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ORIGINAL PAPER

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INFLUENCE OF DIFFERENT ROOTSTOCKS ON BASIC NUTRIENTS, SELECTED MINERALS, AND PHENOLIC COMPOUNDS OF APPLE CV. 'ŠAMPION'

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ABSTRACT

The research objective was to determine and compare dry matter, crude protein, mineral elements (total), dietary fiber, easily hydrolysable sugars, vitamin C, and minerals (K, Na, Mg, Cu) as well as the content of epicatechin and phenolic acids (homovanillic, chlorogenic, caffeic, coumaric, and ferulic) in the flesh and peel of 'Šampion' cv. apples grafted on 4 different rootstocks types: M.26, P2, M.9, and P22 in 2014–2015. The apples from trees grafted on the P22 rootstock had the highest content of dry matter, crude protein, fiber, easily hydrolysable sugars, and vitamin C. The highest mineral compound concentration was exhibited by apples from trees growing on rootstocks P22, P2, and M.9. The highest concentration of phenolic acids was determined in the peel of fruits from trees growing on P22 and M.9. The observations confirm that rootstocks characterized by the lowest growth rate (P22, M.9) ensure the highest accumulation of nutrients in 'Šampion' fruits.

Key words: nutrients, phenols, vitamin C, apple, rootstocks

INTRODUCTION

Consumers' awareness of the nutritional value of food products is still increasing; hence, they make informed choices while purchasing goods [Paul and Rana 2012, Rubio 2014]. Currently, the objective of food (including fruit) production is to achieve not only high performance but also the highest quality level of commodities [Lin and Wals 2008, Dehnen-Schmutz et al. 2010]. At present, high-quality fruits are not only those that meet the strict requirements for the size, shape, or coloration but also fruits that ensure consumption safety and nutritional values [Wolfe and Lui 2003]. The use of such factors of production technology as e.g. different types of rootstocks contributes to satisfactory production outputs and opens the way for predetermination of the nutritional value of fruits by modulation of their chemical composition [Bassal 2009, Cantuarias-Avilés et al. 2011, Kiczorowska and Kiczorowski 2011].

Therefore, the objective of the present research was to determine and compare the following basic nutrients: dry matter, crude protein, fiber, easily hydrolysable sugars (NFE), vitamin C, mineral elements (total), K, Na, Mg, and Cu as well as the content of epicatechin and phenolic acids (homovanillic, chlorogenic, caffeic, coumaric and ferulic) in the flesh and peel of 'Šampion' cultivar apples grafted on chosen rootstocks types: M.26, P2, M.9, and P22 and harvested in 2014 and 2015.

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MATERIALS AND METHODS

The investigations were conducted on 'Šampion' cultivar apples, harvested in 2014–2015, from apple trees growing on chosen rootstocks types: semi dwarf M.26, and dwarf P2, M.9, and P22. The apples were collected in a productive orchard located in Podkarpackie Province southeast of Poland. 60 kg N ha^{-1} in the form of urea were supplied under the apple trees during the blooming time and 100 kg K_2O ha^{-1} as KCl were supplemented in late autumn. The orchard protection was managed according to the Orchard Protection Agenda (apple trees). Harvest dates (20–22.09.2014 and 22–23.09.2015) were determined based on evaluation of apples, i.e. fruit size, degree of peel coloration, blush size, ease of fruit removal from a short shoot, flesh firmness, and starch index (IS) [Campbell and Marini 1992, Rutkowski 2003]. In order to determine IS, 10 apples were collected from each tree. Next, the apples were cut perpendicular to the core axis, immersed for 1 minute in a solution (10g J_2 + 40g KJ in 1l of an aqueous solution), and allowed to dry. The cut and stained fruit slices were presented to 3 individuals, who compared them with standard tables [Rutkowski 2003] and estimated the starch index in the scale from 1 to 10.

The determination of the starch index involved calculation of the total surface area of the slice [Rutkowski 2003]:

$$IS = 10 \cdot (1 - \frac{Pw}{Pc})$$

where:
IS – starch index,
Pw – starch pattern
Pc – starch pattern area

The mean temperature in the growing season was higher in 2015 than in 2014. The spring was by 2°C warmer (March – 5.9°C, April – 11.4°C, May – 15.1°C) in 2014, and June and July were characterized by ca. 1°C and August by 4°C higher temperatures (18.5, 21.7, and 22.9°C, respectively). Similar temperatures, on average 9.7°C, were noted during the apple harvest period. Higher precipitation rates were reported in 2014. Until May, the mean precipitation rates were similar in both years of the experiment. In the other months of the vegetation period, the rainfall was by ca. 200 mm higher in 2014 than in 2015, with the exception of August, when the difference reached 300 mm [WIOŚ 2017].

The samples were collected randomly from 3–4 trees, with three replications of 5 kg each. Prior to the chemical analyses, the apples were kept in cold storage (3–4°C). The fruits were peeled manually with a special knife, which allowed sampling research material with the same thickness from all the peeled fruits. Peel from one apple with an approximate thickness of 0.3 mm weighed ca. 10 g. Approximately 30 g of flesh were sampled from one fruit.

The basic chemical composition of the specimens obtained was determined in compliance with standard procedures [AOAC 2000]: dry matter (method 985.14), total minerals (method 920.153), N-compounds (method 928.08), and easily hydrolysable sugars (calculated on the total basic chemical composition). The group of simple and structural easily hydrolysable carbohydrates was determined in the nitrogen-free extract fraction (NFE) [Dz.U. 2004], and L-ascorbic acid was determined using an enzymatic test kit (No. 10 409 677 035 – test – combination for 21 determinations), Boehringer Mannheim/r-Biopharm [Henniger 1981, Czerwiecki and Wilczyńska 1999].

The resultant ash was solubilized on crucibles using 6 mol l^{-1} of spectrally pure hydrochloric acid (POCH, Poland). Na and K were analyzed with flame atomic emission spectroscopy (FAES) using a flame photometer (Pye Unicam SP 2900, Cambridge, UK) at a wavelength of λ = 589.0 and λ = 766.5 nm, respectively. Mg and Cu concentrations were determined with flame atomic absorption spectroscopy (FAAS) using a SOLAAR 939/959 spectrophotometer (Unicam, Cambridge, UK). Magnesium was determined at λ = 285.2 nm and copper at λ = 324.8 nm, [PN-EN ISO 6869:2002]. In the case of Na and K determinations, cesium chloride (Merck, Poland) was added to the standards and samples as an ionization buffer at a concentration of 0.2% w/v. Mg was analyzed by addition of 0.4% w/v lanthanum oxide (Merck, Poland), which is a correction buffer that allows binding of the analyzed element to the matrix.

A standard solution of β-naphthol (POCH, Gliwice, Poland) was prepared by dissolving 43.2 mg of the compound in 100 ml methanol (Lachema, Brno, Czech Republic) and used as an internal standard in the quantitative analyses. An epicatechin standard solution was prepared by dissolving 56.8 mg of (–)-epicatechin (Sigma–Aldrich, Meinheim, Germany) in 50 ml methanol, and the catechin standard by dissolving 25.1 mg of (+)-catechin (Sigma–Aldrich, Meinheim, Germany) in 25 ml of methanol. The peel and flesh from each apple were transferred into a pre-weighed flask, spiked with 100 µl of β-naphthol, and extracted three times with methanol (20 ml) in an ultrasonic bath (25°C) for 1 h. The solvent was evaporated in a rotating evaporator to a final volume of 6 ml. The final volumes of crude extracts ranged from 3.6 ml to 5.3 ml.

Homogenized freeze-dried fruit samples (0.5 g) were extracted with 5 × 5 ml methanol in an Ultra-Turrax T25 (IKA, Werke, Janke & Kunkel) homogenizer. For the determination of simple polyphenols, aliquots of the methanolic apple extracts (0.1 ml) were transferred to GC vials and 50 µl of an internal standard was added (3-(4-hydroxyphenyl)-1-propanol solution, 19.2 µg ml^{-1}); next, the sample was evaporated to dryness under nitrogen and derivatized by addition of 250 µl BSTFA at 70°C for 20 min. An aliquot (1 µl) of the derivatized sample was injected into the gas chromatograph at a split ratio 1 : 20. Analysis of the samples was performed by an Agilent (Wallborn, Germany) HP series GC 6890N coupled with a HP 5973 MS detector (EI, 70 eV), a split–splitless injector, and an HP 7683 autosampler in an HP-5 MS capillary column (5% phenyl – 95% methyl siloxane, 30 m × 0.25 mm × 250 µm). 3-(4-hydroxyphenyl)-1-propanol was used as an internal standard. Internal standard quantification was performed based on a series of 9 standard mixtures of polyphenolic compounds containing the same quantity of the internal standard as that of the samples.

Measures of location, i.e. mean, standard deviation, and bottom and upper quartile, were calculated for the collected data. The normality and homogeneity of variance data of the chemical composition were tested using the Shapiro-Wilk and Brown-Forsythe tests, respectively. The non-parametric Kruskal-Wallis test (a non-parametric equivalent of one-way analysis of variance) was used to analyze differences in the element concentrations in the apple peel and flesh. Detailed comparisons between the groups were conducted using the post hoc Dunn test. All statements of significance were based on a probability of <0.05. All calculations were performed with statistical software package Statistica version 10 [StatSoft 2010].

RESULTS

The present investigations showed that the highest crude protein ($P < 0.05$) content was characteristic for fruits harvested from trees planted on P22 and M.9 (in the peel 0.70 g 100 g^{-1} f.m. and 0.62 g 100 g^{-1} f.m.; in the flesh 0.30 g 100 g^{-1} f.m. and 0.28 g 100 g^{-1} f.m., respectively) (tab. 1). The significantly highest mean content ($P < 0.05$) of dietary fiber was detected in smaller sized apples obtained from trees grafted on dwarfing rootstocks P22 (peel – 2.60 g 100 g^{-1} f.m.; flesh – 0.45 g 100 g^{-1} f.m.), P2, and M.9 (average in the peel 1.96 g 100 g^{-1} f.m. and 0.35 g 100 g^{-1} f.m.). The concentration of easily hydrolysable sugars was the highest ($P < 0.05$) in the peel and flesh of the P22 and semi-dwarf M.26 fruits (average in the peel 14.64 g 100 g^{-1} f.m., flesh 10.59 g 100 g^{-1} f.m.). In turn, the lowest content of easily hydrolysable sugars was determined in apples from trees produced on the vigorously growing rootstock P2 (average in the peel 13.28 g 100 g^{-1} f.m.; in the flesh 9.44 g 100 g^{-1} f.m.).

The highest vitamin C content ($P < 0.05$) in the studied material was determined in apples obtained from trees grafted on dwarfing rootstocks P22 (peel 12.21 mg 100 g^{-1} f.m. and flesh 10.60 mg 100 g^{-1} f.m.) (tab. 1). In contrast, the lowest vitamin C ($P < 0.05$) level was noted in fruits collected from trees planted on rootstocks M.26 and P2 (average in the peel 8.10 mg 100 g^{-1} f.m. and in the flesh – 7.11 mg 100 g^{-1} f.m.).

In most cases, there was a higher ($P < 0.05$) concentration of mineral elements (total) in the peel of the analyzed apples than in their flesh (tab. 2). In the case of apples collected from trees growing on rootstock P22 (0.39 g 100 g^{-1} f.m. – peel, 0.14 g 100 g^{-1} f.m. – flesh), the highest amount of mineral

Table 1. The content of basic nutrients in the peel and flesh of 'Šampion' cultivar apples growing on different rootstocks (g 100 g^{-1} f.m.)

Rootstock	M.26			P2			M.9			P22			P-value	
Harvest year	2014	2015	$\bar{x}$	2014	2015	$\bar{x}$	2014	2015	$\bar{x}$	2014	2015	$\bar{x}$		
Number of samples	12	14	26	15	12	27	14	17	31	17	13	30	A[a]	B[b]
Dry matter														
Peel	16.75	17.15	16.95	15.68	16.78	16.23	17.29	17.10	17.19	17.97	18.26	18.11	0.187	0.089
SD[c]	±0.23	±0.15	±0.25	±0.12	±0.08	±0.18	±0.17	±0.16	±0.25	±0.15	±0.13	±0.14		
Q25[d]-Q75[e]	15.97–17.45					15.02–16.98			17.05–17.68			17.79–18.43		
Flesh	11.79	12.08	11.93	11.19	11.29	11.24	12.40	12.22	12.31	12.02	12.56	12.29	0.097	0.134
SD[c]	±0.15	±0.12	±0.01	±0.14	±0.12	±0.12	±0.28	±0.20	±0.15	±0.09	±0.07	±0.11		
Q25[d]-Q75[e]	11.56–12.15					11.08–11.39			12.12–12.48			11.98–12.68		
Crude protein														
Peel	0.50	0.48	0.49c	0.54	0.49	0.51	0.60	0.65	0.62	0.68	0.72	0.70	0.026	0.145
SD[c]	±0.13	±0.15	±0.15	±0.25	±0.23	±0.20	±0.24	±0.26	±0.12	±0.19	±0.15	±0.12		
Q25[d]-Q75[e]	0.44–0.52					0.48–0.55			0.58–0.67			0.65–0.73		
Flesh	0.21	0.19	0.20	0.18	0.21	0.19	0.26	0.30	0.28	0.28	0.33	0.30	0.043	0.034
SD[c]	±0.14	±0.12	±0.26	±0.23	±0.27	±0.30	±0.32	±0.24	±0.12	±0.15	±0.16	±0.26		
Q25[d]-Q75[e]	0.17–0.23					0.17–0.22			0.26–0.31			0.25–0.35		
Fiber														
Peel	1.61	1.58	1.59	1.98	2.08	2.03	1.92	1.88	1.90	2.01	2.12	2.60	0.037	0.178
SD[c]	±0.26	±0.25	±0.12	±0.13	±0.17	±0.07	±0.08	±018	±0.16	±0.14	±0.15	±0.25		
Q25[d]-Q75[e]	1.57–1.63					1.98–2.10			1.86–2.03			1.99–2.15		

Flesh	0.35	0.33	0.34	0.33	0.28	0.30	0.38	0.42	0.40	0.42	0.48	0.45	0.034	0.029
SD[c]	±0.15	±0.18	±0.28	±0.16	±0.19	±0.25	±0.18	±0.15	±0.18	±0.19	±0.23	±0.38		
Q25[d]-Q75[e]	0.31–0.36					0.26–0.36			0.36–0.45			0.41–0.49		
Easily hydrolyzed sugars														
Peel	14.21	14.65	14.44	12.78	13.76	13.28	14.34	14.18	14.26	14.81	14.90	14.85	0.018	0.347
SD[c]	±0.15	±0.20	±0.26	±0.14	±0.28	±0.26	±0.12	±0.14	±0.15	±0.18	±0.18	±0.15		
Q25[d]-Q75[e]	14.19–14.68					12.75–13.78			14.16–14.38			14.77–14.93		
Flesh	10.92	11.21	11.06	9.41	9.48	9.44	10.44	10.13	10.28	9.96	10.31	10.13	0.023	0.245
SD[c]	±0.18	±0.19	±0.20	±0.15	±0.28	±0.26	±0.19	±0.16	±0.15	±0.29	±0.30	±0.33		
Q25[d]-Q75[e]	10.95–11.23					9.38–9.49			10.10–10.50			9.95–10.36		
Vitamin C (mg 100 g^{-1} f.m.)														
Peel	6.98	9.53	8.25	7.35	8.56	7.95	9.56	11.06	10.31	11.56	12.87	12.21	0.039	0.028
SD[c]	±0.45	±0.56	±0.34	±0.46	±0.39	±0.20	±0.27	0.25±	±0.61	±0.48	±0.57	±0.67		
Q25[d]-Q75[e]	6.78–9.67					7.10–8.69			9.48–11.16			11.54–13.06		
Flesh	5.80	7.89	6.89	7.12	7.56	7.34	8.26	11.24	9.75	10.23	10.98	10.60	0.019	0.041
SD[c]	±0.28	±0.36	±0.35	±0.25	±0.21	±0.27	±0.19	±0.49	±0.36	±0.27	±0.14	±0.19		
Q25[d]-Q75[e]	5.87–8.02					7.10–7.64			8.15–11.31			10.16–11.03		

[a] Significant differences between the content of nutrients in apples growing on the same rootstock in 2014 and 2015, $P < 0.05$
[b] Significant differences between the average content of nutrients in apples growing on the different rootstocks, $P < 0.05$
[c] Standard deviation
[d] Quartile bottom
[e] Quartile upper

Table 2. The content of chosen mineral elements in the peel and flesh of 'Šampion' cultivar apples growing on different rootstocks

Rootstock	M.26			P2			M.9			P22			P-value	
Harvest year	2014	2015	$\overline{X}$	2014	2015	$\overline{X}$	2014	2015	$\overline{X}$	2014	2015	$\overline{X}$		
Number of samples	12	14	26	15	12	27	14	17	31	17	13	30	A[a]	B[b]
Mineral elements – total (g 100 g^{-1} f.m.)														
Peel	0.32	0.35	0.33	0.28	0.35	0.31	0.33	0.29	0.31	0.37	0.42	0.39	0.029	0.037
SD[c]	±0.14	±0.15	±0.09	±0.16	±0.07	±0.15	±0.18	±0.28	±0.30	±0.23	±0.19	±0.016		
Q25[d]-Q75[e]	0.31–0.36					0.28–0.33			0.28–0.36			0.35–0.44		
Flesh	0.32	0.36	0.34	0.28	0.33	0.30	0.33	0.38	0.35	0.37	0.45	0.41	0.033	0.018
SD[c]	±0.18	±0.17	±0.35	±0.34	±0.20	±0.18	±0.16	±0.17	±0.19	±0.30	±0.13	±0.14		
Q25[d]-Q75[e]	0.30–0.36					0.26–0.33			0.30–0.39			0.35–0.49		
K (mg g^{-1} f.m.)														
Peel	1.16	1.21	1.18	1.03	1.11	1.07	0.99	0.93	0.96	1.26	1.19	1.23	0.183	0.216
SD[c]	±0.19	±0.20	±0.15	±0.16	±0.26	±0.30	±0.68	±0.12	±0.15	±0.29	±0.25	±0.25		
Q25[d]-Q75[e]	0.14–1.21					1.02–1.11			0.92–0.93			1.19–1.26		
Flesh	0.61	0.65	0.63	0.54	0.58	0.56	0.94	0.96	0.95	1.15	1.14	1.15	0.274	0.034
SD[c]	±0.18	±0.12	±0.21	±0.09	±0.16	±0.04	±0.25	0.16±	±0.38	±0.20	±0.36	±0.47		
Q25[d]-Q75[e]	0.61–0.67					0.54–0.59			0.93–0.97			1.14–1.16		
Na (mg 100 g^{-1} ·f.m.)														
Peel	0.68	0.75	0.71c	1.58	1.69	1.63	1.35	1.39	1.37	1.45	1.56	1.50	0.178	0.019
SD[c]	±0.15	±0.13	±0.16	±0.18	±0.20	±0.08	±0.20	±0.16	±0.14	±0.08	±0.20	±0.16		
Q25[d]-Q75[e]	0.64–0.78					1.54–1.70			1.34–1.41			1.43–1.59		
Flesh	0.82	0.79	0.80	1.12	1.23	1.17	0.69	0.78	0.73	1.42	1.56	1.49	0.044	0.036
SD[c]	±0.14	±0.13	±0.15	±0.16	±0.13	±0.16	±0.18	±0.16	±0.17	±0.18	±0.19	±0.09		
Q25[d]-Q75[e]	0.75–0.85					1.06–1.17			0.65–0.83			1.38–1.57		

Mg (mg g^{-1} f.m.)														
Peel	0.129	0.136	0.132	0.123	0.139	0.131	1.115	0.103	0.109	0.139	0.147	0.143	0.245	0.028
SD[c]	±0.15	±0.13	±0.20	±0.15	±0.13	±0.20	±0.09	±0.08	±0.19	±0.13	±0.16	±0.15		
Q25[d]-Q75[e]	0.126–0.137				0.1.19–0.143			0.097–0.121				0.125–0.153		
Flesh	0.038	0.043	0.041	0.029	0.035	0.032	0.019	0.028	0.023	0.036	0.048	0.042	0.019	0.042
SD[c]	±0.18	±0.19	±0.15	±0.16	±0.20	±0.29	±0.30	±0.28	±0.18	±0.18	±0.14	±0.19		
Q25[d]-Q75[e]	0.03–0.04				0.02–0.03			0.01–0.03				0.03–0.05		
Cu (μg g^{-1} f.m.)														
Peel	0.57	0.49	0.50	0.53	0.56	0.54	0.68	0.72	0.70	0.62	0.67	0.64	0.027	0.037
SD[c]	±0.15	±0.13	±0.16	±0.08	±0.20	±0.20	±0.18	±0.09	±0.16	±0.14	±0.13	±0.12		
Q25[d]-Q75[e]	0.45–0.59				0.51–0.57			0.66–0.73				0.60–0.69		
Flesh	0.19	0.22	0.20	0.15	0.19	0.17	0.22	0.29	0.25	0.21	0.27	0.24	0.041	0.016
SD[c]	±0.18	±0.13	±0.20	±0.28	±0.27	±0.17	±0.19	±0.15	±0.13	±0.16	±0.18	±0.14		
Q25[d]-Q75[e]	0.18–0.24				0.11–0.20			0.19–0.31				0.20–0.29		

[a] Significant differences between the content of nutrients in apples growing on the same rootstock in 2014 and 2015, $P < 0.05$
[b] Significant differences between the average content of nutrients in apples growing on the different rootstocks, $P < 0.05$
[c] Standard deviation
[d] Quartile bottom
[e] Quartile upper

Table 3. The content of epicatechin and phenolic acids the peel and flesh of 'Šampion' cultivar apples growing on different rootstocks (mg kg^{-1} f.m.)

Rootstock	M.26			P2			M.9			P22			P-value	
Harvest year	2014	2015	$\bar{x}$	2014	2015	$\bar{x}$	2014	2015	$\bar{x}$	2014	2015	$\bar{x}$		
Number of samples	12	14	26	15	12	27	14	17	31	17	13	30	A[a]	B[b]
Epicatechin														
Peel	10.45	10.86	10.65	9.87	11.56	10.71	13.45	12.58	13.01	13.26	14.89	14.07	0.023	0.049
SD[c]	±0.12	±0.16	±0.15	±0.20	±0.08	±0.21	±0.18	±0.19	±0.30	±0.17	±0.16	±0.23		
Q25[d]-Q75[e]	10.39–10.92					9.79–11.68			12.46–13.35			13.22–14.92		
Flesh	1.22	1.29	1.25	0.87	0.92	0.89	1.56	1.64	1.60	1.33	1.45	1.39	0.167	0.034
SD[c]	±0.15	±0.19	±0.19	±0.89	±0.67	0.16±	±0.35	±0.25	±0.39	±0.17	±0.18	±0.27		
Q25[d]-Q75[e]	1.19–1.32					0.81–0.95			1.52–1.67			1.30–1.46		
Phenolic acids														
Homovanillic acid														
Peel	48.56	52.67	50.61	49.58	52.13	50.99	62.45	61.03	61.74	56.78	55.89	56.33	0.234	0.018
SD[c]	±1.59	±1.65	±2.02	±1.26	±2.06	±3.00	±1.03	±1.02	±1.15	±1.03	±2.08	±2.456		
Q25[d]-Q75[e]	47.89–53.02					49.23–53.06			59.94–63.26			55.03–57.12		
Flesh	17.56	17.89	17.72	19.56	20.05	19.80	25.48	27.23	26.35	22.35	25.16	23.75	0.134	0.031
SD[c]	±3.26	±3.15	±4.05	±2.05	±2.15	±2.98	±1.59	±2.06	±2.03	±3.25	±3.15	±3.01		
Q25[d]-Q75[e]	17.48–17.92					19.46–20.12			25.16–26.89			22.01–26.03		
Chlorogenic acid														
Peel	115.26	113.12	114.19	124.19	127.23	125.71	259.45	264.23	261.84	198.23	215.04	206.63	0.208	0.046
SD[c]	±1.15	±1.16	±2.19	±1.09	±2.08	±3.16	±1.18	±1.67	±2.14	±2.08	±1.04	±2.16		
Q25[d]-Q75[e]	112.98–115.48					124.06–127.68			257.89–268.26			197.56–216.04		
Flesh	68.49	73.56	71.02	78.56	81.25	79.90	110.28	124.02	117.15	98.56	87.25	92.90	0.046	0.037
SD[c]	±0.26	±0.06	±0.06	±0.05	±0.02	±0.14	±0.19	±0.09	±0.08	±0.15	±0.18	±0.16		

Q25[d]-Q75[e]	68.24 – 73.89				77.59–82.09			109.78–124.23				87.06–99.02		
Caffeic acids														
Peel	9.87	10.27	10.07	6.89	7.54	7.21	12.18	13.08	12.58	10.24	11.08	10.66	0.028	0.019
SD[c]	±0.25	±0.39	±0.48	±0.25	±0.48	±0.47	±0.35	±0.25	±0.58	±0.23	±0.36	±0.54		
Q25[d]-Q75[e]	9.67–10.35				6.57–7.58			12.02–13.45				10.19–11.12		
Flesh	5.89	4.56	5.22	6.89	7.56	7.22	8.25	7.98	8.11	8.56	8.78	8.67	0.037	0.025
SD[c]	±0.157	0.265±	±0.157	±0.236	±0.358	±0.268	±0.458	±0.157	±0.254	±0.205	±0.147	±0.241		
Q25[d]-Q75[e]	4.34–5.92				6.78–7.63			7.91–8.21				8.43–8.84		
Coumaric acids														
Peel	0.78	0.86	0.82	0.64	0.58	0.61	1.20	1.10	1.15	0.98	1.23	1.08	0.037	0.014
SD[c]	±0.12	±0.18	±0.25	±0.09	±0.04	±0.07	±0.18	±0.26	±0.19	±0.24	±0.39	±0.34		
Q25[d]-Q75[e]	0.75–0.88				0.53–0.66			1.07–1.25				0.84–1.27		
Flesh	0.05	0.07	0.06	0.08	0.09	0.08	0.18	0.23	0.20	0.11	0.15	0.13	0.019	0.036
SD[c]	±0.08	±0.03	±0.09	±0.04	0.06±	±0.08	±0.23	±0.05	±0.08	±0.45	±0.12	±0.97		
Q25[d]-Q75[e]	0.04–0.07				0.07–0.09			0.17–0.24				0.10–0.15		
Ferulic acids														
Peel	0.009	0.008	0.008	0.006	0.007	0.006	0.010	0.012	0.011	0.009	0.010	0.009	0.117	0.164
SD[c]	±0.012	±0.008	±0.035	±0.024	±0.057	±0.097	±0.023	±0.015	±0.024	±0.034	±0.045	±0.054		
Q25[d]-Q75[e]	0.008–0.009				0.006–0.008			0.008–0.013				0.008–0.016		
Flesh	–	–	–	–	–	–	0.003	0.004	0.003	-	0.002	0.002	0.245	0.098
SD[c]	±0.0	±0.0	±0.0	±0.0	±0.0	±0.0	±0.023	±0.014	±0.018	±0.005	±0.013	±0.009		
Q25[d]-Q75[e]		-			–			–		0.001–0.007			–	

[a] Significant differences between the content of nutrients in apples growing on the same rootstock in 2014 and 2015, $P < 0.05$
[b] Significant differences between the average content of nutrients in apples growing on the different rootstocks, $P < 0.05$
[c] Standard deviation
[d] Quartile bottom
[e] Quartile upper

compounds (in total) was determined. The highest ($P < 0.05$) level of potassium and sodium was found in apples from trees grown on rootstocks P22 (average 1.19 g 100 g^{-1} f.m. and 1.49 g 100 g^{-1} f.m., respectively), M.26 (K average 0.90 g 100 g^{-1} f.m.), and P2 (Na average 1.40 g 100 g^{-1} f.m.). A similar level of the magnesium content was detected in most of the analyzed plant material. The exception was the fruits produced by apple trees grafted on the M9 rootstock whose peel and flesh contained 20% and 40% less ($P < 0.05$) magnesium, respectively. Interesting variability of the research results was noted for copper. Its highest ($P < 0.05$) amount was determined in fruits harvested from trees planted on the M.9 and P22 rootstocks (average 0.67 g 100 g^{-1} f.m. in the peel, 0.25g 100 g^{-1} f.m. in the flesh), whereas the lowest level was detected in apples growing on the M.26 and P2 rootstocks (average in the peel 0.52 g 100 g^{-1} f.m., in the flesh 0.18 g 100 g^{-1} f.m.).

In the studied apples, the content of epicatechins and phenolic acids was also determined (tab. 3). The highest ($P < 0.05$) epicatechin concentration was found in the fruits from trees grafted on the M.9 and P22 rootstocks (average: peel 13.54 g 100 g^{-1} f.m., flesh 1.49 g 100 g^{-1} f.m.). Among phenolic acids, chlorogenic acid dominated and its content in the total amount of phenolic acids was estimated at as much as 7% in both the peel and flesh. In most of the investigated combinations, a significantly higher level of all the phenolic acids was determined in the edible parts (peel and flesh) of apples picked from trees grafted on rootstocks M.9 (homovanillic acid – 61.74 and 26.35 g 100 g^{-1} f.m., chlorogenic acid – 261.84–117.15 g 100 g^{-1} f.m., caffeic acid – 12.58 and 8.11 g 100 g^{-1} f.m., coumaric acid – 1.15 and 0.20 g 100 g^{-1} f.m., respectively) and P22 (homovanillic acid – 56.33 and 23.75 g 100 g^{-1} f.m., chlorogenic acid – 206.63–92.90 g 100 g^{-1} f.m., caffeic acid –10.66g 100 g^{-1} f.m., coumaric acid – 1.08 g 100 g^{-1} f.m., respectively). No statistical analysis was performed only in the case of ferulic acid due to the too small quantities detected, merely close to the detection limit of the applied method (especially in the case of the flesh). Higher intensity ($P < 0.05$) of accumulation of chlorogenic acid (M.9, P22), caffeic acid (M.26), and coumaric acid (P22) in the apples was observed in 2015 and greater accumulation of caffeic and coumaric acids (M.9) was reported in 2014.

DISCUSSION

Dry matter content reflects the nutrient concentration in apples, as it shows the capacity of apple trees for nutrient uptake and is the top determinant of the apple flavor and nutritional value [Solomakhin and Blanke 2010]. Fruit trees with defined variety properties grown under the same meteorological and agrotechnical conditions may exhibit a varied composition of fresh fruits [Kiczorowska and Kiczorowski 2007a, b]. Control of their quality in terms of their physical traits can be achieved by selecting an appropriate type of the rootstock. Apples produced by trees on low-vigor rootstocks are characterized by slightly lower size than fruits from trees grafted on semi-dwarfing rootstocks that show high vigor [Lin and Wals 2008, Yuri et al. 2011]. Predetermination of their nutritional value is considerably more difficult but still possible. The tendency of higher dry matter content in apples produced by trees grafted on the chosen dwarfing rootstocks (M.9, P22) was also observed in the present research, which is likely to be connected with the features of the rootstocks themselves. At a similar nutrient uptake, their concentration proves higher in small-sized fruits than in big ones, where nutrients collected are diluted to some extent [Serra et al. 2010].

The crude protein concentration determined in the examined apple skins was up to two times higher in comparison to the content in the flesh. The crude protein level in fruits is related to soil nitrogen availability, apple tree capacity for nitrogen uptake, as well as fruit size [Pacholak et al. 2004, Nesme et al. 2009]. At equal amounts of nitrogen applied, a varied nitrogen level may be found in apples due to the rate of nutrient intake by apple trees and, to some extent, by the rootstock type [Motosugi et al. 1995, Feliciano et al. 2010].

Apples prove to be one of the excellent sources of such chemical substances as hemicellulose, cellulose, pectin, lignin etc. that constitute the major components of dietary fiber. In the peel of the examined

apples, the crude fiber content was found to be up to over five-fold higher than in the flesh. In their investigations of Portuguese traditional and exotic apple varieties, Serra et al. [2010] determined the average content of crude fiber ranging from 3% (traditional) to 2.3% (exotic). However, they found a 2-fold lower concentration of this nutrient in the flesh than in the peel. The total fiber content has been reported in the literature to be as low as 0.80% or between 1.8% and 2.3% [Gorinstein et al. 2001]. The sugar content in apples can vary depending on different conditions: apples exposed to sunlight tend to have higher total sugar content than fruits located in shaded parts of the tree, and the differences can be statistically significant in some harvest periods. The cultivar type has usually a more marked effect on sugars than the storage time [Remorini et al. 2008, Feliciano et al. 2010, Solomakhin and Blanke 2010]. In the present study, rootstock effects on the easily hydrolyzed sugars amount were observed. Similar effects concerning both apples and other fruits were reported by a number of authors [Motosugi et al. 1995, Giorgi et al. 2005].

The content of vitamin C in fruits can be influenced by various factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures. The higher the intensity of light during the growing seasons, the greater the vitamin C content is in plant tissues. Vitamin C content in many crops can be increased with less frequent irrigation. Temperature management after harvest is the most important factor to maintain vitamin C in fruits [Lee and Karder 2000]. Such an effect was observed in the present study as well. In the case of apples produced in 2015, which was warmer, a greater ($P < 0.05$) concentration of vitamin C was determined. However, in this study, an effect of the rootstock type on the content of vitamin C in apples was also observed. This impact was significantly marked in the apples flesh from apples harvested from trees growing on the M.9 and P22 rootstocks.

The 'Šampion' cultivar is distinguished from other varieties by its higher abundance of minerals [Pacholak et al. 2004, Kiczorowska and Kiczorowski 2007a, b]. Literature provides reports of the impact of the rootstock used on the supply of mineral elements in apple trees, which to some extent may be reflected in the mineral content in fruits. Amiri et al. [2014] demonstrated that the use of the M.26 and M.7 rootstocks contributed to greater accumulation of magnesium and copper in Golden Delicious cv. and Royal Gala cv. trees, respectively. The great levels of magnesium accumulation in apple trees grafted on the M.26 rootstock have also been confirmed in the study carried out by Fallahi and Mohan [2000]. In investigations of the Šampion'cv., Andziak et al. [2004] found the highest K and Ca concentration in apples from trees grafted on P2 and M.26 rootstocks. Similarly, Motosugi et al. [1995], who analyzed the quality of 'Fuji' cv. apples, as well as Zhu and Welander [1999], who conducted research on 'Gravenstein' cv. apples, noted the highest mineral accumulability in the fruits from apple trees produced on semi-dwarf rootstock M.26. A contrasting tendency in mineral accumulation was reported by Fallahi et al. [1985] in the studies on 'Golden Delicious' cv. apples from trees grown on rootstocks types: M.1, MM106, M.7, OAR1, and M.26. Remorini et al. [2008] indicate that a rootstock inducing both weak and vigorous growth is the best tool to achieve an optimal nutritional value in peach skin and flesh. This observation seems to be confirmed in the present study.

Additionally, apples contain phenolic derivatives, which are highly valuable dietary and health-enhancing compounds. They exhibit such beneficial properties as antioxidant and anti-carcinogenic activity and can prevent ischemic heart disease, etc. [Mejía et al. 2006, McCann et al. 2007, Serra et al. 2010]. The authors highlight that the 2–3 fold higher concentration of these substances in the peel than in the fruit flesh is of primary importance from the nutritional and medical point of view [Kosmala and Kołodziejczyk 2006, Vieira et al. 2011]. The same effect of the higher content of phenolic compounds in the skin has been confirmed in the present paper. Among the currently marketed apples, the 'Elstar' variety proves to be the most abundant in phenolic compounds [Wojdylo et al. 2008]. However, earlier studies conducted by the author of the present paper indicate that 'Šampion' cultivar apples are also rich in these substances [Malik et al. 2009].

The content of phenolic compounds in fruits is affected by a number of factors, among others, genetic traits, species, and variety which, importantly, can be modified by the environmental conditions (weather). Investigations conducted in productive orchards confirm that the use of semi-dwarf rootstocks provides potential for an increase in the concentration of phenolic substances in fruits [Jakubek et al. 2009]. Agronomic factors that may regulate the level of phenolic compounds in fruits, especially in the fruit skin, also include optimal fertilization [Awad and de Jager 2002]. Particularly valuable in terms of stimulating the phenolic acids synthesis in apples was the M9 rootstock. The harvested fruit from the trees growing on this rootstock were characterized by a particularly high concentration of almost all the investigated phenolic acids.

CONCLUSIONS

1. Rootstocks characterized by the lowest growth rate ensure the highest accumulation of nutrients in 'Šampion' cv. fruits

2. P22 has proved to be the most effective rootstock in accumulation of essential nutrients, vitamin C, minerals (total, K, Na, Mg, Cu), and epicatechin as well as homovanillic and coumaric acids in apples.

3. High levels of phenolic compounds and epicatechin were detected in apples produced by trees grafted on the M.9 rootstock.

3. The concentration of nutrients was higher in apples produced in 2015 than in 2014.

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