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RELATIONSHIPS OF THE CAPSAICINOID CONTENT BETWEEN THE FRUIT PARTS OF HOT PEPPER (*Capsicum annuum* L.)

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Abstract. Capsaicinoids are secondary metabolites characteristic of plants of the genus *Capsicum* and are only found in the pepper fruit. Their biosynthesis occurs mainly in the placental cells as well as in the interlocular septa of the fruit. In the present study, the content of capsaicinoids (capsaicin and dihydrocapsaicin) was determined by HPLC in two hot pepper cultivars ('Orkan' and 'Bronowicka Ostra'), identifying the percentage of these compounds in the whole fruit and in the following fruit parts: placenta, seeds, pericarp, and pedicle. Significantly more capsaicinoids were found in 'Bronowicka Ostra' peppers (262 mg·kg^{-1}) compared to 'Orkan' peppers (175 mg·kg^{-1}). The highest capsaicin and dihydrocapsaicin content was found in the placenta (401 mg·kg^{-1}). The capsaicin content in the whole fruit and its parts is on average more than 10 times higher in relation to the dihydrocapsaicin content. Fruit harvest time contributes to the differences in the capsaicinoid content in the individual fruit parts. A higher capsaicin content was shown in the pericarp and seeds, while the dihydrocapsaicin content was higher in the pedicle of peppers harvested at the later date.

Key words: Solanaceae, fruit, cultivar, harvest time, secondary metabolites

INTRODUCTION

In morphological and anatomical terms, the fruits of plants of the family Solanaceae, including pepper (*Capsicum annuum* L.), are true berries. The center of origin and variety of the species *C. annuum* is Mexico [Vera-Guzmán et al. 2011, Kraft et al. 2014]. The pepper fruit is characterized by high morphological variation, primarily in

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terms of its size and shape. These traits are determined by the affiliation to a specific species and botanical variety [Pabón-Mora and Litt 2011, Weryszko-Chmielewska and Michałojć 2011, Giuffrida et al. 2013, Zhigila et al. 2014]. Most of the currently grown sweet and hot pepper cultivars are classified in 5 botanical varieties of the species *Capsicum annuum*: var. *annuum*, var *grossum*, var. *abbreviatum*, var. *accuminatum*, and var. *glabriusculum* [Zhigila et al. 2014]. In the tropical and subtropical regions where wild forms of pepper are collected and introduced into cultivation, continuous research is conducted to identify and evaluate the chemical composition of their fruits [Vera-Guzmán et al. 2011]. When evaluating the morphological characteristics and biological value of the fruits of 32 pepper lines, Wahyuni et al. [2011] found that these genotypes represented four species of the genus *Capsicum*: *C. annuum*, *C. frutescens*, *C. chinense*, and *C. baccatum*. The fruits of the lines that contained capsaicinoids were characterized by small weight and size. In hot pepper cultivars classified as chili peppers ('Cayenne', De Arbol', 'Hot Siberian'), the fruits are very small and their fresh weight ranges from 0.4 to 4.5 g [Mueller-Seitz 2008].

The hot pepper fruit is characterized by a hot and pungent taste which is determined by the content of capsaicinoids. These are biologically active substances classified as alkaloids and specific only to plants of the genus *Capsicum*; their presence determines the pharmacological usefulness and culinary use of hot peppers [Reyes-Escogido et al. 2011]. In plants of the genus *Capsicum*, capsaicinoid biosynthesis occurs in the fruit and is mainly located in the placental cells as well as in the interlocular septa [Pandhair and Sharma 2008, Nowaczyk et al. 2006a, b]. From the placental epidermis, these metabolites are transferred to the pericarp, seeds, and pedicle. In most cultivars, however, the highest capsaicinoid content is found in the placenta [Cisneros-Pineda et al. 2007, Wahyuni et al. 2011, Rahman and Inden 2012, Ben Mansour-Gueddes 2012]. Only a trace content of these substances can be found in the vegetative organs (leaves and stems) [Estrada et al. 2002]. The production of capsaicinoids starts immediately after fruit set. Some epidermal cells of the placenta take on the character of secretory glands in which capsaicinoid biosynthesis occurs. Green fruits at the initial ripe stage, which is most frequently 35–50 days after fruit set, contain most capsaicinoids [Contreras-Padilla and Yahia 1998, Estrada et al. 1998, Sung et al. 2005, Ben Mansour-Gueddes 2012, Iqbal and Amjad 2013].

The main components in the capsaicinoid mixture are capsaicin and dihydrocapsaicin which, depending on the cultivar's genotype and affiliation to the species *Capsicum*, can even account for 60 to 90% of the total amount of capsaicinoids [Estrada et al. 1998, Zewide and Bosland 2000, Topuz and Ozdemir 2007, Wahyuni et al. 2011, Wesołowska et al. 2011, Giuffrida et al. 2013]. In chemical terms, capsaicin is a derivative of vanillylamine, an amide-type compound classified as an alkaloid. Other capsaicinoids: homocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and pelargonic acid vanillylamide, are identified in smaller amounts in hot pepper [Topuz and Ozdemir 2007, Wesołowska et al. 2011, Meckelmann et al. 2013]. Capsaicinoids have a strongly irritating effect on the skin and mucous membranes, causing local hyperaemia and a burning pain, and therefore the medicinal raw material from the hot pepper fruit (*Capsici fructus*) is used as a calefacient and an anti-inflammatory agent. The active compounds of pepper also affect the functioning of the digestive system by increasing gas-

tric juice secretion and improving the functioning of the stomach and intestinal peristalsis [Reyes-Escogido et al. 2001]. Hot peppers in fresh and dried form are also of great culinary importance as a seasoning, because they increase the value and attractiveness of dishes due to their unique taste qualities [Tsuchiya 2001, Kohlmünzer 2007].

The content of capsaicinoids in hot peppers and their quantitative relationships are primarily determined by the genetic traits of a given cultivar [Zewide and Bosland 2000, Ben Mansour-Gueddes 2012, Giuffrida et al. 2013]. They can also be modified by thermal conditions [Contreras-Padilla and Yahia 1998, Rahman and Inden 2012, González-Zamora et al. 2013], fertilization [Estrada et al. 1998, Rahman and Inden 2012], water deficit during the fruit set period [Estrada et al. 1999, Sung et al. 2005], ripeness stage, and harvest time [Gnayfeed et al. 2001, Gibbs and O'Garro 2004, Kirschbaum-Titze et al. 2002, Mueller-Seitz et al. 2008]. The aim of the present study was to determine and compare the capsaicinoid content in the seeds, placenta, pericarp and pedicle of the fruits of two hot pepper cultivars harvested at fully ripe stage. The experiment also evaluated the effect of fruit harvest time on capsaicinoid content.

MATERIALS AND METHODS

Capsaicinoid content was determined in the fruits of two Polish hot pepper cultivars: Orkan and Bronowicka Ostra. Peppers were obtained from a field experiment conducted in 2007 which investigated the effect of production method on the earliness, quantity and quality of fruit yield. The field experiment was carried out in a private horticultural farm near Lublin (51.35°N, 22.35°E) on a soil with a pH of 6.7, derived from loess deposits on Cretaceous marls, and with an organic matter content of 1.8%. In the autumn, the field was fertilized with manure at a rate of 30 $t \cdot ha^{-1}$. In the spring, mineral fertilization was applied to supplement the content of nutrients to the following levels ($mg \cdot dm^{-3}$): N – 100; P – 75; K – 210; Ca – 1600. The pepper crop was top-dressed twice with Florovit (0.5%) and twice with calcium nitrate (1%). Seedlings were planted in the field in the last days of May, at a spacing of 0.35 × 0.67 m. The area of each plot, where 40 plants were grown, was 9.4 m^2. Peppers were harvested at fully ripe stage and when turning red, successively every 9–10 days. The pepper harvest was carried out from the third 10-day period of August to the second 10-day period of October. Fruits picked during the first 10 days of September and the first 10 days of October were used for analysis. At these harvest dates, 30 fruits of each cultivar were randomly collected in 4 replicates and the fruit fresh weight was determined (g). Next, in each replicate 20 fruits were randomly selected for further investigation and the fresh weight (g) of the individual parts of the fruit (seeds, placenta, pericarp, and pedicle) was determined and their percentages in the fresh weight of the whole fruit were calculated (%). The randomly selected samples of the whole fruit and of the excised fruit parts, from each treatment and replicate, were dried in a special-purpose microprocessor-controlled drying oven at a temperature of 60°C. The fruit drying period lasted 3 days. Subsequently, the dry matter content in the whole fruit and in the individual fruit parts was determined (%).

The determination of the content of capsaicinoids (capsaicin and dihydrocapsaicin) in the whole fruit and in the fruit parts was carried out by HPLC in 3 replicates accord-

ing to the procedure described by Collins et al. [1995]. The dried fruits were ground immediately before the extraction of capsaicinoids. The samples of the dried fruits and their parts with a weight of 1.5 g were quantitatively transferred to 50 ml round-bottom flasks and immersed in 15 ml of acetonitrile (CAN). The extraction was carried out in a water bath at 80°C for 4 hours, shaking by hand every hour. The obtained supernatant was filtered through Waters-Millex 0.45 ym filters. A liquid chromatograph with a Li Chrom C_{18} column (250 × 4.6 mm) filled with silica gel was used for qualitative and quantitative analysis of the capsaicinoids. HPLC-grade methanol was used as an eluent. The standards used: 8-methyl-N-vanillyl-6-none mamide (capsaicin) and N-vanillylnonemamide (dihydrocapsaicin), were manufactured by Sigma-Aldrich.

The obtained results were statistically analyzed by two-way analysis of variance (cultivar × fruit harvest time). The significance of differences was determined by Tukey's multiple comparisons at a significance level of 5%. To show the correlations of the capsaicinoid content (capsaicin and dihydrocapsaicin) in the whole hot pepper fruit and in its excised parts, correlation coefficients were calculated at a significance level of 5 and 1%.

RESULTS AND DISSCUSSION

The hot pepper cultivars evaluated in this study significantly differed in the fruit fresh weight (tab. 1). The average fruit weight of the cultivar Orkan was 34.6 g, whereas for cv. Bronowicka Ostra it was on average 21.3 g. Fruits of most of the hot pepper cultivars grown in Poland for pharmacopoeial material and for culinary purposes usually have an average fresh weight in the range from 10 to 50 g [Golcz and Kujawski 2004a, b, Orłowski et al. 2004, Nowaczyk et al. 2006a, b, 2009]. Comparing the yield structure and biological value of hot peppers harvested once, Buczkowska and Łabuda [2015] showed that the fruits of the same cultivars were characterized by a slightly higher weight. In a study on evaluation of the yield of several hot pepper cultivars, on the other hand, Golcz and Kujawski [2004a] obtained 'Orkan' peppers with a much lower weight (20.8 g). This shows that environmental growing conditions can have a modifying effect on the fruit weight of a specific hot pepper cultivar.

Among the analyzed parts of the hot pepper fruit, the pericarp had the highest fresh weight, on average 27.8 g ('Orkan') and 15.6 g ('Bronowicka Ostra'). The average percentage of this fruit part in the fresh weight of the whole fruit was respectively 80.4% ('Orkan') and 72.3% ('Bronowicka Ostra'). The weight of the other fruit parts was much smaller and accounted for the following percentages of the total fruit weight, on average: in the case of cv. Orkan placenta – 8.1%, seeds – 6.9%, pedicle – 4.6%, while in the case of cv. Bronowicka Ostra it was respectively 9.4, 10.3, and 7.1%. In a study on the morphology and chemical composition of chili peppers conducted in Mexico, Cisneros-Pineda et al. [2007] found comparable proportions for the percentage of the pericarp, placenta and seeds in the total fruit weight. Likewise, in a study on evaluation of the capsaicinoid content in the individual fruit parts of hot pepper Mueller-Seitz et al. [2008] showed the pericarp to have the highest percentage (80%) in the total fruit weight.

Table 1. Fruit fresh weight and fruit dry matter content of hot pepper depending on the harvest time

Cultivar		Part of fruit	Fresh weight (g) harvest time			Dry matter (%) harvest time		
			1st dec. September	1st dec. October	mean	1st dec. September	1st dec. October	mean
Orkan		pericarp	29.8	25.8	27.8	11.28	15.88	13.58
		placenta	2.4	3.2	2.8	12.50	1312	12.81
		seeds	2.5	2.3	2.4	56.00	63.48	59.74
		peduncle	2.0	1.2	1.6	17.50	19.17	18.34
		whole fruit	36.7	32.5	34.6	14.70	16.55	15.63
Bronowicka Ostra		pericarp	16.1	15.0	15.6	13.98	15.20	14.59
		placenta	1.6	2.4	2.0	11.25	11.67	11.46
		seeds	2.4	2.0	2.2	49.58	56.60	53.09
		peduncle	2.0	1.0	1.5	20.00	21.00	20.05
		whole fruit	22.1	20.4	21.3	18.19	19.11	18.65
Mean		pericarp	22.9	20.4	21.7	12.63	15.54	14.09
		placenta	2.0	2.8	2.4	11.88	12.49	12.18
		seeds	2.5	2.2	2.3	52.79	60.04	56.41
		peduncle	2.0	1.1	1.6	18.75	20.09	19.42
		whole fruit	29.4	26.5	28.0	16.45	17.83	17.14
$NIR_{0.05}$	Cultivar (A)	pericarp			2.53			n.s.
		placenta			0.42			n.s.
		seeds			n.s.			4.274
		peduncle			n.s.			n.s.
		whole fruit			2.98			1.376
	Harvest time (B)	pericarp			n.s			1.252
		placenta			0.42			n.s.
		seeds			n.s.			n.s.
		peduncle			0.31			n.s.
		whole fruit			n.s.			n.s.
	Interaction (A × B)	pericarp			4.88			2.125
		placenta			n.s.			n.s.
		seeds			n.s.			5.278
		peduncle			0.60			n.s.
		whole fruit			5.76			3.732

n.s. – the difference between the average statistically insignificant

Harvest time was demonstrated to have an effect on the fresh weight of the analyzed parts of the fruit of the hot pepper cultivars Orkan and Bronowicka Ostra (tab. 1). Peppers harvested in the first 10 days of October were characterized by a significantly higher fresh weight of the placenta and a lower weight of the pedicle compared to those harvested in the first 10 days of September.

The evaluated cultivars were found to vary in terms of fruit dry matter content (tab. 1). A higher dry matter content was determined in cv. Bronowicka Ostra peppers (on average 18.65%) than in cv. Orkan peppers (on average 15.63%). The determined

dry matter content is similar to that found in the studies conducted under the conditions of Spain [Ayuso et al. 2008] and Italy [Giuffrida et al. 2013], and higher compared to the results of the research carried out in Turkey [Topuz and Ozdemir 2007]. In turn, some previous studies [Buczkowska and Najda 2002, Golcz and Kujawski 2004a, Buczkowska and Łabuda 2015] found a lower level of dry matter in the fruit of the pepper cultivars studied. This indicates that the dry matter content in the pepper fruit is mainly dependent on the cultivar's genotype, but it can also be modified by environmental growing conditions.

In the fruit parts analyzed, the highest dry matter content was found in the seeds: on average 59.74% ('Orkan') and 53.09% ('Bronowicka Ostra') (tab. 1). The dry matter content in the other fruit parts proved to be much lower and, depending on the harvest time and cultivar, it was as follows on average: placenta 11.25–13.12%; pericarp 11.28–15.88%; and pedicle 17.50–21.00%. Similar relationships were demonstrated by Cisneros-Pineda et al. [2007]. More dry matter was found in all the fruit parts in peppers harvested in the first 10 days of October (tab. 1). Harvest time was found to have a significant effect on dry matter content only for the dry matter content in the pericarp.

The hot pepper cultivars studied significantly differed in terms of the capsaicinoid content in the fruit (tab. 2). Cv. Bronowicka Ostra peppers accumulated significantly more capsaicin (on average 240 $mg \cdot kg^{-1}$) in relation to the content of this alkaloid in cv. Orkan peppers (on average 162 $mg \cdot kg^{-1}$). The capsaicin contents in peppers of the Polish cultivars found in this study were lower than or comparable to the results of previous studies [Golcz and Kujawski 2004a, b, Perucka and Materska 2003, Buczkowska et al. 2013, 2015]. Capsaicinoid biosynthesis is dependent on the genotype [Zewide and Bosland 2000, Topuz and Ozdemir 2007] as well as on environmental factors [Estrada et al. 1999, Rahman and Inden 2012], including light conditions [Gangadhar et al. 2012]. Due to this, the content of these compounds (11.0–1445.5 $mg \cdot kg^{-1}$) and the related pungency of the fruits of various pepper cultivars vary strongly [Zewide and Bosland 2000, Topuz nad Ozdemir 2007, Ayuso et al. 2008, González-Zamora et al. 2013]. When determining the capsaicin content in the individual parts of the fruit, most capsaicin was found in the placenta of the hot pepper fruit (tab. 2). The average capsaicin content in this fruit part was 375 $mg \cdot kg^{-1}$ of dry matter. A significantly higher content of capsaicin was determined in the placenta of cv. Bronowicka Ostra peppers harvested both in the first 10 days of September (454 $mg \cdot kg^{-1}$) and in the first 10 days of October (466 $mg \cdot kg^{-1}$) compared to its content in the placenta of cv. Orkan peppers (respectively 273 $mg \cdot kg^{-1}$ and 296 $mg \cdot kg^{-1}$). The capsaicin content in the placenta of the fruit of cv. Bronowicka Ostra proved to be on average 40% higher compared to the other parts of the fruit. The average content of this compound was as follows: pedicle 172 $mg \cdot kg^{-1}$; pericarp 173 $mg \cdot kg^{-1}$; and seeds 174 $mg \cdot kg^{-1}$. Significant differences in the capsaicin content in the pericarp were shown between the evaluated cultivars. Cv. Bronowicka Ostra was determined to have more of this compound (on average 183 $mg \cdot kg^{-1}$) compared to cv. Orkan (on average 164 $mg \cdot kg^{-1}$). More capsaicin was found in the whole fruit in the case of peppers harvested at the later date. Harvest time was found to have a significant effect on capsaicin content only in relation to the content of this alkaloid in the seeds and in the pericarp (tab. 2). The obtained results are in agreement with those found by other authors [Cisneros-Pineda et al. 2007, Wahyuni et al. 2011, Ben

Mansour-Gueddes et al. 2012], indicating the source of capsaicin biosynthesis (placenta) and further transport of this compound to the other parts of the fruit.

Table 2. Capsaicin and dihydrocapsaicin content in the fruit parts of hot pepper depending on the harvest time

Cultivar	Part of fruit	Capsaicin (mg·kg^{-1})			Dihydrocapsaicin (mg·kg^{-1})		
		harvest time			harvest time		
		1st dec. September	1st dec. October	mean	1st dec. September	1st dec. October	mean
Orkan	pericarp	153	175	164	14	16	15
	placenta	273	296	271	19	19	19
	seeds	130	227	179	6	10	8
	peduncle	193	144	168	12	14	13
	whole fruit	158	165	162	12	14	13
Bronowicka Ostra	pericarp	160	205	183	15	20	18
	placenta	454	478	466	32	34	33
	seeds	145	195	170	8	11	10
	peduncle	129	223	176	12	20	16
	whole fruit	221	260	240	20	24	22
Mean	pericarp	157	190	173	14	18	16
	placenta	364	387	375	26	26	26
	seeds	137	211	174	7	11	9
	peduncle	161	183	172	12	17	15
	whole fruit	190	212	201	16	19	17
	pericarp			10.9			n.s.
Cultivar (A)	placenta			24.0			4.8
	seeds			n.s.			n.s.
	peduncle			n.s.			n.s.
	whole fruit			13.3			2.9
$NIR_{0.05}$	pericarp			10.9			n.s.
Harvest time (B)	placenta			n.s.			n.s.
	seeds			11.4			n.s.
	peduncle			n.s.			3.0
	whole fruit			n.s.			2.9
Interaction (A × B)	pericarp			21.4			n.s.
	placenta			47.0			9.4
	seeds			22.7			n.s.
	peduncle			22.7			5.9
	whole fruit			26.2			6.8

n.s. – the difference between the average statistically insignificant

The dihydrocapsaicin content in the whole fruit of the hot pepper cultivars evaluated was on average 17 mg·kg^{-1} and it was more than 11 times lower compared to the capsaicin content (tab. 2). Significant differences were shown in the dihydrocapsaicin content in the whole fruit between the evaluated cultivars. The average content of this alkaloid in the fruit of cv. Bronowicka Ostra was 22 mg·kg^{-1}, while for cv. Orkan it was 13 mg·kg^{-1}. The dihydrocapsaicin content was similar to that found in previous studies

[Buczkowska et al. 2013, Buczkowska and Łabuda 2015]. In evaluating the content of this alkaloid in the fruit parts, most dihydrocapsaicin was found in the placenta of cv. Bronowicka Ostra peppers (on average 33 mg·kg^{-1}), while its least amount was determined in the seeds of cv. Orkan (on average 8 mg·kg^{-1}). Depending on the cultivar and harvest time, the dihydrocapsaicin content in the individual fruit parts was as follows: pericarp 14–20 mg·kg^{-1}; placenta 19–34 mg·kg^{-1}; seeds 6–11 mg·kg^{-1}; pedicle 12–20 mg·kg^{-1}; and whole fruit 12–24 mg·kg^{-1}. Peppers harvested at the later date were determined to have a higher dihydrocapsaicin content. Harvest time was found to have a significant effect on the content of this compound only in the case of its content in the whole fruit and in the pedicle part. The dihydrocapsaicin content determined in this study and its ratio to capsaicin corroborate with the results of other authors [Cisneros-Pineda et al. 2007, Wahyuni et al. 2011, Ben Mansour-Gueddes et al. 2012] and confirm the thesis that in quantitative terms it is the second largest component of the capsaicinoid mixture of pepper.

Table 3. Quantitative relationship between capsaicin and dihydrocapsaicin in the fruit parts of hot pepper depending on the harvest time

Cultivar	Part of fruit	Harvest time		
		1st dec. September	1st dec. October	Mean
Orkan	pericarp	10.9	10.9	10.9
	placenta	14.4	15.6	15.0
	seeds	21.7	22.7	22.4
	peduncle	16.1	10.3	12.9
	whole fruit	13.2	11.8	12.5
Bronowicka Ostra	pericarp	10.7	10.3	10.8
	placenta	14.2	14.1	14.1
	seeds	18.1	17.1	17.0
	peduncle	10.8	11.1	11.0
	whole fruit	11.1	10.8	10.9
Mean	pericarp	11.2	10.6	10.8
	placenta	14.0	14.9	14.4
	seeds	19.6	19.2	19.3
	peduncle	13.4	10.8	11.5
	whole fruit	11.9	11.2	11.8

Based on the quantitative relationships between the capsaicin content and dihydrocapsaicin content (tab. 3), the ratio of these compounds in the individual fruit parts, depending on the cultivar and harvest date, was shown to be from 10.3:1 to 22.7:1, respectively. Compared to capsaicin, most dihydrocapsaicin was found in the pericarp. In this fruit part, the ratio of capsaicin to dihydrocapsaicin ranged from 10.3:1 to 10.7:1. The least amount of dihydrocapsaicin, on the other hand, was determined in the seeds for which the quantitative ratio for these two capsaicinoids was from 17.1:1 to 22.7:1. It was found that the quantitative relationships between capsaicin and dihydrocapsaicin

in the fruit placenta were dependent on the cultivar and harvest time and they were as follows: from 14.4:1 to 15.6:1 ('Orkan') and from 14.1:1 to 14.2:1 ('Bronowicka Ostra'). Similar relationships were demonstrated in previous studies [Buczkowska et. al 2013, Buczkowska and Łabuda 2015] which found that the capsaicin content exceeded more than a dozen times the dihydrocapsaicin content. The results of other authors' research [Zewide and Bosland 2000, Gibbs and O'Garro 2004, Wang and Bosland 2006] reveal that capsaicinoid content and quantitative ratios between individual compounds are determined by genetic traits, but they do not depend on the fruit weight, even though a higher content is found in fruits set in the higher portions of the plant [Estrada et al. 2002, Mueller-Seitz et al. 2008]. Giuffrida et al. [2013] demonstrated that in peppers of 12 cultivars belonging to three *Capsicum* species (*C. annuum*, *C. chinense*, and *C. frutescens*) the percentage of capsaicin was 50.6–83.7%, while the percentage of dihydrocapsaicin was 14.2–38.8% of the total content of these compounds. In chili peppers grown in Mexico, Cisneros-Pineda et al. [2007] showed a much lower percentage of capsaicin compared to dihydrocapsaicin; the same relationship was shown in Peruvian forms of this type by Meckelmann et al. [2013] as well as for peppers of soft-flesh *Capsicum* spp. genotypes [Nowaczyk et al. 2006a, b, Nowaczyk et al. 2009]. On the other hand, Kirschbaum-Titze et al. [2002] found a similar content of capsaicin (45–52%) in relation to dihydrocapsaicin (36–40%) in cayenne pepper. Estrada et al. [1998] also showed a comparable content of capsaicin in relation to dihydrocapsaicin in cv. Padrón in which the quantitative ratio, depending on mineral fertilization applied, ranged from 1.00:1.00 to 1.88:1.00.

The evaluated cultivars differed in terms of capsaicinoid content. More capsaicin and dihydrocapsaicin were found in 'Bronowicka Ostra' peppers (on average 240 mg·kg^{-1} and 22 mg·kg^{-1}) than in 'Orkan' (on average 162 mg·kg^{-1} and 13 mg·kg^{-1}). The capsaicinoid content determined in the present study is similar to that found for *C. annuum* in other studies [Golcz and Kujawski 2004b, Topuz and Ozdemir 2007, Ayuso et al. 2008, Pandhair and Sharma 2008, Ben Mansour-Gueddes 2012, Iqbal and Amjad 2013, Buczkowska et al. 2013, 2015]. On the other hand, the fruits of cultivars of the following other species: *C. baccatum*, *C. chinense*, and *C. frutescens*, are characterized by a higher capsaicinoid content [Gibbs and O'Garro 2004, Cisneros-Pineda et al. 2007, Mueller-Seitz et al. 2008, Chinn et al. 2011, Wahyuni et al. 2011, Giuffrida et al. 2013, Meckelmann et al. 2013].

The results on the content of capsaicinoids in the individual parts of the hot pepper fruit found in this study prove that the synthesis of these compounds in hot peppers occurs in the placenta and that this part of the fruit is characterized by their highest content. Nowaczyk et al. [2006a, b, 2009] showed a much higher capsaicinoid content in the paste made from the whole fruit of soft-flesh *Capsicum* spp. genotypes compared to the pericarp. This indicates that the significant increase in the content of these compounds in the paste from the whole fruit was an effect of their release from the placenta and interlocular septa. In their research on 32 hot pepper genotypes, Wahyuni et al. [2011] demonstrated that the capsaicinoid concentration in the placenta and in the seeds was 5 times higher than in the pericarp. In evaluating capsaicinoid accumulation in the individual parts of the fruit of cv. Punjab Lal, Pandhair and Sharama [2008] determined the content of capsaicin in the placenta to be 10 times higher compared to its content in

the pericarp and seeds. While Simonovska et al. [2014] showed only two times higher content of capsaicin in placenta than pericarp. Ben Mansour-Gueddes et al. [2012] found that, depending on the cultivar's genotype, the alkaloid content in the placenta accounts for as much as 71.3–90.0%, whereas in the pericarp for 3.7–18.9% and in the seeds for 6.3–9.7% of the total alkaloid content in the fruit.

Table 4. Correlation coefficients between the content of capsaicinoids in the whole fruit and the fruit parts of hot pepper

		Pericarp	Placenta	Seeds	Peduncle	Whole fruit
Capsaicin	pericarp	×	0.8929**	0.4171	0.9268**	0.8320**
	placenta	0.8929**	×	0.1782	0.4433	0.7380**
	seeds	0.4171	0.1782	×	0.7750**	0.7811**
	peduncle	0.9268**	0.4433	0.7750**	×	0.7524**
	whole fruit	0.8320**	0.7380**	0.7811**	0.7524**	×
Dihydrocapsaicin	pericarp	×	0.5639	0.6632*	0.5676	0.7206**
	placenta	0.5639	×	0.0186	0.1365	0.9695**
	seeds	0.6632*	0.0186	×	-0.0130	0.1088
	peduncle	0.5676	0.1365	-0.0130	×	0.3556
	whole fruit	0.7206**	0.9695**	0.1088	0.3556	×

* – significance level of 5%
** – significance level of 1%

The values of the correlation coefficients for the capsaicinoid content in the whole fruit of the hot pepper cultivars evaluated in this study and in its parts reveal a significantly high positive correlation of the biosynthesis of capsaicin in the placenta and its content in the whole fruit ($r = 0.7380^{**}$) and in the pericarp ($r = 0.8929^{**}$) (tab. 4). The values of the coefficients of correlation between the amount of capsaicin in the whole fruit and in the individual fruit parts: pericarp ($r = 0.8320^{**}$), seeds ($r = 0.7811^{**}$), and pedicle ($r = 0.7524^{**}$), allow us to conclude that the capsaicin content in the whole fruit is determined by the content of this alkaloid in the individual parts of the fruit. As regards the amount of dihydrocapsaicin in hot peppers, the study also revealed a significantly high positive correlation between the biosynthesis of this compound in the placenta and its content in the whole fruit ($r = 0.9695^{**}$) and in the pericarp ($r = 0.7206^{**}$). However, no significant correlation was found between the content of dihydrocapsaicin synthesized in the placenta and its content in the seeds ($r = 0.0186$) and in the pedicle ($r = 0.1365$). The above results as well as the results of other authors [Nowaczyk et al. 2006a, b, Cisneros-Pineda et al. 2007, Pandhair and Sharma 2008, Wahyuni et al. 2011, Ben Mansour-Gueddes 2012, Rahman and Inden 2012] prove that the placenta is the anatomical part of the fruit that determines the pungency of a specific hot pepper cultivar.

CONCLUSIONS

The most efficient source of capsaicinoids (capsaicin and dihydrocapsaicin) in hot peppers grown for the needs of the pharmaceutical industry is the placenta, including the seeds, whereas the pericarp has predominantly seasoning qualities. In the fruits of the evaluated cultivars, capsaicin is the dominant component of the capsaicinoid mixture, since its content is more than 10 times higher compared to the dihydrocapsaicin content. The chemical profile of the capsaicinoids of hot pepper exhibits genetic variation. Fruit harvest time determines the capsaicinoid content in the individual parts of the hot pepper fruit.

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RELACJE W ZAWARTOŚCI KAPSAICYNOIDÓW MIĘDZY CZĘŚCIAMI OWOCÓW PAPRYKI OSTREJ (*Capsicum annuum* L.)

Streszczenie. Kapsaicynoidy są metabolitami wtórnymi charakterystycznymi tylko dla roślin z rodzaju *Capsicum* i występują tylko w owocach papryki. Ich biosynteza zlokalizowana jest głównie w komórkach łożyska oraz w przegrodach nasiennych owoców. W

niniejszej pracy metodą HPLC oznaczono zawartość kapsaicynoidów (kapsaicyny i dihydrokapsaicyny) w całych owocach i w ich częściach: łożysku, nasionach, perykarpie oraz szypułce dwóch odmian papryki ostrej: Orkan i Bronowicka Ostra. Istotnie więcej kapsaicynoidów oznaczono w owocach odmiany Bronowicka Ostra (262 $mg \cdot kg^{-1}$) w porównaniu z owocami odmiany Orkan (175 $mg \cdot kg^{-1}$). Największą zawartość kapsaicyny i dihydrokapsaicyny stwierdzono w łożysku (401 $mg \cdot kg^{-1}$). Zawartość kapsaicyny w całych owocach oraz w poszczególnych ich częściach była średnio ponad 10-krotnie większa porównaniu z zawartością dihydrokapsaicyny. Terminy zbioru owoców miał wpływ na zróżnicowanie zawartości kapsaicynoidów w poszczególnych częściach owoców. Większą zawartość kapsaicyny wykazano w perykarpie oraz nasionach, a dihydrokapsaicyny w szypułkach owoców z późniejszego terminu zbioru.

Słowa kluczowe: Solanaceae, owoc, odmiana, termin zbioru, metabolity wtórne

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