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Seasonal Variation of Qualitative and Quantitative Composition of Phenolic Compounds and Antioxidant Activity in Apple (*Malus domestica* Borkh.) Fruits

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VJ and RB designed the experiments. Authors ML, JV and DK performed the experiments. Authors PV and KG analyzed and discussed the data. Author ML wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to explore the peculiarities of the qualitative and quantitative composition of phenolic compounds and antioxidant activity variation in apple (*Malus domestica* Borkh.) fruit samples during their growth season. The highest total concentration of phenolic compounds (20.97±0.74 mg g^{-1}) was detected during the initial period of the phenological fruit growth stage (31 days after full bloom - DAFB), and the lowest (1.61±0.05 mg g^{-1}) – during the fruit maturation period (143 DAFB). Chlorogenic acid was the predominant compound in apple samples throughout the

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vegetation period – it comprised 24–32.12% of all identified and quantitatively evaluated phenolic compounds in apples. The highest concentration of chlorogenic acid (20.97±0.74 mg g^{-1}) was detected at the beginning of the fruit development period.
In total, 6 quercetin glycosides were detected in apple samples: hyperoside, isoquercitrin, rutin, reynoutrin, avicularin and quercitrin. The highest total concentration of quercetin glycosides (2.75±0.08 mg g^{-1}) was detected at the beginning of the fruit development period (31 DAFB), and the lowest (0.23±0.01 mg g^{-1}) – during the fruit maturation period.
The antioxidant activity in apple sample extracts was analysed by applying 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) spectrophotometric assays. The strongest antiradical and reducing activity (TE_{DPPH} reached 81.02 μmol g^{-1}, and TE_{FRAP} - 715.63 μmol g^{-1}) was detected at the beginning of the fruit development period. The statistical correlation analysis showed a very strong positive correlation between the total amount of the identified phenolic compounds and the reducing activity of the apple extracts (Spearman's correlation coefficient – R=0.927, $p<0.01$). There was also a strong positive correlation between the total amount of phenolic compounds and the antiradical activity of the apple extracts (R=0.770, $p<0.01$). Among individual compounds that have been identified and quantitatively evaluated via high-performance liquid chromatography (HPLC), the strongest correlation with antiradical and reducing activity was observed for quercetin glycosides – isoquercitrin (respectively, R=0.851 and 0.845, $p<0.01$) and hyperoside (R=0.770 and 0.891 $p<0.01$).

Keywords: Apples; phenolic compounds; phloridzin; antioxidant activity; HPLC.

1. INTRODUCTION

Over the recent years, the society has been showing a growing interest in healthy lifestyle, ecological food products, botanical medicines and dietary supplements [1]. In addition to the main function – the supply of the necessary nutrients and energy for the human body – food products may also strengthen the body, have a prophylactic effect, and reduce the risk of diseases [2]. The growing interest in botanical raw materials broadens the possibilities of their use in medicine, cosmetics and food industry. It also stimulates the application of modern methods for the evaluation and description of the resources and chemical composition of botanical raw material, its analysis techniques, and its effect on the human body, as well as the compilation and presentation of recommendations for use.

Apples play an important role in human nutrition – they are among the most widely used fruit in the world [3,4]. Apples are widely used in food industry to produce various food products and drinks (juice, wine, cider), and are also used unprocessed [5]. Apples are an important source of various biologically active substances for the human body [6]. They contain abundant phenolic compounds, organic acids, vitamins (mostly ascorbic acid), macro- and microelements, and dietary fibre [3,7]. These substances are important for the physiological processes in the body, and their deficiency results in the body's susceptibility to disease, which, in turn, leads to greater morbidity.

Phenolic compounds are an important group of botanical biologically active compounds that have an organism-strengthening and disease-preventing effect [8]. The significant correlation between the consumption of food products rich in polyphenolic compounds and a reduced risk of cardiovascular diseases, degenerative diseases and cancer has been proven in a number of epidemiological studies [9,10,11]. Phenolic compounds have a strong antioxidant, antimicrobial, anti-inflammatory, antiviral, antiallergic, and vasodilating effect [12-14]. They suppress lipid peroxidation and platelet aggregation, and reduce permeability and fragility of capillaries [14,15].

The evaluation and comparison of the accumulation of biologically active substances in various organs of a plant during the vegetation period is relevant, as such studies promote better understanding of the regularities in the accumulation of biologically active compounds. These studies are also important in searching for botanical raw materials accumulating high amounts of phenolic compounds, and ensuring the quality of such materials and the products manufactured from them.

We conducted a study on the composition and antioxidant activity of phenolic compounds in apple cultivars grown in Lithuania [16,17]. It is

relevant to analyse the variation of the phytochemical composition of phenolic compounds in apples during their development and maturation periods. The results of this study provide new knowledge about the variations in the quantitative composition and antioxidant activity of individual phenolic compounds in the fruit of apple cultivars grown in Lithuanian climatic conditions during their different phenological growth stages. This will provide an opportunity for supplying the consumers with high-quality apples and their products with pre-determined phenolic compound composition, and also will allow for planning the isolation of individual phenolic compounds accumulated in these apples.

2. MATERIALS AND METHODS

2.1 Plant Material

Studied fruit samples of the 'Ligol' apple cultivar. The 'Ligol' cultivar (a winter cultivar bred in Poland) is one of the main cultivars in commercial apple orchards in Lithuania. The apple trees were grown in the experimental orchard (block 2, row 4, trees 21-40) of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania (55°60′ N, 23°48′ E). The altitude of Babtai town is 57 m above the sea level. The trees were trained as slender spindle. Pest and disease management were carried out according to the rules of the integrated plant protection. The experimental orchard was not irrigated. Tree fertilization was performed depending on the results of the soil and leaf analysis. Nitrogen was applied before flowering at the rate of 80 kg ha^{-1}, and potassium was applied after harvest at the rate of 90 kg ha^{-1}. Soil conditions of the experimental orchard were the following: clay loam, pH – 7.3, humus – 2.8%, P_2O_5 – 255 mg kg^{-1}, and K_2O – 230 mg kg^{-1}.

The temperature during the vegetation was close to the annual averages with the exception of a hot period at the beginning of August. The amount of precipitations was not evenly distributed, with some dry periods in June, August, and October. Overall, there were no extremes that could have affected the normal development of apple trees. Daily minimum temperatures indicate autumn frosts, which were recorded 6 nights at the end of September – the beginning of October. Such long cold periods might interrupt leaf activity.

Apples were collected at monthly intervals right after the June drop and at weekly intervals during the final fruit ripening period beginning on September 9 (Table 1). The sample consisted of 20 fruits taken from the mid part of the outer canopy.

Apples were cut into slices of equal size (up to 1 cm in thickness), and the stalks and the seeds were removed. The apple slices were immediately frozen in a freezer (at –35°C) with air circulation. The apple samples were lyophilized with a ZIRBUS sublimator 3×4×5/20 (ZIRBUS technology, Bad Grund, Germany) at the pressure of 0.01 mbar (condenser temperature, −85°C). The lyophilized apple slices were ground to fine powder (about 100 μm) by using a knife mill Grindomix GM 200 (Retsch, Haan, Germany).

Loss on drying before the analysis was determined by drying the apple lyophilisate in a laboratory drying oven to complete the evaporation of water and volatile compounds (temperature: 105°C; the difference in weight between measurements: up to 0.01 g) and by calculating the difference in raw material weight before and after drying [19]. The data were recalculated for absolute dry lyophilisate weight.

Table 1. Sampling dates and phenological growth stages (Based on Meier et al. 2001 [18])

Date	Day of the year	DAFB	BBCH stages	Phenological growth stage
June 17	168	31	74	
July 15	196	59	75	
Aug. 12	224	87	76	Fruit development
Sept. 9	252	115	77	
Sept. 16	259	122	79	
Sept. 23	266	129	81	
Sept. 30	273	136	85	
Oct. 7	280	143	87	Fruit maturation
Oct. 14	287	150	87	
Nov. 04	308	171	89	

2.2 Chemicals

All solvents, reagents, and standards used were of analytical grade. Acetonitrile and acetic acid were obtained from Sigma-Aldrich GmbH (Buchs, Switzerland), and ethanol - from Stumbras AB (Kaunas, Lithuania). Hyperoside, rutin, quercitrin, phloridzin, procyanidin B1, procyanidin B2, and chlorogenic acid standards were purchased from Extrasynthese (Genay, France), reynoutrin, (+)-catechin and (−)-epicatechin - from Sigma-Aldrich GmbH (Buchs, Switzerland), and avicularin, procyanidin C1, and isoquercitrin - from Chromadex (Santa Ana, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical, sodium acetate trihydrate, iron (III) chloride hexahydrate, and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich (Steinheim, Germany). In the study, we used deionized water produced by using the Crystal E HPLC (Adrona SIA, Riga, Latvia) water purification system.

2.3 Extraction

The extraction of apple samples was carried out under conditions analogous to those described by Liaudanskas et al. [20].

2.4 High Performance Liquid Chromatography

The phenolic compounds of the apple samples were separated, identified, and quantified by applying HPLC - similarly to the technique described in previous studies [17,20].

2.5 Determination of Antioxidant Activity

2.5.1 DPPH• free radical scavenging assay

The DPPH• free radical scavenging activity was determined by applying the method proposed by Brand-Williams et al. [21]. The DPPH• solution in 96.3% v/v ethanol (3 mL, 6×10^{-5} M) was mixed with 10 μL of the apple ethanol extract. A decrease in absorbance was determined at a wavelength of 515 nm after keeping the samples for 30 minutes in the dark.

2.5.2 FRAP assay

The ferric reducing antioxidant power (FRAP) assay was performed as described by Benzie and Strain [22]. The working FRAP solution included TPTZ (0.01 M dissolved in 0.04 M HCl), $FeCl_3\bullet6H_2O$ (0.02 M in water), and acetate buffer (0.3 M, pH 3.6) at the ratio of 1:1:10. A volume of 3 mL of a freshly prepared FRAP reagent was mixed with 10 μL of the apple leaf extract. An increase in absorbance was recorded after 30 minutes at a wavelength of 593 nm.

2.5.3 Calculation of antioxidant activity of the apple ethanol extracts

The antioxidant activity of the extracts was calculated from the Trolox calibration curves and was expressed as μmoL Trolox equivalent (TE) per one gram of absolutely dry weight (DW). The TE was calculated according to the following formula: TE = c×V/m (μmol g^{-1}); where c – the concentration of Trolox established from the calibration curve (in μM); V – the volume of apple fruit or leaf extract (in L); and m – the weight (precise) of lyophilized leaf powder (in g).

2.6 Methods of Statistical Data Processing

All data in the text and the figures are presented as the mean ± standard deviation calculated from three consecutive tests. The correlation was evaluated by Spearman's analysis. Differences at $p < 0.05$ were considered to be statistically significant. Calculations were made using computer software Microsoft Office Excel 2003 and SPSS 20.0 (Chicago, USA).

3. RESULTS AND DISCUSSION

Changes in the qualitative and quantitative composition of secondary metabolites in apples during their development and maturation periods determine the organoleptic properties of the apples [23] and influence their biological effect. Compared to the main apple growing regions, Lithuanian climatic conditions are characterized by cooler summers, greater variations between daytime and night-time temperatures, and earlier fall frosts. This results in a shorter vegetation period and an earlier and more intensive coloration of the apples [24,25]. It is thus relevant to study the variation in the quantitative composition of phenolic compounds in the apples grown in Lithuania during the vegetation period of the apple trees, to compare the obtained results with those published by other researchers, and to identify the time periods when the apples contain the highest amounts of flavonoids and phenolic acids.

Apple is the main fruit crop in commercial orchards in Lithuania. Apple plantations occupy

more than 2000 ha, or more than 90% of all commercially grown fruit trees. Annual apple yields vary from 80 000 to 120 000 t. Most apples are grown for fresh consumption, but a significant part (around 25-30%) is suited for processing. Due to the cultivar selection, Lithuanian apple juice concentrate is distinguishable for its high acidity. Investigating the mode of the accumulation of bioactive substances could help enrich other processed apple products as well.

The cultivar (Cv.) 'Ligol' is one of main apple varieties grown in Lithuania and occupies more than 20% of new apple plantations. Cv. 'Ligol' is the most productive variety in Lithuania producing up to 80 t ha^{-1} yields. 'Ligol' fruits are very large, tasty, and have a good appearance, but they require special care in older orchards. Mature trees, if not well pruned and trained, produce a high proportion of discoloured fruits with somewhat worse flavour. At the same time, 'Ligol' trees, if not properly thinned, are over-cropping and produce much smaller fruits. All these second-class fruits could be a good source for processing purposes.

We studied changes in the dynamics of the quantitative composition of phenolic compounds in the samples of the 'Ligol' apple cultivar during the development and the maturation periods of the fruit. Phenolic compounds of different groups were identified in the ethanol extracts of the studied apple samples – quercetin glycosides (rutin, hyperoside, isoquercitrin, reynoutrin, avicularin, quercitrin), dihydrochalcones (phloridzin), monomeric and oligomeric flavan-3-ols ((+)-catechin, (−)-epicatechin, procyanidin B1, procyanidin B2 and procyanidin C1), and phenolic acids (chlorogenic acid). The chromatogram of the ethanol extract of the apple samples is presented in Fig. 1.

Flavonols (quercetin glycosides) were the most abundant group of phenolic compounds identified in apple samples. Quercetin glycosides together with compounds of the flavan-3-ol group are most responsible for the antioxidant effect of the complex of phenolic compounds in apples [26]. Flavonols possess anticancer properties due to antioxidant mechanisms and by inducing caspase-3 activity [27]. Quercetin and its derivatives affect cytochrome P450 1A1 enzymes participating in carcinogenesis [28,27].

The total amount of quercetin glycosides comprised 11.8−16.43% of all identified and quantitatively evaluated phenolic compounds. The highest concentrations of hyperoside (0.762±0.025 mg g^{-1}), quercitrin (1.014±0.043 mg g^{-1}) and avicularin (0.739±0.030 mg g^{-1}) were detected in apple samples collected at the beginning of the development period (31 DAFB). Subsequently, during the development and maturation of the apples, the amounts of these compounds were decreasing. The lowest amounts of quercitrin and avicularin were detected in apple samples collected during the maturation period of the apples (143 DAFB) (Fig. 2). At the end of the vegetation period, when the apples reached the consumption-level of maturation (171 DAFB), the concentration of hyperoside was 0.177±0.006 mg g^{-1}, the concentration of quercitrin – 0.139±0.006 mg g^{-1}, and the concentration of avicularin – 0.091 ± 0.004 mg g^{-1} (Fig. 2).

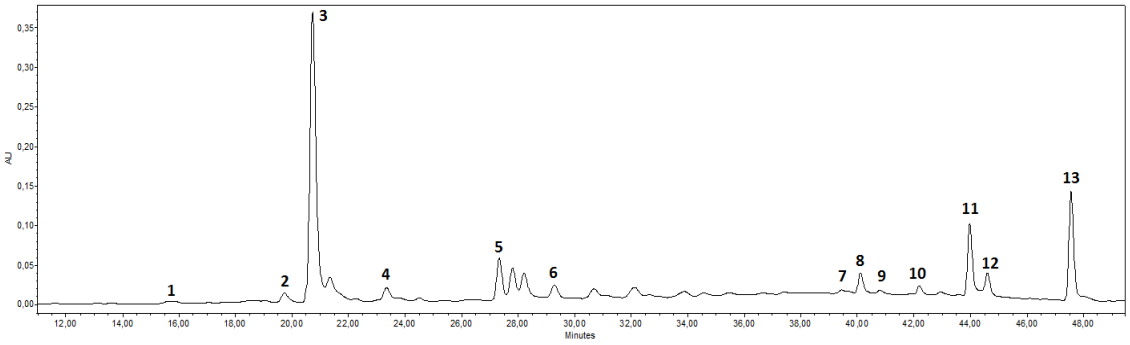

Fig. 1. Chromatogram of the ethanol extract of the 'Ligol' apple sample (λ = 280 nm). Numbers indicate the peaks of analytes: 1: Procyanidin B1, 2: (+)-catechin, 3: Chlorogenic acid, 4: Caffeic acid, 5: (–)-epicatechin, 6: Procyanidin C1, 7: rutin, 8: Hyperoside, 9: Isoquercitrin, 10: Reynoutrin, 11: Avicularin, 12: Quercitrin and 13: Phloridzin

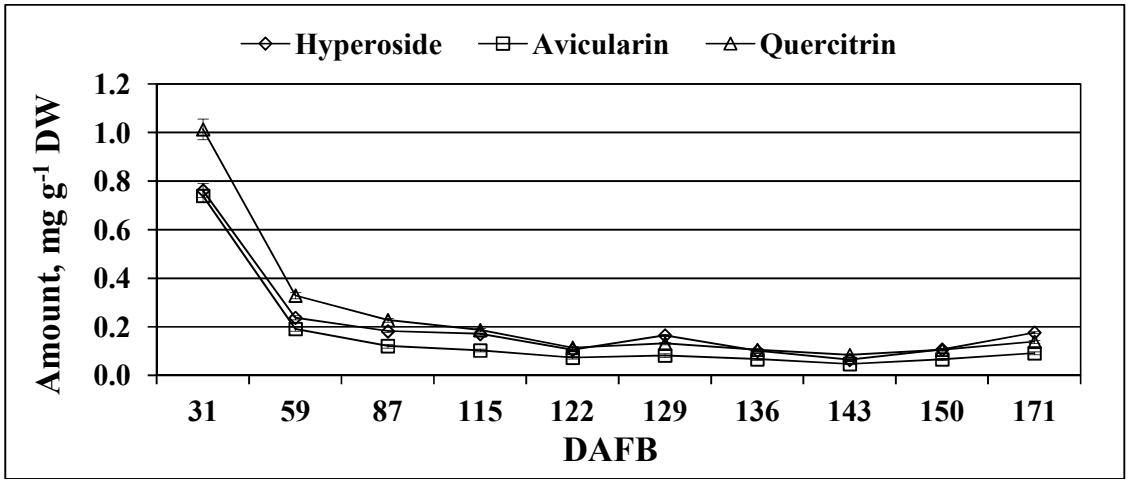

Fig. 2. Variation in the quantitative composition of quercitrin, hyperoside and avicularin in apple samples throughout the vegetation period

The concentrations of isoquercitrin, rutin and reynoutrin detected in apple samples were lower than those of other quercetin glycosides. The variation in the quantitative composition of these compounds throughout the vegetation period is presented in Fig. 3. The highest concentrations of rutin (0.041±0.002 mg g^{-1}), isoquercitrin (0.104±0.004 mg g^{-1}) and reynoutrin (0.092±0.005 mg g^{-1}) were detected at the beginning of the phenological stage of the development of the apples (31 DAFB). The lowest concentrations of these compounds were detected during the phenological stage of the apple maturation (143 DAFB). When apples reached the consumption-level of maturation (on the 308th day of the year), the detected concentration of rutin was 0.029±0.001 mg g^{-1}, the concentration of isoquercitrin – 0.033±0.001 mg g^{-1}, and the concentration of reynoutrin – 0.019 mg g^{-1} (Fig. 3). The concentration of rutin was the lowest of all identified compounds of the quercetin glycoside group throughout the vegetation period. The results of our study on the variation in the quantitative composition of quercetin glycosides were supported by those obtained by other researchers: samples of mature apples contained lower concentrations of quercetin glycosides, compared to samples obtained at the beginning of the development period [29,30].

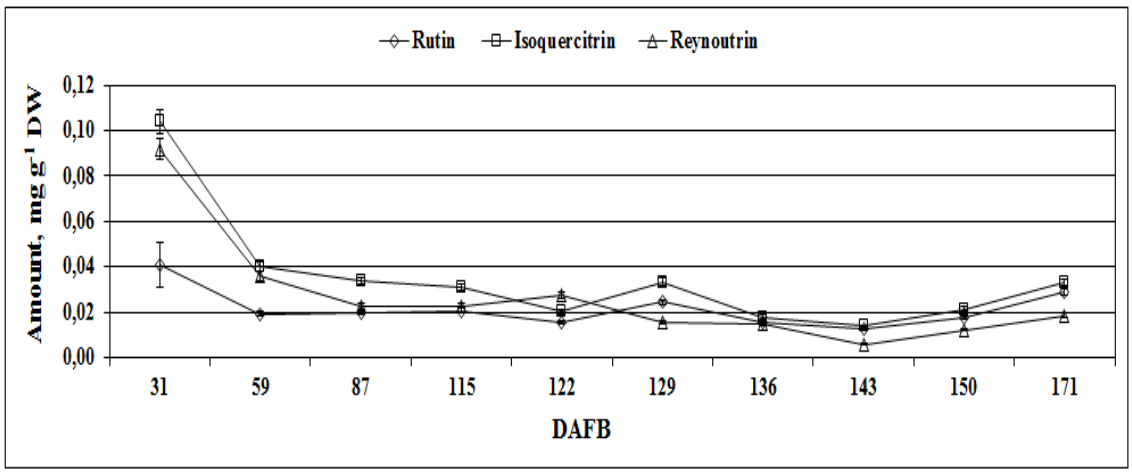

Fig. 3. Variation in the quantitative composition of rutin, isoquercitrin and reynoutrin in apple samples throughout the vegetation period

During the study, we identified and quantitatively evaluated monomeric flavan-3-ols – (+)-catechin and (–)-epicatechin. They comprised 9.5–16.7% of all the identified phenolic compounds. The highest concentrations of (+)-catechin and (–)-epicatechin were detected at the beginning of the phenological stage of fruit development (31 DAFB) – 0.690±0.027 mg g^{-1} and 1.875±0.078 mg g^{-1} respectively (Fig. 4). During the fruit development, the concentrations of (+)-catechin and (–)-epicatechin were decreasing. The concentrations of (+)-catechin detected at the beginning of the phenological stage of apple maturation (129 DAFB) were 19 times lower, and the concentration of (–)-epicatechin – 8.3 times lower than those detected at beginning of the fruit development period (on the 168th day of the year). Changes in the concentrations of these compounds during apple maturation (129–171 DAFB) were only slight. The lowest concentrations of (+)-catechin and (−)-epicatechin were detected in apple samples collected on 143 DAFB (Fig. 4). Similar regularities were observed by Awad et al. [29] who analysed the changes in the quantitative composition of catechins in apple peel samples.

We also identified and quantitatively evaluated 3 oligomeric flavan-3-ols (procyanidins) in the extracts of the studied apple samples – procyanidin B1, procyanidin B2, and procyanidin C1. Various studies confirm that flavan-3-ols, especially procyanidins, have strong antioxidant properties [31,4,32] and can act as radical scavengers [33], may inhibit certain oxidases (lipoxygenase cyclooxygenase, myeloperoxidase, NADPH oxidase, and xanthine oxidase), or may promote antioxidant enzymes (superoxide dismutase and catalase) [34]. Procyanidins isolated from apples have an antineoplastic effect [35], a strong antioxidant effect [26], an anti-inflammatory effect [36], a cholesterol- and triglyceride-lowering effect [37], and a positive effect on the cardiovascular system [38,39], and therefore they are among the most promising phenolic compounds in apples. Toda et al. [40] suggested that apple products containing significant amounts of procyanidins have a neuroprotective effect by suppressing amyloid-β-protein aggregation. The oligomeric procyanidins identified in apple samples comprised 10.2–28.6% of all the identified and quantitatively evaluated compounds. The analysis of the quantitative composition of apple samples showed that throughout the vegetation period, the concentration of procyanidin B1 ranged from 0.015 mg g^{-1} to 0.239 mg g^{-1}, the concentration of procyanidin B2 – from 0.335 mg g^{-1} to 1.853 mg g^{-1}, and the concentration of procyanidin C1 – from 0.007 mg g^{-1} to 0.045 mg g^{-1} (Fig. 5). The highest concentrations of these compounds were detected in apple samples collected at the beginning of the phenological stage of fruit development (31 DAFB), while the lowest concentrations of procyanidin B1 and procyanidin B2 were detected in apple samples c ollected during the fruit maturation period (143 DAFB). At the end of the vegetation period, when apples reached the consumption-level maturation, the detected concentration of procyanidin B1 was 0.118±0.005 mg g^{-1}, the concentration of procyanidin B2 – 0.645±0.029 mg g^{-1}, and the concentration of procyanidin C1 – 0.011 mg g^{-1}.

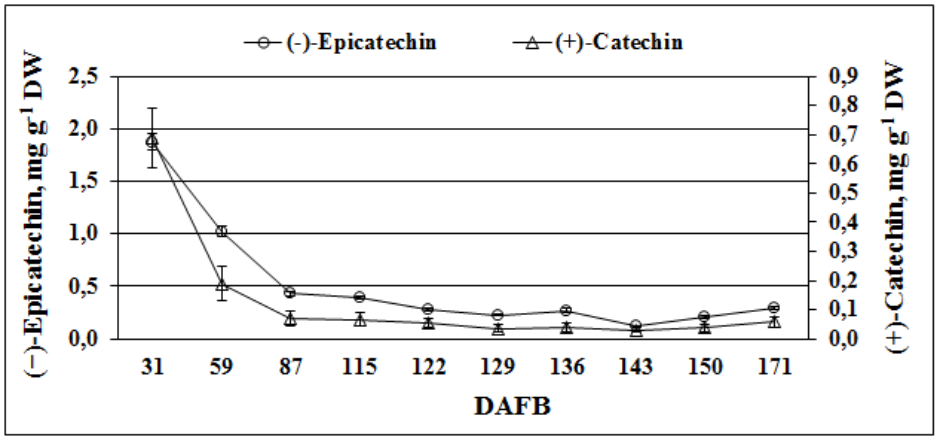

Fig. 4. Variation in the quantitative composition of (+)-catechin and (–)-epicatechin in apple samples throughout the vegetation period

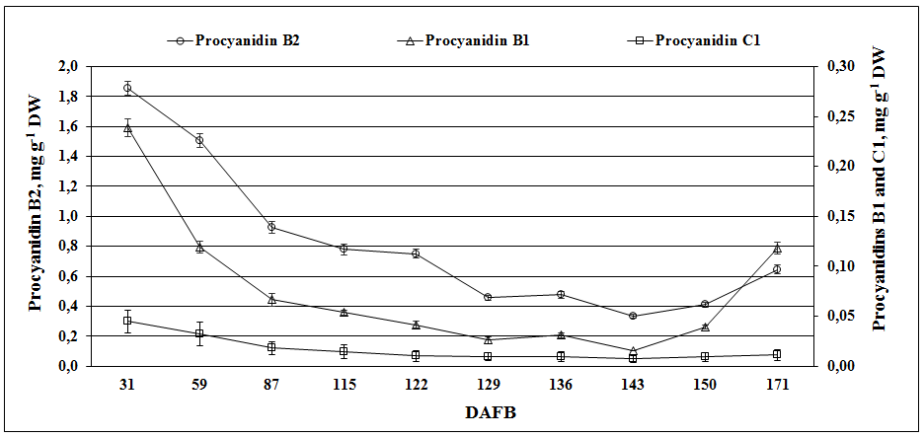

Fig. 5. Variation in the quantitative composition of procyanidin B1, procyanidin B2, and procyanidin C1 in apple samples throughout the vegetation period

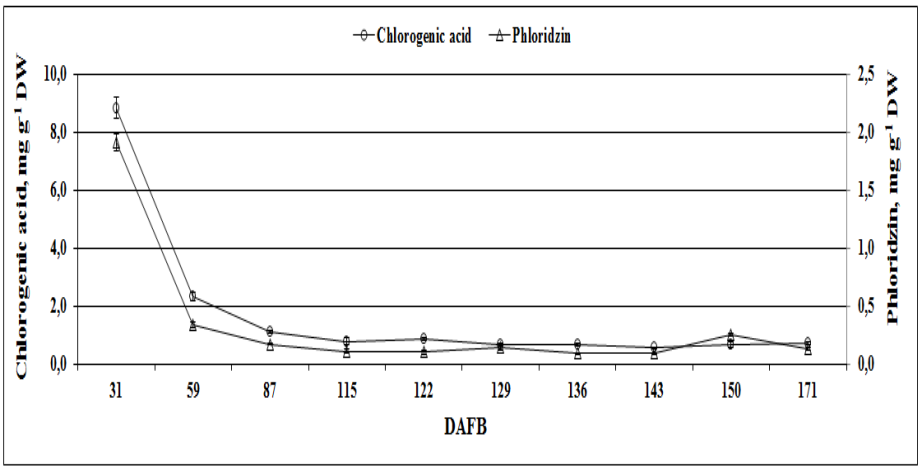

Fig. 6. Variation in the quantitative composition of phloridzin and chlorogenic acid in apple samples throughout the vegetation period

A compound of the dihydrochalcone group, phloridzin, was detected in apple samples. Qualitative and quantitative analysis of phloridzin is of great importance as this compound can be regarded as a chemotaxonomic marker for the identification of apple cultivars as well as products made from apples [41-43]. In the studied samples, this compound comprised 3.44–11.25% of all identified and quantitatively evaluated phenolic compounds. The highest concentrations of phloridzin (1.916±0.074 mg g^{-1}) were detected in apple samples collected at the beginning of the fruit development period (31 DAFB). The concentration of phloridzin (0.334±0.016 mg g^{-1}) detected in apple samples collected during apple development, after the shedding of apple embryos (59 DAFB), was 5.74 times lower than that detected on 31 DAFB. The lowest concentration of this compound was detected on 136 and 143 DAFB – 0.089±0.003 mg g^{-1}. When apples reached the consumption-level maturation (171 DAFB), the detected concentration of phloridzin was 0.134±0.010 mg g^{-1} (Fig. 6). Similar regularities in the variation of the compounds of the dihydrochalcone group during the phenological stages of fruit development and maturation were observed by Renard et al. who studied the phytochemical composition of apple peels and flesh [44].

Chlorogenic acid is one of the most important derivatives of cinnamic acid, characterized by notable antioxidant activity and detected in almost all apple cultivars [45,46,47]. Chlorogenic acid was the predominant compound throughout the vegetation period. It comprised 24–32.12% of all identified phenolic compounds. The results confirm the findings of other studies indicating

that chlorogenic acid in apples is one of the most common phenolic compounds [31,4,48]. The highest concentrations of chlorogenic acid (8.844±0.381 mg g^{-1}) were detected in apple samples collected at the beginning of the fruit development period (on the 168th day of the year). On the 196th day of the year, during fruit development, the amount of this compound decreased 3.81 times. The lowest concentrations of chlorogenic acid (0.56±0.03 mg g^{-1}) were detected during the fruit maturation period (143 DAFB). When apples reached the consumption-level maturation, the concentration of this acid reached 0.73±0.04 mg g^{-1}. Similar regularities in the variation of the quantitative composition of chlorogenic acid in apple peel samples throughout the vegetation period were observed by Awad et al. These authors indicated that the highest amounts of chlorogenic acid were accumulated in apple peel samples collected at the beginning of the fruit development period [29].

The analysis of change dynamics in the quantitative composition of all identified phenolic compounds during the apple development and maturation periods showed that the highest amounts of these compounds were accumulated at the beginning of the fruit development period. As the apples were maturing, the concentrations of phenolic compounds were decreasing. There is a general agreement that the concentration of phenolic compounds is very high in young fruits and then rapidly decreases during fruit development. This regularity is characteristic of apples as well as of other fruit [49,50, 51,29,52,53].

3.1 Evaluation of the Antioxidant Activity of Apple Sample Extracts

The benefit of phenolic compound-accumulating botanical raw materials for human health has been proven by abundant data from a number of studies [54-56]. Antioxidant activity is one of the most important biological characteristics of phenolic compounds closely related to their antineoplastic, anti-inflammatory, and cardioprotective effect [57,58,59].

We conducted the evaluation of the variation in antiradical and reduction activity of apple extracts throughout the vegetation period (Figs. 7 and 8). The strongest antiradical and reduction effect was observed in apple samples collected at the beginning of the development period (31 DAFB) (TE_{DPPH} reached 81.02 μmol g^{-1}, and TE_{FRAP} - 715.63 μmol g^{-1}, respectively).

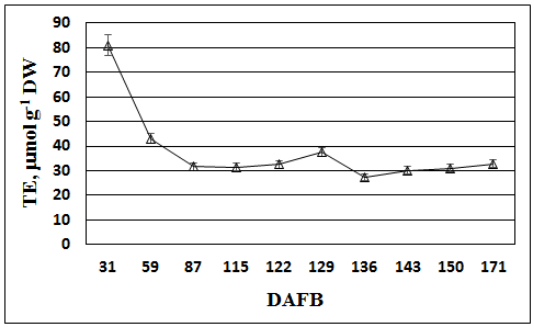

Fig. 7. Variation in antiradical activity of apple sample extracts throughout the vegetation period

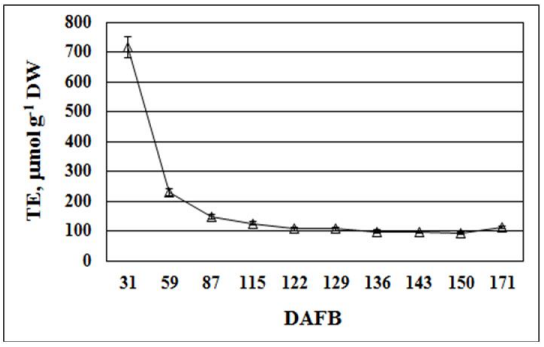

Fig. 8. Variation in reduction activity of apple sample extracts throughout the vegetation period

As apples were developing and maturing, the antioxidant activity was decreasing. The weakest antiradical activity (TE_{DPPH}=27.42 μmol g^{-1}) was observed in the extracts of apple samples collected on 136 DAFB, and the weakest reduction activity (92.15 μmol g^{-1}) – in the extracts of apple samples collected on 150 DAFB. When apples reached the consumption-level maturation, the antiradical activity of apple extracts reached 32.85 μmol $g^{-1,}$ and their reduction activity reach 112.57 μmol g^{-1} (Figs. 7 and 8).

3.2 Results of the Correlation Analysis

The statistical correlation analysis revealed a very strong correlation between the total amount of phenolic compounds identified via the HPLC technique and the reduction activity of apple extract (Spearman's correlation coefficient R=0.927, $p<0.01$). In addition, a strong

correlation was found between antiradical activity of the analysed apple extracts and the total amount of quantitatively evaluated phenolic compounds ($R=0.770$, $p<0.01$).

Among individual compounds that have been identified and quantitatively evaluated via HPLC, the strongest correlation with antiradical and reduction activity was observed for quercetin glycosides – isoquercitrin ($R=0.851$ and 0.845 respectively, $p<0.01$), hyperoside ($R=0.770$ and 0.891 $p<0.01$), avicularin ($R=0.770$ and 0.976 $p<0.01$), and quercitrin ($R=0.784$ and 0.967 $p<0.01$). Other previously published studies also reported strong correlative connection between phenolic content in apple samples and the antioxidant activity of plant extracts evaluated by different assays [30,60]. HPLC post-column assays should be performed to evaluate the variation in antioxidant activity of individual phenolic compounds.

4. CONCLUSION

The results of the evaluation of variations in the quantitative composition of phenolic compounds in apples showed that the highest amounts of all identified phenolic compounds were accumulated in apples at the beginning of the phenological stage of fruit development (31 DAFB). Over the vegetation period, as the apple mass was increasing, the concentration of phenolic compounds was decreasing. During the fruit development and maturation periods, the total concentration of the quantitatively evaluated phenolic compounds ranged from 20.97±0.74 mg g^{-1} (31 DAFB) to 2.96±0.12 mg g^{-1} (171 DAFB). Research data have shown that the total concentration of phenolic compounds in mature apples suitable for picking and further storage ranges from 1.61±0.05 mg g^{-1} (143 DAFB) to 2.96±0.12 mg g^{-1} (171 DAFB).

During the fruit development period, the quantitative composition of quercetin glycosides could be arranged in the following descending order:rutin<reynoutrin<isoquercitrin<avicularin<hyperoside<quercitrin. At the end of the vegetation period, the quantitative composition and the ratio of quercetin glycosides changed to form the following descending sequence: rutin <isoquercitrin<avicularin<reynoutrin<quercitrin<hyperoside. Chlorogenic acid was the predominant compound among the identified individual phenolic compounds throughout the vegetation period.

The strongest antiradical ($TE_{DPPH}=81.02$ μmol g^{-1}) and reduction ($TE_{FRAP}=715.63$ μmol g^{-1}) activity was observed in the extracts of apple samples collected at the beginning of the phenological stage of fruit development (31 DAFB). The biologically active compound complex that accumulated in apples has an antioxidant effect, which makes this botanical raw material beneficial for human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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