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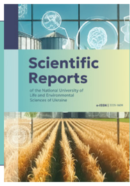

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Bioactive compounds in red-fleshed apple germplasm

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Abstract. Red-fleshed apple cultivars of the *Malus domestica* Niedzwetzkyana Group have attracted increasing attention due to their distinctive pigmentation and elevated levels of health-promoting phytochemicals. This study aimed to evaluate the biochemical composition and variability of major bioactive compounds in red-fleshed apple cultivars grown in the forest-steppe zone of Ukraine. Eleven cultivars were investigated over three growing seasons (2023-2025). The content of ascorbic acid, total polyphenols, anthocyanins, flavonols, and chalcones was determined using standard spectrophotometric and titrimetric methods. The results revealed substantial biochemical diversity among the studied genotypes. The content of ascorbic acid ranged from 5.06 to 11.41 mg/100 g fresh weight and did not differ significantly among cultivars. In contrast, pronounced differences were observed in the accumulation of phenolic compounds. The highest total polyphenol concentrations were recorded in cultivars 'Jurgen' (636.50 mg/100 g) and 'Firecracker' (621.63 mg/100 g). Anthocyanin and chalcone contents showed considerable variation, with the highest values detected in 'Dr Campbell' (31.86 mg/100 g and 22.20 mg/100 g, respectively). Statistical analysis yielded a coefficient of determination ($R^2 = 0.81$), suggesting that chalcone concentration accounts for 81% of the observed variability in anthocyanin levels. The cultivars 'Era' and 'Jurgen' demonstrated the highest flavonol accumulation (79.10 mg/100 g and 65.90 mg/100 g, respectively). Overall, 'Dr Campbell', 'Cranberry', and 'Jurgen' were identified as the most promising genotypes due to their favourable combination of

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high phenolic content and pigment accumulation. The results highlight the significant nutritional and breeding potential of red-fleshed apple cultivars and support their wider introduction into horticultural production and functional food development in Ukraine

Keywords: *Malus domestica* Niedzwetzkyana Group; ascorbic acid; polyphenols; anthocyanins; flavonols; chalcones

Introduction

Apples (*Malus domestica* (Suckow) Borkh.) are among the most widely cultivated and consumed fruit crops in the world and represent an important component of a healthy human diet. According to the Food and Agriculture Organization of the United Nations (FAO) (2025), global apple production exceeds 97 million tonnes annually, while Ukraine produces more than 1 million tonnes. Apples are the most popular fruit in Ukraine and are valued not only for their taste but also for their nutritional and biological properties.

The health-promoting value of apples is largely associated with their rich content of biologically active compounds, including polyphenols, flavonoids, and vitamin C. These substances contribute to fruit colour, flavour, and aroma while exhibiting strong antioxidant activity (Asma *et al.*, 2023). Polyphenols and related compounds have been associated with anti-inflammatory, cardioprotective, and other health-promoting effects (Catalán *et al.*, 2022). In apples, many of these compounds are concentrated in the peel; however, because consumers generally eat more flesh than peel, their intake of beneficial compounds may be limited (Juhart *et al.*, 2022).

A special group of apple cultivars accumulates anthocyanins not only in the peel but also in the fruit flesh (Bu *et al.*, 2025). These red-fleshed apples belong to *Malus domestica* f. *niedzwetzkyana* (Dieck) Mezhenskyj, commonly referred to as the Niedzwetzkyana Group (Mezhenskyj *et al.*, 2024). They attract increasing interest because of their unique pigmentation and high content of bioactive compounds. The presence of anthocyanins in the flesh significantly increases the content of flavonoids, making these cultivars of particular interest for both nutrition and breeding. Studies by J. Juhart *et al*. 2022) have shown that red-fleshed cultivars may contain higher levels of anthocyanins, flavonols, and dihydrochalcones compared with traditional white-fleshed varieties such as 'Golden Delicious'. The red coloration of apple flesh is determined by the anthocyanin biosynthetic pathway. Genomic studies by Z. Li *et al*. (2023) and S. Keller-Przybylkowicz *et al*. (2025) have identified numerous regulatory genes, particularly transcription factors of the MYB family, which, together with other structural genes, control anthocyanin accumulation in the fruit. Flavonoids formed through this pathway also play an important role in plant protection and stress resistance, while demonstrating antioxidant and antibacterial properties, as stated by Y. Chen *et al*. (2023). These bioactive compounds specifically exhibit hepatoprotective effects by reducing oxidative stress and modulating the composition of intestinal microorganisms. In particular, the study demonstrated that these extracts effectively reduce the levels of malondialdehyde in liver tissues, thereby preventing lipid peroxidation and cellular damage. Furthermore, the researchers observed that the flavonoids promote the growth of beneficial probiotic bacteria, such as *Lactobacillus*, which reinforces the host's overall immunological resilience.

In the 2020's, interest in red-fleshed apples has increased significantly due to their distinctive appearance and potential health benefits. Many cultivars are marketed internationally under collective trademarks such as Redlove®, Lucy™, Kissabel®, and Red Moon®. Despite this growing popularity, such varieties are not yet included in the State Register of Plant Varieties of Ukraine, although they are already cultivated by some farms and private growers (Pyshcholka & Mezhenskyj, 2026).

The Forest-Steppe region provides a unique environmental matrix for studying secondary metabolism in *Malus* species. The interplay between specific soil compositions and fluctuating temperature regimes during the ripening phase plays a crucial role in the phenotypic expression of genetic traits. Eleven cultivars were investigated over three growing seasons (2023-2025). This study provided a multi-year evaluation of bioactive compounds in red-fleshed apple cultivars under Ukrainian Forest-Steppe conditions. This multi-year approach was essential to mitigate the influence of seasonal climatic anomalies and to establish a reliable baseline for the nutraceutical profiling of these genotypes. To achieve this, the research focused on several interrelated tasks: first, to quantify and compare the concentrations of primary antioxidants, including ascorbic acid and various phenolic fractions, across eleven distinct cultivars. Furthermore, the study aimed to evaluate the environmental stability and interannual variability of these phytochemicals over the 2023-2025 growing seasons to identify the most resilient genotypes. Finally, a detailed analysis was conducted to establish the correlations between different flavonoid groups, specifically investigating the metabolic dependence of anthocyanin accumulation on chalcone concentrations within the fruit mesocarp.

Materials and Methods

The study used 11 cultivars: 'Cranberry', 'Dr Campbell' ('Simontornya Veralma'), 'Era' (Redlove®Era), 'Firecracker', 'Jurgen', 'Marisa' (Baya®Marisa), 'Maypole', 'Mott`s Pink', 'Red Katty', 'Sirena' (Redlove®Sirena), and 'Weirouge' (Fig. 1). Trees were grown in the collection orchard of the Educational, Research and Production Laboratory of Genetic Resources, Introduction and Breeding of Rare Fruits and Ornamental Plants at the Professor V.L. Symyrenko Department of Horticulture of the National University of Life and Environmental Sciences of Ukraine (NULES). This orchard is located at the Agronomic Research Station of the NULES in Pshenychne village, Bila Tserkva district, Kyiv region, Ukraine (50°05'24.6"N 30°12'55.1"E). It is part of the Forest-Steppe natural zone. According to the Köppen climate classification system, the region is characterised by a typical warm-summer humid continental climate (Dfb). The research was conducted on soil classified as meadow chernozem. The adult trees were cultivated at a spacing of 5 × 4 m, with care involving standard horticultural practices such as inter-row cultivation and mechanical weed control. Irrigation was not used. Notably, the study complied with the ethical principles of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1976). Fruit harvesting was performed in mid-September in 2023-2025, with the fruits being picked upon reaching the stage of technical maturity. The analysis was carried out by the Laboratory of Fruit Storage and Processing Technology at the Institute of Horticulture of the National Academy of Agricultural Sciences of Ukraine. Ascorbic acid was determined by grinding samples in a porcelain mortar with glass and transferring them to a 100 ml volumetric flask containing a 2% oxalic acid : 1% HCl mixture (80:20, v/v). The extract was filtered and titrated with 2.6-dichlorophenolindophenol using Tillman's method. Results were expressed as mg/100 g fresh mass. Total polyphenol content was determined using the Folin–Denis method. Samples were macerated with ethanol, filtered through a Büchner funnel, and washed with ethanol until colorless. Extracts reacted with Folin-Denis reagent, followed by saturated sodium carbonate. Optical density was measured at 640 nm after 1 hour. Calibration was done using chlorogenic acid standards, and results were expressed as mg/100 g fresh mass. Anthocyanin and chalcone content were determined by spectrophotometry using alcoholic extracts from 3.5% HCl-acidified homogenates, measured at 530 nm and 364 nm, respectively. Results were expressed as mg/100 g raw mass. Flavonol content was measured by absorption spectrophotometry using a flavonol complex with a 3% aluminium chloride solution. The absorption was measured at 410 nm using a ULAB 102UV spectrophotometer. Data were expressed as mg/100g raw weight. Statistical analysis involved descriptive statistics such as mean values, standard deviation (SD), and coefficient of variation (CV, %). Microsoft Excel 2019 was used for statistical analyses and chart construction. ANOVA was used to test differences among means, and Pearson correlation coefficients were calculated for normally distributed data. Fisher's least significant difference (LSD) test identified significant differences between means at a 95% confidence level ($p \leqslant 0.05$).

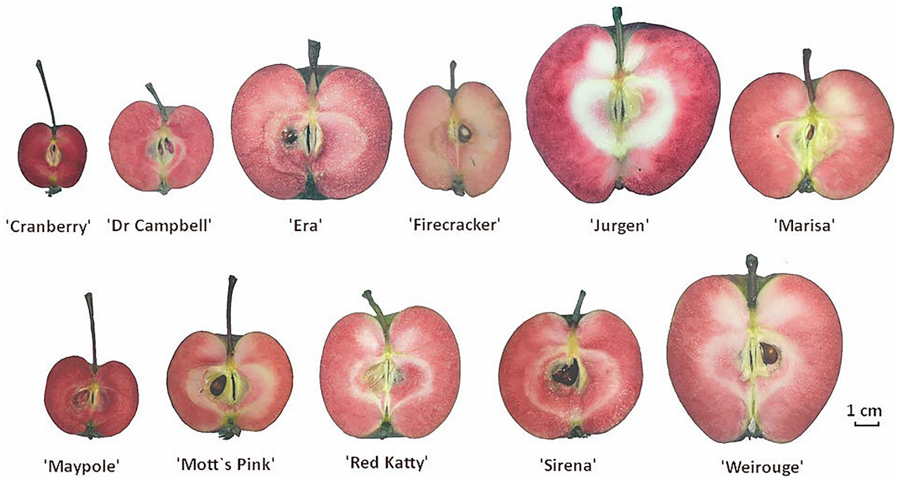

Figure 1. *Red-fleshed cultivars of Malus domestica Niedzwetzkyana Group*

Source: developed by the authors

Results and Discussion

The analysis of the biochemical composition of the cultivars is a key step in identifying their unique properties and potential. The content of the main bioactive compounds is presented in Table 1. The ascorbic acid content of red-fleshed apples varied from 5.06 mg/100 g in 'Red Katty' to 11.41 mg/100 g in 'Cranberry', with an average value of 7.12 mg/100 g. No statistically significant differences were detected among the cultivars for this trait. When comparing these red-fleshed cultivars with modern Ukrainian apple cultivars, the latter contain an average of 5.7 mg/100 g of ascorbic acid, according to L. Shevchuk *et al.* (2021). Only the cultivar 'Harant' shows a comparable level to 'Cranberry' in terms of ascorbic acid content. In general, apples are not considered a major dietary source of this vitamin. As shown by J. Juhart *et al.* (2022), the ascorbic acid content in the traditional, white-fleshed apple cultivar 'Golden Delicious' is only 0.9 mg/100 g, compared to 2.6 mg/100 g in the red-fleshed 'Marisa'. The relative stability of ascorbic acid across the 11 cultivars suggests that this vitamin serves as a basal antioxidant component, likely regulated by highly conserved metabolic pathways. However, the lack of significant differences between cultivars ($P > 0.05$) does not diminish their importance; rather, it indicates that the health benefits of red-fleshed apples are consistently supported by a reliable vitamin C base, regardless of the intensity of red pigmentation. This finding is crucial for processors who require a predictable nutritional profile for standardised juice or dried fruit production.

Table 1. *The phytochemicals of red-fleshed apple (2023-2025, $\bar{x}$ ± SD, mg/100 g)*

Cultivar	Ascorbic acid	Total polyphenols	Anthocyanins	Flavonols	Chalcones
'Cranberry'	11.41 ± 3.44	428.13 ± 141.01c	21.98 ± 8.09b	27.05 ± 12.09fg	15.47 ± 4.45bc
'Dr Campbell'	6.32 ± 1.38	582.73 ± 36.17ab	31.86 ± 5.72a	46.01 ± 14.57cde	22.20 ± 7.83a
'Era'	5.89 ± 1.34	216.73 ± 44.46d	4.70 ± 2.23c	79.10 ± 12.73a	6.60 ± 1.49d
'Firecracker'	7.78 ± 2.84	621.63 ± 50.52a	4.87 ± 4.37c	40.20 ± 3.56def	10.07 ± 4.45cd
'Jurgen'	6.05 ± 1.05	636.50 ± 96.99a	9.07 ± 5.26c	65.90 ± 6.08ab	8.30 ± 2.08d
'Marisa'	8.33 ± 1.50	212.73 ± 29.01d	9.08 ± 2.39c	14.50 ± 0.85g	6.40 ± 2.44d
'Maypole'	6.74 ± 0.40	487.43 ± 30.99bc	22.75 ± 10.82b	26.40 ± 4.53fg	16.60 ± 10.47b
'Mott's Pink'	7.12 ± 0.89	189.67 ± 29.60d	6.18 ± 2.10c	61.05 ± 14.78bc	5.33 ± 1.50d
'Red Katty'	5.06 ± 2.60	175.43 ± 33.95d	4.10 ± 1.74c	20.40 ± 2.97g	5.33 ± 2.15d
'Sirena'	8.04 ± 1.94	186.53 ± 17.84d	5.24 ± 1.97c	57.65 ± 10.25bc	5.50 ± 1.57d
'Weirouge'	5.56 ± 3.90	187.57 ± 38.58d	6.15 ± 1.13c	31.30 ± 13.29efg	5.03 ± 0.90d
Average	7.12 ± 2.51	356.83 ± 195.36	11.45 ± 9.72	42.70 ± 21.66	9.73 ± 6.78
CV, %	35.2	54.7	84.9	50.7	69.7

Note: different letters within a column indicate statistically significant differences according to the LSD test ($p \leq 0.05$)
Source: compiled by the authors

While ascorbic acid levels are relatively modest, red-fleshed apples stand out significantly in terms of their polyphenolic profile. In contrast, as noted by D. Bars-Cortina *et al.* (2017) and S. Yuste *et al.* (2022), apples are an important source of total polyphenols with strong antioxidant properties. While modern Ukrainian apple cultivars contain an average of 206 mg/100 g of total polyphenols in their fruits, according to L. Shevchuk *et al.* (2021), the red-fleshed cultivars examined in this study contain, on average 356.83 mg/100 g, which is approximately 1.5 times higher. The cultivars 'Jurgen', 'Firecracker', and 'Dr Campbell' exhibited the highest accumulation of total polyphenols, containing 636.50 mg/100 g, 621.63 mg/100 g, and 582.73 mg/100 g, respectively. These values are higher than those reported by M. Jalali *et al.* (2024) for red-fleshed apples in Iran and D. Bars-Cortina *et al.* (2017) for red-fleshed apples in Europe.

Apple peel colour is determined by pigments such as chlorophyll, carotenoids, and anthocyanins (Ban *et al.*, 2007; Liu *et al.*, 2013). Differences in pigment composition and their regulatory mechanisms explain the wide colours observed among apple cultivars, ranging from green to deep purple (Bu *et al.*, 2025). In red-skinned apples, anthocyanins accumulate in large quantities in the peel, whereas in other cultivars their content is negligible. Studies have shown that in red apples, anthocyanins are mainly localised in several epidermal and hypodermal cell layers, while the mesocarp usually remains unpigmented (van Nocker *et al.*, 2012). In contrast, red-fleshed apples are characterised by anthocyanin accumulation within the fruit flesh, which is associated with the activity of specific regulatory genes controlling anthocyanin biosynthesis (Espley *et al.*, 2007).

Researchers who have studied red-fleshed apples report considerable variation in anthocyanin content in both the peel and the flesh. According to X. Wang *et al.* (2015), anthocyanin content in the peel ranges from 22.7 mg/100 g to 120.3 mg/100 g, and in the flesh from 0.8 mg/100 g to 38.1 mg/100 g, depending on the genotype. P. Bouillon *et al.* (2024) reported values ranging from 0.2 mg/100 g to 30.9 mg/100 g of anthocyanins in the fruit flesh. Among the cultivars examined in this study, 'Dr Campbell' showed the highest anthocyanin content, averaging 31.86 mg/100 g, which corresponds closely to the upper values reported by X. Wang *et al.* (2015) and P. Bouillon *et al.* (2024). 'Dr Campbell' is followed by the cultivars 'Maypole' and 'Cranberry' with anthocyanin contents of 22.75 mg/100 g and 21.98 mg/100 g, respectively. All other studied cultivars formed a third group with significantly lower anthocyanin levels, ranging from 4.10 mg/100 g to 9.08 mg/100 g. Overall, the coefficient of variation for anthocyanin content is the highest in this study at 84.9%. This high variability likely reflects their sensitivity to environmental factors and their role as intermediate metabolites in the anthocyanin biosynthetic pathway. Specifically, the accumulation of these compounds is profoundly influenced by the interplay between solar radiation and ambient temperature during the pre-harvest period. High light intensity, particularly in the UV-B spectrum, acts as a primary inducer of the MYB10 transcription factor. According to H. Bu *et al.* (2025), this process is further mediated by a complex network of transcription factors, such as MdHY5 and MdBBX20, which enhance anthocyanin accumulation by binding to MYB promoters specifically under conditions of high UV radiation and low temperatures. Furthermore, the thermal regime plays a decisive role: while moderate day temperatures combined with cool nights significantly stimulate anthocyanin synthesis, prolonged periods of high heat (above 30°C) are known to inhibit pigment accumulation or even trigger their degradation. In the context of the Ukrainian Forest-Steppe, the interannual fluctuations in precipitation and temperature during August and September create a diverse environmental pressure that explains the instability of chalcone and anthocyanin concentrations across different seasons.

M. Jalali *et al.* (2024) reported that red-fleshed genotypes in Iran exhibit significant variability in anthocyanin content, ranging from 2.1 mg/100 g to 18.9 mg/100 g. In Spain, D. Bars-Cortina *et al.* (2017) found that four red-fleshed cultivars contained from 0.93 mg/100 g to 4.93 mg/100 g of anthocyanins. A. Ceci *et al.* (2021) reported an anthocyanin content of 7.5 mg/100 g. Given the significant differences in eco-geographical growing conditions and the heterogeneity of plant material across various studies, a direct comparison of absolute metabolite concentrations appears problematic. A more objective approach involves analysing general trends and patterns of trait variability.

As noted by R. Tsao *et al.* (2003), chalcones represent one of the common groups of flavonoids. They serve as primary precursors in the biosynthesis of anthocyanins (Lautenbach *et al.*, 2025). Apples are considered a major dietary source of these compounds (Bars-Cortina *et al.*, 2018). Chalcones generally account for approximately 2-6% of the total polyphenols (Vrhovsek e*t al.*, 2004). In the mesocarp of commercial apple cultivars with white flesh, their average content is 19 mg/100 g (Tsao *et al.*, 2003). In contrast, M. Jalali *et al.* (2026) reported lower chalcone content in the mesocarp of red-fleshed apples, averaging 5.63 mg/100 g. In the red-fleshed cultivars examined in this study, the average content was 9.73 mg/100 g, ranging from 5.03 mg/100 g to 22.20 mg/100 g in 'Dr Campbell', which also showed the highest anthocyanin concentration.

According to X. Zhang *et al.* (2020), flavonols constitute a relatively high proportion of the polyphenolic compounds in red-fleshed apples. Regarding the flavonoid profile, Y. Ren *et al.* (2026) identified eight distinct classes of flavonoids based on their chemical structures. In terms of structural diversity, the most abundant class was flavonols, accounting for 40.29% of the identified compounds, followed by flavones (9.42%), flavanones (12.23%), chalcones (9.35%), flavanols (7.19%), flavonoid carbonosides (5.76%), flavanonols (5.04%), and anthocyanidins (0.72%). In studies by X. Wang *et al.* (2015), it was shown that the highest content of flavonols (mainly quercetin derivatives) is found in the peel of red-fleshed apples, ranging from 18.2 mg/100 g to 89.8 mg/100 g, while their content in the mesocarp ranges from 5.3 mg/100 g to 14.5 mg/100 g. In the red-fleshed cultivar 'XJ4', the most abundant flavonol (quercetin) was 23.087 mg/100 g. Research by M. Jalali *et al.* (2026) reported an average flavonol content of 4.21 mg/100 g, with a maximum value of 24.55 mg/100 g. In the present study, the highest flavonol content was observed in the cultivar ‘Era’ (79.10 mg/100 g), followed by ‘Jurgen’ and ‘Mott’s Pink’, with values of 65.90 mg/100 g and 61.05 mg/100 g, respectively. Such variations in absolute flavonol concentrations compared to international studies may be attributed to the complex nature of genotype × environment interactions. This is consistent with the findings of B.T. Geleta *et al.* (2025), who demonstrated that while the genotype is the primary driver of variance for fruit weight and total phenolic content, the environment exerts a more profound influence on the total flavonoid content and vitamin C levels. In the present study, the relatively higher level of flavonols in cultivars such as ‘Era’ and ‘Jurgen’ suggests a strong genetic determination of this trait. Conversely, the high variability of anthocyanins (CV = 84.9%) compared to flavonols aligns with the observation that certain flavonoid classes are more susceptible to environmental shifts, reflecting the cumulative impact of specific hydrothermal conditions during the ripening period in the Ukrainian Forest-Steppe. Flavonols, mainly represented by quercetin glycosides, are known for their high thermal stability compared to anthocyanins. The fact that flavonol levels did not correlate with the red pigment concentration implies that these two groups of flavonoids can be enhanced independently. To better identify relationships among the studied traits, data from different years were pooled to increase the sample size for correlation analysis (Table 2).

Table 2. *Pearson correlation coefficients between traits (r)*

Trait	Ascorbic acid	Total polyphenols	Anthocyanins	Chalcones	Flavonols
Ascorbic acid	1				
Total polyphenols	0.036	1			
Anthocyanins	0.178	**0.495**	1		
Chalcones	0.160	**0.591**	**0.904**	1	
Flavonols	-0.109	0.117	-0.172	-0.128	1

Note: significant coefficients ($p \leq 0.05$) are shown in bold ($n = 33$)
Source: compiled by the authors

A strong and highly significant correlation was observed between anthocyanins and chalcones ($r = 0.904$), confirming the pivotal role of chalcones as essential biosynthetic precursors. This relationship aligns with the metabolic model proposed by X. Wang *et al.* (2024), which suggests that the efficiency of the conversion from chalcones to downstream flavonoids is a limiting factor for intense fruit pigmentation. The correlation coefficients between total polyphenols and their components (anthocyanins and chalcones) are lower, but remain statistically significant. In contrast, flavonols behaved as a relatively independent group of compounds, the accumulation of which did not show a relationship with other polyphenols. Similarly, the ascorbic acid content was not correlated with pigments or total polyphenol content, suggesting that this trait is largely genetically determined. The observed peak concentration in ‘Dr Campbell’ likely reflects a high metabolic flux through the flavonoid pathway, ensuring a consistent supply of substrates for anthocyanin synthesis even under fluctuating environmental conditions. Furthermore, the discrepancy between these results and the lower averages reported by M. Jalali *et al.* (2026) may be attributed to the specific genetic background of the investigated cultivars and their differential response to the hydrothermal regime of the Ukrainian Forest-Steppe. Such findings support the hypothesis that high chalcone accumulation is a prerequisite for achieving superior anthocyanin levels in red-fleshed genotypes, providing a biochemical marker for selecting high-pigment varieties. The comparison of coefficients of variation for the main antioxidant compounds revealed significant cultivar differences (Fig. 2).

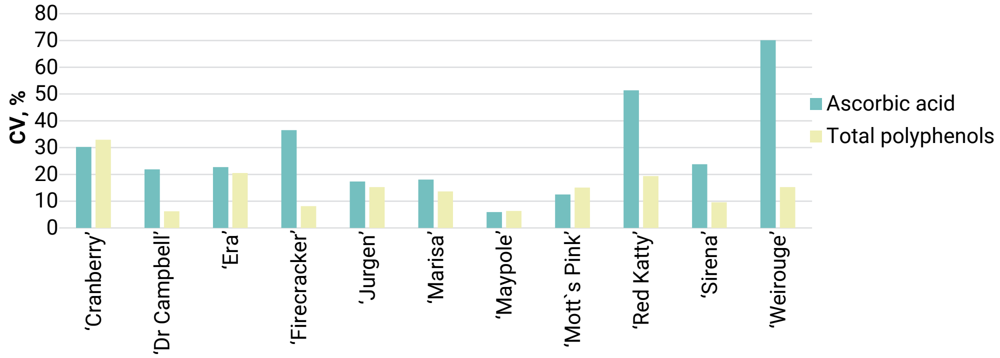

Figure 2. *Variability (CV, %) of ascorbic acid and total polyphenol content in apple fruits (2023-2025)*
Source: compiled by the authors

The 'Maypole' proved to be the most stable in terms of ascorbic acid and total polyphenol content. This indicates a low dependence of antioxidant accumulation on the weather conditions of a specific year. The cultivars 'Mott's Pink' and 'Marisa' also showed relatively low variability in these traits. 'Dr Campbell' was characterised by high antioxidant accumulation while maintaining relatively stable polyphenol levels. Total polyphenols were generally more stable than ascorbic acid, the content of which showed greater sensitivity to environmental conditions, with CV values ranging from approximately 6% in 'Maypole' to 70% in 'Weirouge'. A comparative analysis of the coefficients of variation revealed differences in the stability of secondary metabolite accumulation among the studied cultivars (Fig. 3).

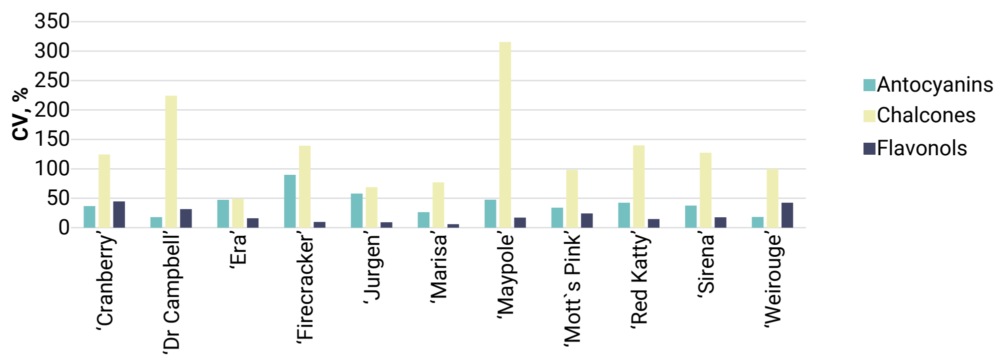

Figure 3. *Stability of anthocyanin, chalcone, and flavonol accumulation across different growing seasons*
Source: compiled by the authors

Among the components of the pigment complex, flavonols exhibited the highest stability, showing the lowest coefficients of variation, which indicates a lower dependence on annual weather conditions. In contrast, anthocyanins showed moderate variability, whereas chalcones demonstrated the highest instability, with coefficients of variation exceeding 100% in several cultivars. This high variability likely reflects their sensitivity to environmental factors and their role as intermediate metabolites in the anthocyanin biosynthetic pathway. It is hypothesised that such instability in these parameters is driven by the dynamics of temperature and humidity, as well as their interaction with other abiotic environmental factors. The higher variability of anthocyanins compared to their precursors, chalcones, may be attributed to the cumulative effect of environmental conditions on the final stages of biosynthesis throughout the fruit ripening period. Among the studied cultivars, 'Dr Campbell' combined high anthocyanin accumulation with relatively stable expression of this trait, whereas 'Era' showed a more balanced profile, with similar variability of anthocyanins, chalcones, and flavonols (about 40-50%). Overall, the results indicate that different groups of flavonoids differ in environmental stability: flavonols are the most stable, anthocyanins show moderate variability, and chalcones are the most sensitive to interannual environmental changes. Figure 4 illustrates a strong proportional relationship between chalcone and anthocyanin content, confirming chalcones as the metabolite precursors in the anthocyanin biosynthetic pathway, which supports the findings of J. Lautenbach *et al.* (2025).

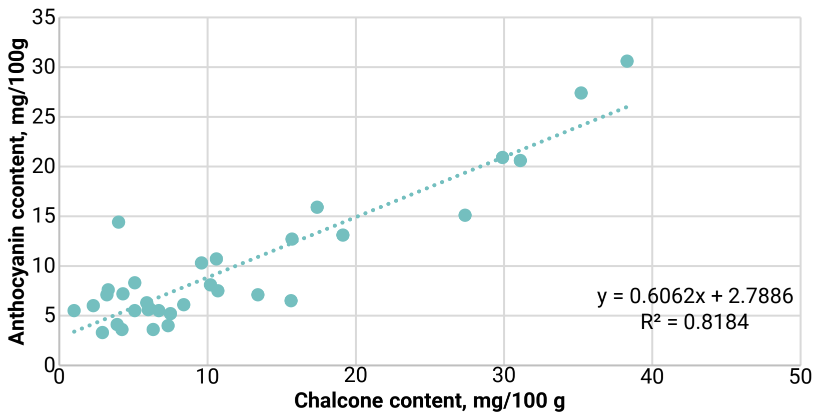

Figure 4. *Correlation between chalcone and anthocyanin content in red-flesh apples (2023-2025)*
Note: the dashed line represents the linear regression equation (R^2 = 0.81)
Source: compiled by the authors

These two groups of pigments function as a single mechanism. The calculated coefficient of determination (R^2 = 0.81) indicates that approximately 81% of the variation in anthocyanin content can be explained by the differences in chalcone concentration. The dashed line in Figure 4 represents the linear regression model, confirming that the anthocyanin accumulation is closely associated with chalcone concentration in the fruit mesocarp.

Thus, flavonols demonstrated the highest stability among the components of the pigment complex, showing minimal variability, which indicates that they are less affected by weather conditions. Anthocyanins showed moderate variability, whilst chalcones proved to be the most unstable, likely due to their sensitivity to environmental factors and their role in the anthocyanin biosynthesis pathway. The high variability of chalcones and anthocyanins is influenced by temperature, humidity and other abiotic factors, with this effect being most pronounced during fruit ripening. The 'Dr Campbell' variety showed high anthocyanin accumulation with stable expression, whilst the 'Era' variety demonstrated more balanced variability among different types of flavonoids. The strong correlation between the content of chalcones and anthocyanins supports the hypothesis that chalcones are precursors in the anthocyanin biosynthesis pathway.

Conclusions

The primary objective of this study was to evaluate the biochemical diversity and nutritional potential of red-fleshed apple cultivars of the Niedzwetzkyana Group under the environmental conditions of the Ukrainian Forest-Steppe. Through a comprehensive analysis of secondary metabolites, the research aimed to identify genotypes with superior antioxidant properties and establish the metabolic relationships between key flavonoid classes. The results obtained fully confirm the achievement of this goal, providing a detailed characterisation of the phytochemical profiles of eleven specialised cultivars. The study involved a systematic quantification of ascorbic acid, total polyphenols, anthocyanins, chalcones, and flavonols over multiple growing seasons. It was found that red-fleshed apples significantly outperform traditional white-fleshed cultivars in terms of total phenolic content, with an average concentration of 356.83 mg/100 g.

The analysis revealed that 'Jurgen', 'Firecracker', and 'Dr Campbell' are the most promising genotypes for functional food production, exhibiting peak polyphenol levels exceeding 580 mg/100 g. A critical finding of the research was the extreme variability of anthocyanin accumulation (CV = 84.9%), which was shown to be highly sensitive to interannual hydrothermal fluctuations. In contrast, flavonol concentrations remained relatively stable, with 'Era' and 'Jurgen' consistently maintaining high levels of quercetin glycosides. Correlation analysis established a robust positive link between chalcones and anthocyanins (r = 0.904), while the coefficient of determination ($R^2 = 0.81$) confirmed that chalcone availability is the decisive factor for intense flesh pigmentation. Conceptualising these results, the data suggest that the biosynthetic pathway in red-fleshed apples is driven by a high metabolic flux from chalcone precursors to downstream anthocyanins, which is both genetically determined and environmentally regulated. This implies that while the maximum pigment potential is fixed by the genotype, the actual expression of the "red-flesh" trait is a result of complex genotype-by-environment interactions. Such findings deepen the understanding of flavonoid metabolism in niche fruit crops and provide a clear biochemical rationale for the selection of parent material in breeding programmes. The identified independence of flavonol and ascorbic acid accumulation further indicates that these traits can be improved simultaneously without pleiotropic constraints.

Future research should focus on evaluating the stability of these bioactive compounds during long-term storage and industrial processing to ensure the delivery of high-quality functional products to consumers.

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Conflict of Interest

None.

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Біоактивні сполуки в генетичному матеріалі яблук з червоним м'якушем

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Анотація. Сорти *Malus domestica* Niedzwetzkyana Group з червоним м'якушем привертають все більше уваги завдяки своєму характерному забарвленню та підвищеному вмісту фітохімічних речовин, корисних для здоров'я. Метою цього дослідження було оцінити біохімічний склад і мінливість основних біоактивних сполуку червоном'якушних сортів яблук, вирощених у лісостеповій зоні України. Протягом трьох вегетаційних періодів (2023-2025 рр.) було досліджено 11 сортів. Уміст аскорбінової кислоти, загальних поліфенолів, антоціанів, флавонолів та халконів визначали за допомогою стандартних спектрофотометричних і титрометричних методів. Результати виявили значне біохімічну різноманітніття серед вивчених генотипів. Уміст аскорбінової кислоти коливався від 5,06 до 11.41 мг/100 г свіжої маси і не відрізнявся істотно між сортами. Натомість спостерігалися суттєві відмінності в накопиченні фенольних сполук. Найвищі концентрації загальних поліфенолів зафіксовано в сортах 'Jurgen' (636,50 мг/100 г) та 'Firecracker' (621,63 мг/100 г), що с відчить про їхній сильний антиоксидатний потенціал. Уміст антоціанів і халконів демонстрував значну варіабельність, причому найвищі значення виявлено в сорту 'Dr Campbell' (відповідно 31,86 мг/100 г і 22,20 мг/100 г). Статистичний аналіз показав коефіцієнт детермінації ($R^2 = 0,81$), який свідчить про те, що концентрація халконів пояснює 81 % спостережуваної мінливості рівнів антоціанів. Сорти 'Era' і 'Jurgen' продемонстрували найвище накопичення флавонолів (відповідно 79,10 мг/100 г і 65,90 мг/100 г). Загалом, сорти 'Dr Campbell', 'Cranberry' та 'Jurgen' були визначені як найперспективніші генотипи завдяки поєднанню високого вмісту фенолів і накопиченню пігментів. Отримані результати підкреслюють значний харчовий та селекційний потенціал червоном'якушних сортів яблук та підтверджують доцільність їх ширшого впровадження у садівництво та розробку функціональних продуктів харчування в Україні

Ключові слова: *Malus domestica* Niedzwetzkyana Group; аскорбінова кислота; поліфеноли; антоціани; флавоноли; халкони