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Antioxidant properties of pepper (*Capsicum annuum* L.) depending on its cultivar and fruit colouration

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Abstract: Due to its nutritional, health-promoting and taste-related values, new cultivars are introduced every year. The aim of the study was to assess the biological value of Polish and Bulgarian cultivars of pepper grown in moderate climate conditions and collected at different degrees of maturity. Ascorbic acid, carotenoids, polyphenols, chlorophyll *a*, chlorophyll *b*, total chlorophyll and antioxidant activity (DPPH) were determined in air-dried fruit. The investigation included five Polish cultivars: Roberta, Marta Polka, Etiuda, Trapez, and Cyklon and five Bulgarian cultivars: Bulgarski Ratung, Sivriya, Kurtovska Kapiya, Delikates, and Dzuliunska Shipka. Its design involved randomised sub-blocks, with three replications comprising nine plants each. The area of a single plot was 1.44 m^2 (1.2 m × 1.2 m). The study confirmed the strong antioxidant properties of pepper grown in the field, without cover, and under temperate climate conditions. The vegetable is rich in vitamin C, polyphenols, carotenoids, chlorophyll pigments, and shows high antioxidant activity. However, the biological value of pepper is cultivar-dependent and is also determined by the fruit colouration degree. The coloured fruits are richer in vitamin C and carotenoids than the green ones, and when matured, they have greater antioxidant capacity. Green fruits contain more chlorophyll *a*, chlorophyll *b* and total chlorophyll than the coloured ones. The fruits of cv. Etiuda were the richest in vitamin C, of cv. Sivriya in polyphenols, of cv. Dzuliunska Shipka in carotenoids, and of cv. Trapez in total chlorophyll. Cv. Sivriya showed the strongest antioxidant properties.

Keywords: Solanaceae; phytochemical; weather conditions; nutrition

Capsicum annuum L. belongs to the Solanaceae family and is native to South America (Singh et al. 2009). It is classified as a vegetable of very high biological value (Jadczak et al. 2010). *Capsicum annum* is the most widely grown species of pepper in the world, it encompasses e.g., sweet and hot pepper (Padilha et al. 2015), and it is highly palatable. Recent years have brought about a rise in consumer and researcher awareness on health-promoting properties of vegetables and their role in the human diet (Krochmal-Marczak et al. 2017). They are an invaluable source of e.g., exogenous antioxidants necessary to balance off free radicals (Howard et al. 2000, Perucka et al. 2004). Many of those compounds are only present in plants (Kosiorek et al. 2013). The fruits of both sweet and hot pepper cultivars provide e.g. vitamins C, E and beta-carotene, chlorophylls, polyphenols or lycopene that show antioxidant activity and protect the body against the harmful effects of free radicals (De Marino et al. 2006, Topuz and Ozdemir 2007, Nazarro et al. 2009, Buczkowska and Michałojć 2012, Baenas et al. 2019), which translates into the prevention of cardiovascular and neoplastic diseases, neurological disorders (Navarro et al. 2006, Sun et al. 2007), and diabetes (Howard et al. 2000, Perucka et al. 2004). Proper growth and development of *Capsicum annum* require warm and sunny weather and the appropriate amount of water, which is rather challenging in a temperate climate. However, despite these requirements, field cultivation of *Capsicum annum*, mainly

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for processing purposes, grows in popularity. There are numerous cultivars intended for field cultivation without covers that provide yield satisfactory in terms of size and quality. The biological value of pepper depends on e.g., genetic factors, climatic conditions or maturation degree (Deep et al. 2007, Golcz et al. 2009, Nazarro et al. 2009). Pepper fruits can be eaten at various stages of maturity (Navarro et al. 2006). Their maturation degree affects their biological composition (Conforti et al. 2006), and the content of antioxidants (Howard et al. 2000). The global market offers numerous cultivars of *Capsicum annum* (Conforti et al. 2006), but new, most valuable cultivars that can be grown in different places around the world are constantly being sought (Żurawik et al. 2020). Considering the above remarks, we decided to assess the antioxidant properties of Polish and Bulgarian cultivars of pepper grown in moderate climate conditions and collected at different stages of maturity.

MATERIAL AND METHODS

A field experiment aimed at comparing the antioxidant properties of selected sweet and hot pepper cultivars was conducted in 2016–2018 at the Vegetable Research Station in Dołuje (14°41'E, 53°43'N) belonging to the West Pomeranian University of Technology in Szczecin. The investigation included five Polish cultivars: Roberta, Marta Polka, Etiuda, Trapez – sweet cultivars, and Cyklon – hot cultivar, and five Bulgarian cultivars: Bulgarski Ratung, Sivriya, Kurtovska Kapiya, Delikates – sweet cultivars, and Dzuliunska Shipka – hot cultivar. The seeds of Polish cultivars were purchased in Poland, while those of Bulgarian cultivars were imported from Bulgaria as part of them to cooperate with the Agricultural University in Plovdiv.

The experimental design involved randomised subblocks with three repetitions. The area of a single plot was 1.44 m^2 (1.2 m × 1.2 m).

Pepper seedlings were produced from seeds sown into seed trays in the third decade of March (2016 – 21 March, 2017 – 25 March, 2018 – 20 March) and cultivated in a heated greenhouse. After four weeks, the seedlings were transferred into pots 10 cm diameter filled with a vegetable substrate of pH 5.4–6.0 and supplemented with macronutrient fertiliser NPK (14-16-18) + Mg (5) at 0.6 kg/m^3 and micronutrient fertiliser at 0.2 kg/m^3 (Sterlux BN-72/0520-11, Hollas, Pasłęk, Poland). During growth the seedlings were fed once with a liquid fertiliser Florovit N 3.0%; K 2.0%; Cu 70 mg/L; Fe 400 mg/L, Mn 170 mg/L; Mo 20 mg/L; Zn 150 mg/L (INCO, Warsaw, Poland). Well-developed seedlings with no woody stems and visible first flower buds, on average 25 cm tall, were transferred into the field to their final location in the first week of June (2016 – 5 June, 2017 – 2 June, 2018 – 6 June). They were planted on flatbeds at 40 × 40 cm spacing and covered for two weeks with polypropylene nonwoven fabric weight 17 g/m^2. The fertilisation scheme was based on a previous soil analysis (Table 1) and any deficiencies were supplemented up to the levels recommended for field cultivation of pepper. Before planting, the soil was fertilised with multicomponent mineral fertiliser Azofoska (N 13.6, P 2.8, K 15.9, Mg 2.7, B 0.045, Cu 0.180, Fe 0.17, Mn 0.27, Mo 0.040, Zn 0.045), at a dose of 70.0 g/m^2 (2016), 81.0 g/m^2 (2017), and 95.0 g/m^2 (2018). During vegetation and before flowering, top dressing with Azofoska at 35 g/m^2 was applied. Pepper fruits were harvested three times: in 2016 on 25 August, and 11 and 23 September, in 2017 on 23 August and 10 and 19 September, and in 2018 on 25 August and 7 and 21 September.

We collected well-developed, cultivar-typical green and coloured fruits. From each cultivar and replicate, we randomly selected healthy fruits that were dried in a laboratory oven SLN 115Eco, at 40 °C (POL-ECO AP., Wodzisław Śląski, Poland) and then ground in a WŻ-1 laboratory mill (ZBPP, Bydgoszcz, Poland). Pooled samples prepared this way were used to determine the content of:

– vitamin C as ascorbic acid – by the Tillman's method, consisting in the reduction of a coloured

Table 1. Chemical properties and mineral composition of the soil collected from the experimental field in consecutive years of the study before starting the experiment

Year	pH_{H_2O}	Salinity (g NaCl/L)	Bulk density (kg/L)	Mineral content (mg/L of soil)					
				$N\text{-}NO_3$	P	K	Ca	Mg	Cl
2016	7.4	0.49	1.76	24	60	156	3 549	101	15
2017	7.7	0.19	1.71	17	75	130	3 342	123	16
2018	7.4	0.21	1.74	30	28	79	3 160	125	17

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solution of 2,6-dichlorophenolindophenol to a colourless compound;
– assimilation pigments – carotenoids, chlorophyll *a*, chlorophyll *b*, total chlorophyll by Lichtenthaler and Wellburn method (1983), the resulting extract underwent absorbance at 441, 646, 652 and 663 nm and their content was calculated using pigment specific formulas;
– polyphenols – spectrophotometric method with Folin-Ciocalteu;
– antioxidant activity – by a reduction of free radicals (DPPH (1,1-diphenyl-2-picrylhydrazyl)), according to Yen and Chen (1995), and DPPH inhibition percentage was calculated according to the formula provided by Rossi et al. (2003).

Results of chemical analyses were subjected to Tukey's test and confidence intervals for $\alpha = 0.05$ confidence level were calculated.

RESULTS AND DISCUSSION

Vitamin C is a functional and nutritional compound of pepper fruits, an antioxidant and a biologically active compound (Rietjens et al. 2002, Topuz and Ozdemir 2007). According to Buczkowska and Najda (2002), the mean content of vitamin C in sweet pepper is cultivar independent and amounts to 180 mg/100 g DM (dry matter). In our study, its mean content in the investigated pepper cultivars was 52.4 mg/100 DM lower (Table 2). In the study by Buczkowska and Najda (2002), green fruits of cv. Delphin F_1 pepper contained 120 mg/100 g DM, and red fruits 350 mg/100 g DM of vitamin C. Our experiment confirmed these reports. Regardless of the tested cultivar, the coloured fruits accumulated by 15.8 mg/100 g DM more vitamin C than the green ones. Our findings confirmed those published by Nazarro et al. (2009), who detected different amounts of vitamin C in the extracts of investigated pepper cvs. Teseo and Lampo (0.079 and 0.067 mg/mL, respectively). Golcz and Kozik (2004) revealed that vitamin C content in sweet pepper depended on the fruit maturity. They detected its lowest amounts in green and the highest amounts in red fruits. According to Janda et al. (2015), the content of vitamin C in plant products also depends on the country of their origin. This information was corroborated by the results of our study, as all tested Bulgarian cultivars were poorer in vitamin C than Polish ones. Buczkowska and Najda (2002) claimed that hot pepper fruits at their physiological maturity were richer in vitamin C than sweet peppers. Similar conclusions were published by Golcz and Kozik (2004), who found considerable differences in vitamin content in sweet and hot pepper. These differences were not confirmed in our study, in which the greatest content of vitamin C was found in coloured fruits of sweet cv. Etiuda, and the lowest in hot cv. Dzuliunska Shipka (50.4% less). Low levels of vitamin C were also determined for sweet cv. Kurtovska Kapiya (49.1% less).

Vegetables, including pepper fruits, are a rich source of polyphenols, i.e., compounds that play an important role in the protection against many civilization diseases (Howard et al. 2000, Perucka et al. 2004, Kozłowska and Ścibisz 2012). Kosiorek et al. (2013) demonstrated that polyphenols could be a potential source of plant nutraceuticals, i.e., substances with health-promoting and prophylactic effects. In our study (Table 3), the mean content of polyphenols in the investigated pepper cultivars reached 1 218.4 mg/kg DM, but it differed depending on the cultivar. The cultivar most abundant in polyphenols, irrespective of fruit colouration, was cv. Sivriya, but cvs. Delikates and Bulgarski Ratung were also rich in these compounds. The lowest level of polyphenols was determined for cv. Dzuliunska Shipka, but cvs. Kurtovska Kapiya, Marta Polka and Trapez, Cyklon and Etiuda were also poor in these compounds.

Table 2. The content of ascorbic acid (mg/100 g dry matter) in pepper cultivars depending on fruit maturity (mean for the years 2016–2018)

Cultivar (C)	Fruit colouration (FC)		
	green	coloured	mean
Roberta	245.3	195.1	220.2
Trapez	179.8	226.0	202.9
Marta Polka	160.7	192.4	176.6
Etiuda	207.3	270.3	238.8
Cyklon	168.7	141.0	154.9
Bulgarski Ratung	126.9	173.5	150.2
Sivriya	108.2	156.4	132.3
Kurtovska Kapiya	100.0	142.9	121.5
Delikates	115.7	151.5	133.6
Dzuliunska Shipka	106.4	134.1	120.3
Mean	151.9	178.3	165.1
$LSD_{0.05}$	C – 9.232; FC – 2.535; C/FC – 13.056; FC/C – 22.3		

LSD – least significant difference

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Table 3. The content of polyphenols (mg/100 g dry matter) in pepper cultivars depending on fruit maturity (mean for the years 2016–2018)

Cultivar (C)	Fruit colouration (FC)		
	green	coloured	mean
Roberta	1 253.9	1 260.0	1 256.9
Trapez	1 057.6	1 144.8	1 101.2
Marta Polka	1 097.2	1 103.0	1 100.1
Etiuda	1 113.1	1 182.1	1 147.6
Cyklon	1 096.3	1 180.4	1 138.3
Bulgarski Ratung	1 365.9	1 435.0	1 400.4
Sivriya	1 412.5	1 469.4	1 441.0
Kurtovska Kapiya	1 135.5	1 063.8	1 099.7
Delikates	1 489.9	1 319.8	1 404.8
Dzuliunska Shipka	1 083.7	1 103.3	1 093.5
Mean	1 210.6	1 226.1	1 218.4
$LSD_{0.05}$	C – 81.4; FC – ns; C/FC – 115.1; FC/C – 22.3		

LSD – least significant difference; ns – non-significant differences

Carotenoids are a group of chemicals with strong antioxidant properties and they may positively affect the human body (Russo and Howard 2002). These natural substances counteract free radical damage by inhibiting free radical formation and their conversion into inactive derivatives (Gryszczyńska et al. 2011). Carotenoids are present in large amounts in vegetables, e.g., kale, spinach, red pepper or cabbage. Pepper owes its intense red colour to the presence of carotenoids, including the pigments of capsanthin and capsorubin (Topuz and Ozdemir 2007, de Azevedo-Meleiro and Rodriquez-Amaya 2009). Carotenoid consumption improves the human diet and limits numerous civilization diseases (Eggersdorfer and Wyss 2018). The mean content of carotenoids in our study, irrespective of the cultivar and colouration degree, reached 306.5 mg/kg DM (Table 4). Russo and Howard (2002) and Gryszczyńska et al. (2011) claimed that carotenoid content in plants is determined by species, growing site, sunlight, harvest time and cultivar. In our study, carotenoids were more abundant (on average by 294.9 mg/kg DM) in coloured fruits *vs*. those well developed, but green. Irrespective of maturity degree, the greatest amount of carotenoids were found in the fruits of cv. Dzuliunska Shipka, and the smallest in cvs. Sivriya and Marta Polka. Carotenoid level in green fruits was the highest in cv. Dzuliunska Shipka and the lowest in cv. Sivriya, with a considerable difference of 156.6 mg/kg DM. In the coloured fruits, carotenoids were the most abundant in cv. Dzuliunska Shipka, and the least in cv. Marta Polka, also with a considerable difference of 466.1 mg/kg DM.

Conforti et al. (2006) stated that the green colour of pepper fruits is mostly due to the content of chlorophyll and carotenoids typical of chloroplasts. Our study showed (Table 5) that green fruits contained significantly more chlorophyll *a*, chlorophyll *b* and total chlorophyll than the coloured ones, by 203.2, 78.7 and 316.5 mg/kg DM, respectively. Moreover, Skwaryło-Bednarz and Krzepiłko (2009) reported that the content of chlorophyll *a* was two to four times higher than the content of chlorophyll *b*. This claim was confirmed in our study, where the mean content of chlorophyll *a*, irrespective of cultivar and colouration degree, was 112.0 mg/kg DM and exceeded that of chlorophyll *b* by 2.1 times. The greatest accumulation of chlorophyll *a*, irrespective of the fruit colouration, was detected in cv. Cyklon. High amounts were also found in cvs. Trapez and Kurtovska Kapiya, while cv. Sivriya was the least abundant in this pigment (mean content 118.1 mg/kg DM). Among green fruits, the highest level of chlorophyll *a* was determined in cv. Cyklon, with equally high amounts in cvs. Trapez and Kurtovska Kapiya, and again the lowest level in cv. Sivriya. The differences were substantial and reached 240.0, 238.5 and 222.5 mg/kg DM, respec-

Table 4. The content of carotenoids (mg/kg dry matter) in pepper cultivars depending on fruit maturity (mean for the years 2016–2018)

Cultivar (C)	Fruit colouration (FC)		
	green	coloured	mean
Roberta	125.8	529.3	327.6
Trapez	175.3	473.6	324.5
Marta Polka	119.6	247.2	183.4
Etiuda	188.1	385.2	286.6
Cyklon	195.4	598.8	397.1
Bulgarski Ratung	160.4	468.0	314.2
Sivriya	67.8	284.1	175.9
Kurtovska Kapiya	185.7	381.7	283.7
Delikates	147.5	457.5	302.5
Dzuliunska Shipka	224.4	713.3	468.8
Mean	159.0	453.8	306.4
$LSD_{0.05}$	C – 18.10; FC – 4.97; C/FC – 25.59; FC/C – 4.97		

LSD – least significant difference

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Table 5. The content of assimilation pigments (mg/kg dry matter) in pepper cultivars depending on fruit maturity (mean for the years 2016–2018)

Cultivar (C)	Fruit colouration (FC)	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Roberta	green	165.2	85.6	296.6
	coloured	18.3	9.7	44.3
	mean	91.8	47.6	170.5
Trapez	green	291.0	138.9	486.5
	coloured	11.2	7.8	27.3
	mean	151.1	73.4	256.9
Marta Polka	green	168.1	66.9	269.4
	coloured	13.2	15.2	27.7
	mean	90.6	41.0	148.6
Etiuda	green	248.5	105.3	407.6
	coloured	9.7	12.5	20.4
	mean	129.1	58.9	214.0
Cyklon	green	292.5	77.0	442.3
	coloured	10.3	26.4	30.9
	mean	151.4	51.7	236.6
Bulgarski Ratung	green	203.6	100.0	372.4
	coloured	7.3	13.5	28.9
	mean	105.5	56.7	200.7
Sivriya	green	52.5	22.7	86.8
	coloured	8.2	11.6	23.3
	mean	30.4	17.2	55.1
Kurtovska Kapiya	green	275.0	94.9	425.8
	coloured	10.9	15.2	31.0
	mean	143.0	55.0	228.4
Delikates	green	244.7	175.0	377.3
	coloured	7.3	11.1	21.8
	mean	126.1	93.1	199.5
Dzuliunska Shipka	green	194.7	57.1	278.9
	coloured	7.6	13.3	22.9
	mean	101.2	35.2	150.9
Mean for FC	green	213.6	92.3	344.33
	coloured	10.4	13.6	27.8
$LSD_{0.05}$ C		17.23	9.41	13.34
$LSD_{0.05}$ FC		4.73	2.58	3.66
$LSD_{0.05}$ C/FC		24.36	13.30	18.87
$LSD_{0.05}$ FC/C		4.73	2.58	3.66

LSD – least significant difference

tively. The highest content of chlorophyll *b*, both in green fruit and irrespective of their colouration, was found in cv. Delikates, and the lowest in cv. Sivriya. This cultivar was also the least abundant in total chlorophyll, by 201.8 mg/kg DM lower than in cv. Trapez accumulated the greatest amounts of total chlorophyll irrespective of its fruit colouration. The same tendency was confirmed for green fruits.

Mean antioxidant capacity reached 28.0% inhibition of DPPH (Table 6). In all cultivars, it was sig-

Table 6. Antioxidant capacity as percentage inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals in pepper cultivars depending on fruit ripeness (mean for the years 2016–2018)

Cultivar (C)	Fruit colouration (FC)		
	green	coloured	mean
Roberta	29.81	31.80	30.81
Trapez	24.87	28.10	26.48
Marta Polka	32.38	31.33	31.86
Etiuda	25.11	30.68	27.90
Cyklon	17.68	17.05	17.37
Bulgarski Ratung	38.68	36.77	37.73
Sivriya	47.43	36.22	41.83
Kurtovska Kapiya	23.73	24.77	24.25
Delikates	43.43	29.60	36.52
Dzuliunska Shipka	16.48	18.90	17.69
Mean	29.96	28.52	29.24
$LSD_{0.05}$	C – 1.918; FC – 0.527; C/FC – 2.712; FC/C – 0.527		

LSD – least significant difference

nificantly on average by 1.0% higher in coloured than green fruits. The greatest antioxidant capacity was confirmed for cv. Sivriya, and the smallest for cv. Cyklon and cv. Dzuliunska Shipka. Differences in the antioxidant capacity of pepper were also reported by Nazarro et al. (2009), as it reached 11.7 (% DPPH) for cv. Teseo and 20.5 for cv. Lampo. As far as the antioxidant capacity was concerned, in green fruits, it was the highest in cv. Sivrija and the lowest in cvs. Dzuliunska Shipka and Cyklon. The differences were substantial and reached 30.9% and 29.7% inhibition of DPPH. In coloured fruits, the highest antioxidant capacity was detected in cv. Bulgarski Ratung and cv. Sivriya, while cv. Cyklon and cv. Dzuliunska Shipka showed the lowest value of this parameter.

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