maroon biotypes between AOA and the total amount of phenolics (r = 0.91), AOA and BCs (r = 0.80), and BCs and phenolics (r = 0.90). In the yellow accessions, AOA was associated with chlorophyll *b* (r = 0.85), ascorbic acid (r = 0.83), and phenolic components (r = 0.83), while no relationship between BCs or BCs and AOA was found.

The fractional composition of betalains was analyzed to ascertain that the largest share among BCs in the maroon accessions belonged to betanin (59%) and isobetanin (23%), but in the yellow group isobetanin prevailed (66%, 0.6 mg/100 g FW). The highest content and diversity of BXs were observed in the yellow group, with vulgaxanthin IV (leucine-betaxanthin) dominating among them (80%). As for the maroon cultivars, their BXs were represented mainly (70%) by vulgaxanthin I (glutamine-betaxanthin).

The results of the study made it possible to identify key components associated with high AOA in the biochemical profile of table beets contrasting in their root color. Dynamic changes in AOA and the fractional composition of betalains during the growing season were shown. A conclusion was made about the higher nutritional value of maroon cultivars. It is recommended to include young beet roots in the diet at the age of 60–65 days.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14050999/s1.

Author Contributions: Conceptualization, D.V.S. and N.A.S.; methodology, A.E.S. and V.S.P.; formal analysis, A.E.S. and A.S.M.; investigation, D.V.S., V.S.P. and N.A.S.; resources and data curation, D.V.S.; writing original draft and preparation, D.V.S. and A.E.S.; writing—review and editing, D.V.S. and N.A.S.; visualization, A.S.M. and V.S.P.; supervision and project administration, E.K.K. All authors have read and agreed to the published version of the manuscript.

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