

CAPSAICINOIDS IN HOT PEPPER DEPENDING ON FRUIT MATURITY STAGE AND HARVEST DATE

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Abstract. The pharmacological and culinary usefulness of hot pepper fruits is determined, among others, by the content of capsaicinoids that give hot peppers a pungent burning taste. The content of these compounds in hot pepper is primarily determined by the genetic traits of a cultivar and is modified by various factors: weather conditions during the growing season, fertilization, harvest time, and fruit maturity stage. The aim of the present study was to evaluate the effect of fruit maturity stage: green fruits, fruits turning colour as well as physiologically mature fruits (red), and fruit harvest date: the first and third decade of September, on the content of capsaicinoids in four hot pepper cultivars: 'Cyklon', 'Orkan', 'Rokita', and 'Bronowicka Ostra'. In this study, which was carried out in 2005 and 2006, the content of capsaicinoids (capsaicin and dihydrocapsaicin) was measured by HPLC. The highest amount of capsaicinoids was obtained in the fruits of hot pepper that were harvested at the initial stage of maturity – in green fruits (on average $309 \text{ mg}\cdot\text{kg}^{-1}$), compared to fruits turning red (258) and red fruits ($250 \text{ mg}\cdot\text{kg}^{-1}$). Peppers selected in the third decade of September contained more capsaicinoids (on average $301 \text{ mg}\cdot\text{kg}^{-1}$) than those harvested in the first decade of this month ($243 \text{ mg}\cdot\text{kg}^{-1}$). Fruits of the evaluated cultivars differed in capsaicinoids content; on average it ranged from 212 ('Cyklon') to $326 \text{ mg}\cdot\text{kg}^{-1}$ ('Bronowicka Ostra'). In fruits of all the quantitative ratio of capsaicin to dihydrocapsaicin was at a similar level, ranging between 11.9 and 12.9. The capsaicinoids content in fruits of the investigated cultivars differed between years 2005 (302) and 2006 ($243 \text{ mg}\cdot\text{kg}^{-1}$).

Key words: *Capsicum annuum* L., capsaicin, dihydrocapsaicin, cultivar

INTRODUCTION

Capsaicinoids are a group of secondary metabolites characteristic only of the genus *Capsicum* [Zewide and Bosland 2000, Wang and Bosland 2001, Cisneros-Pineda et al. 2007]. In the group of capsaicinoids occurring in hot pepper in the largest amounts, capsaicin and dihydrocapsaicin are identified; their content is more than 75% of total

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pungency [Zewide and Bosland 2000, Topuz and Ozdemir 2007]. Other compounds have also been identified in smaller amounts: homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin as well as pelargonic acid vanillylamide [Gibbs and O'Garro 2004, Topuz and Ozdemir 2007, Ayso et al. 2008, Wesołowska et al. 2011, Ben Mansour-Gueddes 2012]. The highest amount of capsaicinoids is found in the placenta of the fruit, while their lower content in the pericarp and seeds. The biosynthesis takes place in the placenta where specialised epidermal cells accumulate them in the vacuoles from which they are distributed to seeds and the pericarp as well as even to the vegetative organs of leaves and shoots in smaller amounts [Contreras-Padilla and Yahia 1998, Estrada et al. 2002, Ben Mansour-Gueddes et al. 2012]. That is why products made of whole hot pepper fruits contain more capsaicinoids. They are more pungent in taste compared to those made of the pericarp alone [Nowaczyk et al. 2006a, b, 2009, Nowaczyk 2011, 2012].

Capsaicinoids accumulation in hot pepper is determined by many factors. The accumulation of these compounds is dependent to the greatest extent on the genetic traits of a cultivar [Buczkowska et al. 2001a, 2001b, Golcz and Kujawski 2005, Wang and Bosland 2001, Cisneros-Pineda et al. 2007, Topuz and Ozdemir 2007, Ben Mansour-Gueddes et al. 2012]. Capsaicinoids content is also determined by the stage of fruit maturity at the time of harvest. Fruits at the initial maturation stage (40–50 days after fruit set) have been shown to have a higher content of these compounds [Perucka 1995, Contreras-Padilla and Yahia 1998, Gibbs and O'Garro 2004].

Environmental and weather conditions during the period of fruit set and growth as well as fertilization can have a significant effect on capsaicinoids synthesis in hot pepper [Buczkowska et al. 2001a, b, Zewide and Bosland 2000, Golcz et al. 2004, Golcz and Kujawski 2005].

It is characteristic for hot pepper yielding that several fruits with low unit weight are usually obtained from a single plant [Buczkowska 2001, Buczkowska et al. 2001a, b]. Successive harvests of hot pepper as they grow and ripen require high labour inputs. Our earlier study on agriculture of hot pepper demonstrated that in the cultivation of this plant it is possible to reduce the frequency of harvesting. The frequency of fruit harvesting was not found to affect the quantity and quality of fruit yield, including capsaicinoids content [Buczkowska et al. 2001c].

Capsaicinoids content in hot pepper is also dependent on fruit harvest time. Perucka [1995] identified more of these compounds in fruits harvested in the first and the second decade of September compared to pepper harvested in August.

The aim of the present study was to determine the effect of fruit maturity stage and harvest date on capsaicinoids content in fruits of four hot pepper cultivars.

MATERIAL AND METHODS

The study material consisted of fruits of four Polish hot pepper cultivars: 'Cyklon', 'Orkan', 'Rokita' and 'Bronowicka Ostra'. Peppers at three maturity stages: green fruits, fruits turning colour, and red ripe fruits were used for analytical determinations. They were obtained from an agricultural experiment, conducted in the period 2005–

–2006, which evaluated the effect of harvest date on the quantity and quality of hot pepper yield. Two harvest dates were used in the experiment: the first and the third decade of September.

The study was carried out in a private farm in the village of Zezulin (51.35°N, 22.85°E) near the city of Lublin, on lessive soil derived from chalky marl, with an organic matter content of 1.8% and a pH of 6.7. Winter wheat was the previous crop for hot pepper. In autumn organic fertilization with farmyard manure was applied at a rate of 30 t·ha⁻¹, while in spring soil nutrients were replenished to the following levels (mg·dm⁻³): N – 110; P – 80; K – 220; Mg – 80; Ca – 1600. Plants were fertilized twice with Florovit (0.5%) and twice with calcium nitrate (1.0%) as top dressing. Potted pepper seedlings were grown in a propagation greenhouse. In both years of the study, plants were planted in the field in the third 10-day period of May at a spacing of 0.67 × 0.30 m. This agricultural study was set up as a two-factor experiment in 4 replications. The area of each plot was 4.7 m² (20 plants per plot).

Randomly selected fruit samples of the four hot pepper cultivars were dried in a special microprocessor-controlled drying oven at a temperature of 60°C. The fruit drying period lasted 3 days.

The content of capsaicinoids was measured by HPLC according to the procedure described by Collins et al. [1995]. The dried fruits were ground immediately before the extraction of capsaicinoids. Samples 1.5 g were transferred quantitatively to 50 ml round-bottom flasks and immersed in 15 ml of acetonitrile (ACN). The extraction was carried out in a heated bath at 80°C for 4 hours, shaking it by hand every hour. The obtained supernatant was filtered through 0.45 µm Waters-Millex filters. The qualitative and quantitative analysis of capsaicinoids was performed using a liquid chromatograph with a Li Chrom C₁₈ column (250 × 4.6 mm) filled with silica gel. HPLC methanol was used as an eluent. The standards: 8-methyl-N-vanillyl-6-nonenamide (capsaicin) and N-vanillylnonenamide (dihydrocapsaicin), were manufactured by Sigma-Aldrich. The determinations were performed in 3 replications.

The results obtained from the analytical determinations were statistically analysed as a three-factor experiment (fruit maturity stage × harvest date × cultivar) by analysis of variance. The significance of differences was evaluated with Tukey's multiple confidence intervals at a significance level of 5%.

RESULTS AND DISCUSSION

The main aim of growing hot pepper as raw material for the needs of the pharmaceutical and food industries is to obtain fruits with high capsaicinoids content. The results of this study show that the maturity stage of fruits harvested differentiated capsaicin content in the studied cultivars, but it did not have a statistically significant effect on dihydrocapsaicin accumulation (tab. 2).

More capsaicinoids (capsaicin + dihydrocapsaicin) were found in green fruits (on average 309) compared to fruits turning colour (on average 258) and red physiologically mature fruits (on average 250 mg·kg⁻¹). In her study on the formation of capsaicinoids in hot pepper treated with ethephon, Perucka [1995] identified the highest capsaicinoids

content in fruits of cv. 'Bronowicka Ostra' which were harvested 45 days after flowering, thus at the initial stage of maturation. In fruits of 3 cultivars grown in Mexico, Contreras-Padilla and Yahia [1998] also determined the highest content of these compounds in peppers harvested after 40–50 days after fruit setting.

Table 1. Mean monthly air temperature and sums of rainfalls in the vegetation period of peppers

	Year	Month				
		May	June	July	August	September
Temperature °C	2005	13.2	16.0	19.8	16.9	14.9
	2006	13.6	16.9	21.9	17.4	15.7
	mean for years 1951–2005	13.0	16.2	17.8	17.1	12.6
Sums of rainfalls (mm)	2005	98.0	55.9	109.8	108.7	18.0
	2006	59.5	37.9	6.8	132.6	11.0
	mean for years 1951–2005	57.7	65.7	83.5	68.6	51.6

The present study found a statistically significant effect of fruit harvest date on capsaicin and dihydrocapsaicin content. On average, a higher amount of these capsaicinoids was determined in fruits picked in the third decade of September (on average capsaicin + dihydrocapsaicin 301 mg·kg⁻¹) relatively to peppers harvested in the first decade of this month (on average 243 mg·kg⁻¹). In the studies on yield and chemical composition of hot pepper conducted by Perucka [1995] and Buczkowska et al. [2001a, b, c], fruits of the following cultivars were characterized by the highest capsaicinoids content: 'Bronowicka Ostra', 'Tajfun', 'Cyklon' and 'Orkan'; they were also harvested in the second half of September.

The capsaicinoids content in fruits of the evaluated cultivars varied between years (figs 1–4). Fruits obtained in 2005 were shown to have higher capsaicin and dihydrocapsaicin contents (on average 302 mg·kg⁻¹) compared to those harvested in 2006 (on average 243 mg·kg⁻¹). During the growing season of pepper in the field in 2005 and 2006, large differences in thermal and humidity conditions were observed relatively to the long-term means (1951–2005) (tab. 1). In 2006 very high air temperature was recorded in July (average daily temperature 21.9°C) compared to the long-term mean for this month (17.8°C) as well as a huge rainfall deficit (6.8 mm compared to the long-term mean of 83.5 mm) which was not compensated by regular irrigation of the plants. Such weather conditions affected adversely plant flowering and fruit setting, as well as the initial stages of fruit growth when an intense synthesis of capsaicinoids usually starts [Perucka 1995; Conteras-Podilla and Yahia 1998; Ben Mansour-Gueddes et al. 2012].

Polish hot pepper cultivars evaluated in this study showed high variation in capsaicinoids content in fruits harvested on two dates at all maturity stages (tab. 2, figs 3, 4). On average, more capsaicin was determined in peppers of cvs. 'Bronowicka Ostra' (247–402 mg·kg⁻¹) and 'Rokita' (237–360 mg·kg⁻¹) compared to fruits of cv. 'Orkan'

(188–300 mg·kg⁻¹) and ‘Cyklon’ (163–260 mg·kg⁻¹). A comparable content of capsaicinoids in fruits of the same hot pepper cultivars was determined in the studies on the effect of potassium fertilizer type on yield quantity and quality of hot pepper [Golcz et al. 2004, Golcz and Kujawski 2005]. Whereas a higher content was determined in the research on the effect of frequency of harvesting on yield quantity and quality of hot pepper conducted by Buczkowska et al. [2001a, 2001b, 2001c]. In their research a antioxidant activity of capsaicin and dihydrocapsaicin, some other authors [Perucka and Materska 2003, Materska and Perucka 2005] isolated definitely more capsaicinoids from fruits of the cultivars evaluated in the present study.

Table 2. Content of capsaicin and dihydrocapsaicin in the hot pepper depending on the fruit maturity stage and harvest date (mean 2005–2006)

Cultivar	Fruit maturity stage	Capsaicin (mg·kg ⁻¹ d.m.)			Dihydrocapsaicin (mg·kg ⁻¹ d.m.)		
		harvest date			harvest date		
		1 st dec. September	3 rd dec. September	mean	1 st dec. September	3 rd dec. September	mean
Cyklon	g	188	260	224	16	19	18
	t	163	207	185	14	18	16
	r	173	188	181	14	15	15
	mean	175	218	197	15	17	16
Orkan	g	218	300	259	18	23	21
	t	188	239	214	17	21	19
	r	199	217	208	15	17	16
	mean	202	252	227	17	20	19
Rokita	g	262	360	311	23	27	25
	t	237	301	269	20	26	23
	r	252	271	262	21	21	21
	mean	250	311	281	21	25	23
Bronowicka Ostra	g	294	402	348	24	30	27
	t	247	314	281	26	27	26
	r	264	288	276	22	23	23
	mean	268	335	302	24	27	25
mean	g	241	330	286	20	25	23
	t	209	265	237	19	23	21
	r	222	242	232	18	19	18
Total mean		224	279	252	19	22	21
LSD _{α = 0.05}							
fruit maturity stage (a)				42.4	n.s.		
harvest date (b)				17.9	n.s.		
cultivar (c)				32.0	4.8		
interaction: a × b				39.7	n.s.		
a × c				60.5	n.s.		
b × c				52.4	n.s.		

explanation: fruits: g – green; t – turning; r – red

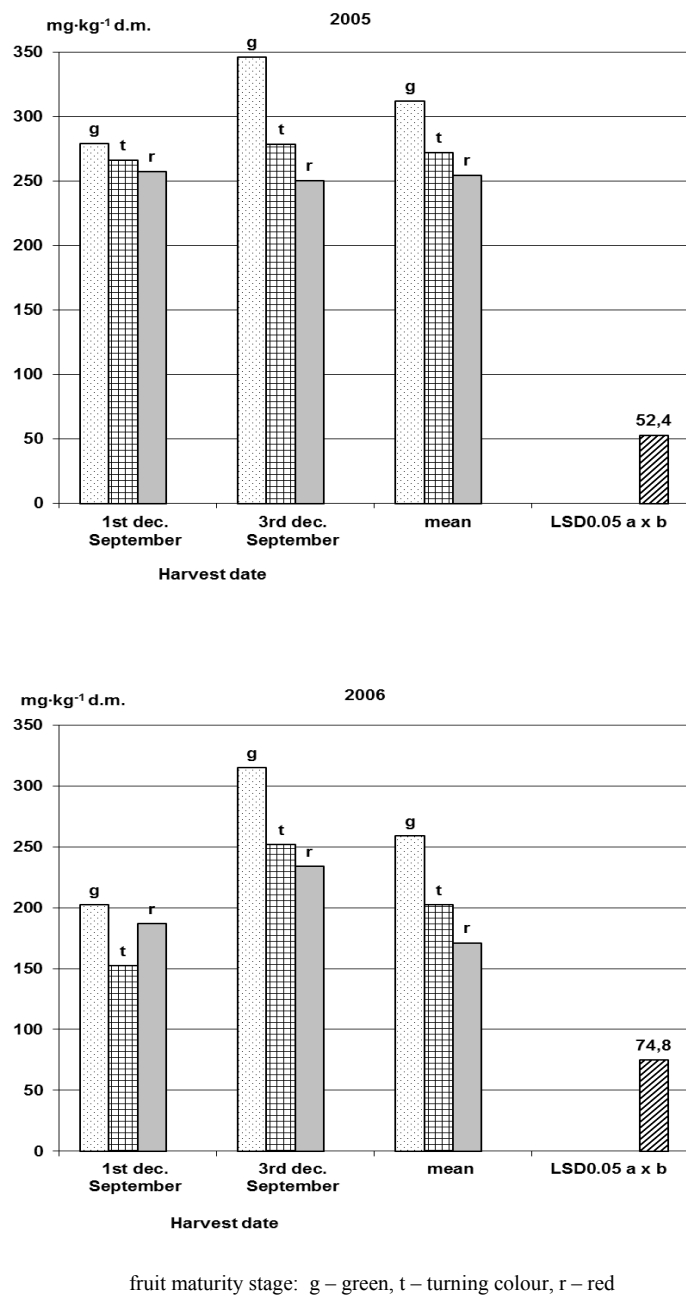


Fig. 1. Content of capsaicin in the hot pepper depending on the fruit maturity stage and harvest date (mean for cultivars)

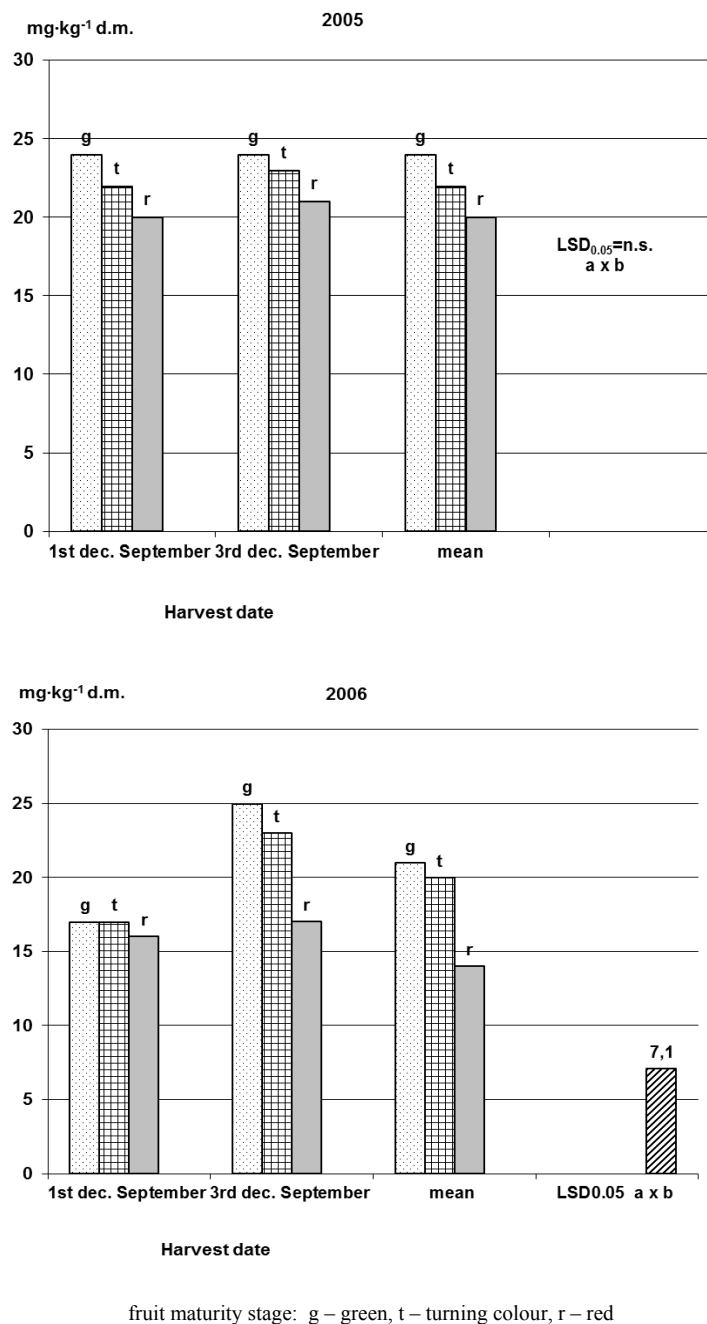


Fig. 2. Content of dihydrocapsaicin in the hot pepper depending on the fruit maturity stage and harvest date (mean for cultivars)

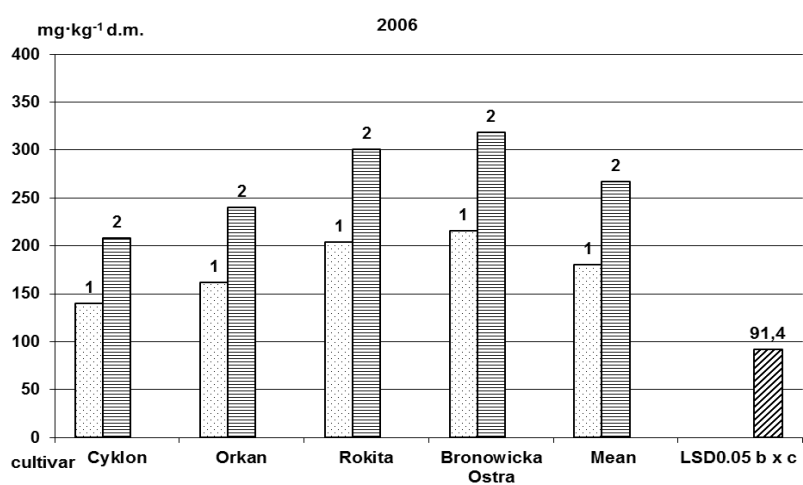
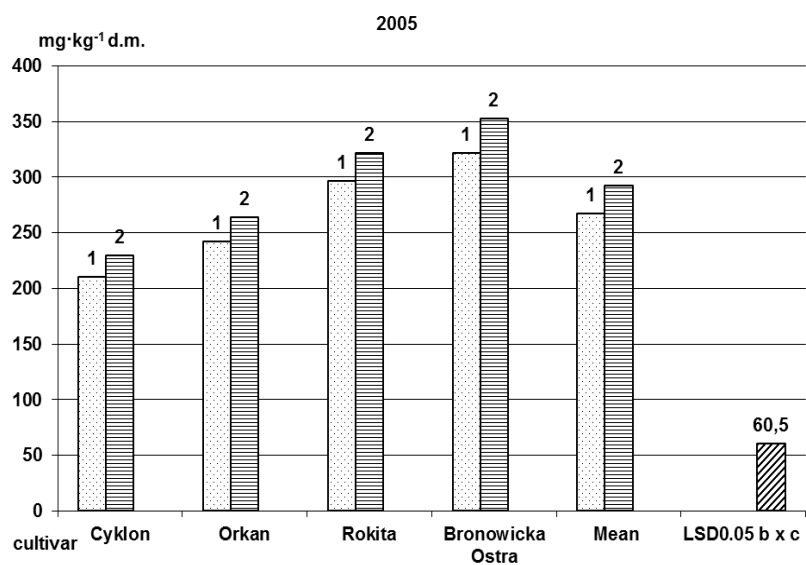
Irrespective of fruit maturity stage, fruits of all evaluated cultivars picked at both harvest dates in 2005 were characterized, on average, by higher capsaicin content (210–352 mg·kg⁻¹) (fig. 3). On average, less capsaicin was isolated from fruits harvested in 2006 (140–318 mg·kg⁻¹). In this year of the study, harvest time was found to affect the content of this compound. On average, more capsaicin was determined in fruits harvested in the third decade of September (267) compared to those harvested in the first decade of September (180 mg·kg⁻¹). The highest difference in capsaicin content, depending on harvest date, was found in fruits of cvs. ‘Bronowicka Ostra’ and ‘Rokita’ (fig. 3).

The investigated cultivars showed statistical differences in the content of the other compound under evaluation, capsaicinoids (tab. 2, fig. 4). Significantly more dihydrocapsaicin was determined in fruits of cvs. ‘Bronowicka Ostra’ (25) and ‘Rokita’ (23) compared to cv. ‘Cyklon’ (16 mg·kg⁻¹). The study also revealed differences in the content of this alkaloid between years. Fruits of all cultivars contained on average more dihydrocapsaicin in the year 2005 in which the time of harvest did not influence the content of this alkaloid. In the second year of the study, fruits harvested at the later date were marked by higher dihydrocapsaicin content, but these differences were statistically insignificant.

Table 3. Quantitative relations between capsaicin and dihydrocapsaicin in the hot pepper depending on the fruit maturity stage (mean 2005–2006)

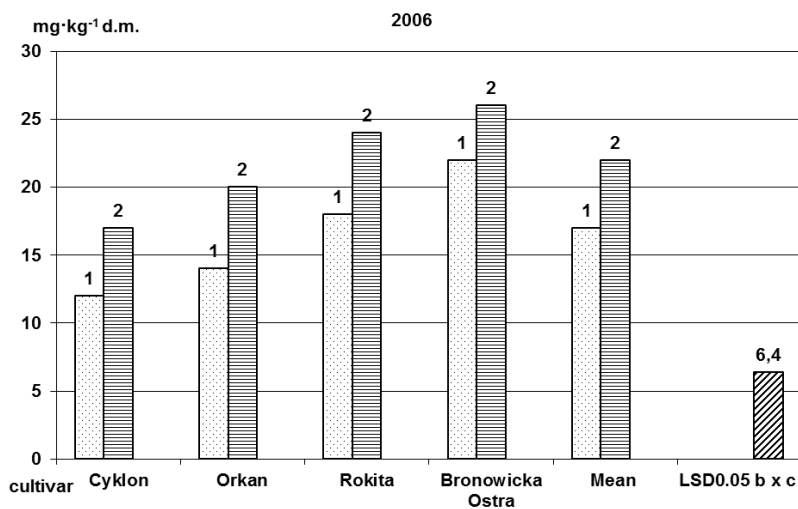
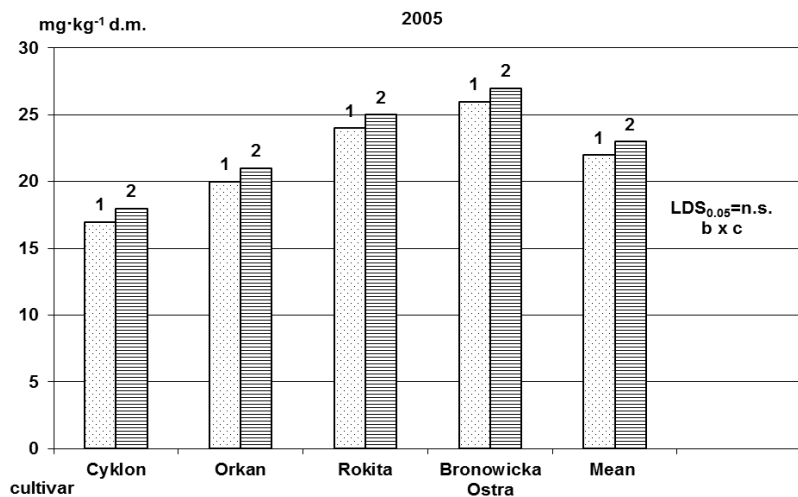
Cultivar	Fruit maturity stage	Harvest date		
		1 st dec. September	3 rd dec. September	mean
Cyklon	g	11.8	13.7	12.7
	t	11.6	11.5	11.6
	r	12.4	12.5	12.5
	mean	11.9	12.6	12.3
Orkan	g	12.1	13.0	12.5
	t	11.1	11.4	11.3
	r	13.3	12.8	13.0
	mean	12.2	12.4	12.3
Rokita	g	11.4	13.3	12.4
	t	11.9	11.6	11.7
	r	12.0	12.9	12.4
	mean	11.8	12.6	12.2
Bronowicka Ostra	g	12.3	13.4	12.9
	t	9.5	11.6	10.5
	r	12.0	12.5	12.3
	mean	11.3	12.5	11.9
Mean	g	11.9	13.2	12.6
	t	11.0	11.5	11.3
	r	12.4	12.7	12.6
Total mean		11.8	12.5	12.2

explanation: fruits: g – green; t – turning; r – red



1 – the first harvest date (1st dec. September)
 2 – the second harvest date (3rd dec. September)

Fig. 3. Content of capsaicin in the fruits of four cultivars hot pepper depending on the harvest date (mean for fruit maturity stage)



1 – the first harvest date (1st dec. September)
2 – the second harvest date (3rd dec. September)

Fig. 4. Content of dihydrocapsaicin in the fruits of four cultivars hot pepper depending on the harvest date (mean for fruit maturity stage)

This study's results on the content of capsaicinoids in hot pepper grown in 2005 and 2006 showed that the accumulation of these secondary metabolites is primarily determined by the genetic traits of a cultivar and environmental factors, in particular weather conditions during the growing season of pepper. The results of this study correspond to the results of the research of Zewide and Bosland [2000] that proved a statistically significant effect of the interaction of genotype traits and environmental conditions on the content of five capsaicinoids in fruits of several breeding lines of hot pepper. The results of earlier experiments, which were conducted in the climatic conditions of our country, on the content of capsaicinoids, also demonstrate the relationship between the accumulation of these secondary metabolites and the genetic traits of a cultivar as well as various environmental factors [Buczowska et al. 2001a, Materska and Perucka 2005, Golcz and Kujawski 2005, Nowaczyk et al. 2009, Wesołowska et al. 2011, Nowaczyk 2012]. In a warm climate, that is, in optimal environmental conditions for growing pepper, the accumulation of capsaicinoids in fruits is primarily determined by the genotype of a cultivar, which has been demonstrated by determining capsaicinoids content in fruits of various hot pepper cultivars and lines in the experiments conducted in Turkey [Topuz and Ozdemir 2007], Tunisia [Ben Mansour-Gueddes et al. 2012] and Mexico [Cineros-Pineda 2007].

Table 3 shows the results concerning the quantitative relations between capsaicin and dihydrocapsaicin in hot pepper harvested at different maturity stages in the first and third 10-day periods of September. Fruits of the cultivars under evaluation were characterized by a very similar quantitative ratio of capsaicin to dihydrocapsaicin; its average value was from 11.9 (cv. 'Orkan') to 12.9 (cv. 'Cyklon'). In analysing the obtained numerical values, it can be stated that fruits harvested at the earlier date contained slightly less capsaicin relative to dihydrocapsaicin (11.8:1) than pepper obtained from the later harvest date (12.7:1). Gibbs and O'Garro [2004] found a much higher content of capsaicin compared to dihydrocapsaicin in fruits of hot pepper cultivars and lines belonging to the species *Capsicum chinense*, as well as Nowaczyk et al. [2006 a,b, 2009] in fruits obtained from soft-flesh lines. The results of other studies prove different quantitative relations between particular capsaicinoids identified in hot pepper and they are evidence that the accumulation of capsaicinoids and their individual fractions is mainly determined by the genotype of a cultivar [Zewide and Bosland 2000, Wang and Bosland 2001].

The results of the present study as well as the results of the research of other authors [Perucka 1995, Gibbs and O'Garro 2004, Contreras-Padilla and Yahia 1998] confirm that hot pepper harvested at the initial stage of maturation, when they are still green, have the highest commercial value in terms of capsaicinoids content. Under the temperate climatic conditions, the length of the growing season of pepper in the field is determined by the time of occurrence of the first autumn frosts. An important issue for horticultural practice is to determine the optimal time of fruits harvest with a view to obtaining a yield of fruits that would contain the highest amount of capsaicinoids. On the basis of this study's results as well as the results of earlier research [Perucka 1995, Buczowska 2001, Buczowska et al. 2001a, b], it can be concluded that the optimum time for double or single harvest of hot peppers is the second half of September [Buczowska et al. 2001a, b]. Taking into account the fact that pepper with the highest

capsaicinoids content are the most useful as raw material for industry, fruit harvest dates for a given cultivar should be selected in such a way so that fruits at the initial stage of maturity, thus green fruits and fruits turning colour, are predominant.

CONCLUSIONS

The highest amount of capsaicinoids (capsaicin and dihydrocapsaicin) was determined in fruits that were harvested at the initial stage of maturity – green fruits (on average $309 \text{ mg}\cdot\text{kg}^{-1}$), compared to fruits turning colour (258) and red fruits ($250 \text{ mg}\cdot\text{kg}^{-1}$). Fruits harvested in the third decade of September contained more capsaicinoids (on average 301) than pepper picked in the first decade of this month ($243 \text{ mg}\cdot\text{kg}^{-1}$). Fruits of the evaluated cultivars differed in capsaicinoids content; on average it ranged from 212 ('Cyklon') to $326 \text{ mg}\cdot\text{kg}^{-1}$ ('Bronowicka Ostra'). In fruits of all evaluated cultivars, the quantitative ratio of capsaicin to dihydrocapsaicin was at a similar level, on average ranging between 11.9 and 12.9. The capsaicinoids content in fruits of the investigated cultivars differed between years: 2005 (on average 302) and 2006 ($243 \text{ mg}\cdot\text{kg}^{-1}$). The most useful as raw material for industry are green fruits and fruits turning colour and those at the initial stage of maturity are predominant in the yield structure.

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KAPSAICYNOIDY W PAPRYCE OSTREJ W ZALEŻNOŚCI OD FAZY DOJRZAŁOŚCI OWOCÓW I TERMINU ZBIORU

Streszczenie. Przydatność farmakologiczną i kulinarną owoców papryki ostrej warunkuje m.in. zawartość kapsaicynoidów, które nadają jej specyficzny, ostry i piekący smak. Zawartość tych związków w papryce ostrej determinowana jest przede wszystkim cechami genetycznymi odmiany oraz modyfikowana różnymi czynnikami: warunkami pogodowymi w okresie wegetacji, stosowanym nawożeniem, terminem zbioru i fazą dojrzałości owoców. W niniejszej pracy oceniono wpływ fazy dojrzałości owoców: owoce zielone, przebarwiający się i dojrzałe fizjologicznie (czerwone) i terminu zbioru owoców: 1. i 3. dekada września na zawartość kapsaicynoidów (kapsaicyny i dihydrokapsaicyny) czterech

odmian papryki ostrej: 'Cyklon', 'Orkan', 'Rokita', 'Bronowicka Ostra'. W badaniach przeprowadzonych w latach 2005 i 2006 zawartość kapsaicynoidów (kapsaicyny i dihydrokapsaicyny) oznaczono metodą HPLC. Najwięcej kapsaicynoidów (kapsaicyny i dihydrokapsaicyny) oznaczono w owocach papryki ostrej, które były zbierane w początkowej fazie dojrzałości – zielone (średnio 309) w porównaniu z przebarwiającymi się (258) oraz czerwonymi (250 mg·kg⁻¹). Więcej kapsaicynoidów zawierały owoce, które zebrano w 3. dekadzie września (średnio 301) niż w 1. dekadzie tego miesiąca (243 mg·kg⁻¹). Owoce ocenianych odmian różniły się pod względem zasobności kapsaicynoidów średnio od 212 ('Cyklon') do 326 mg·kg⁻¹ ('Bronowicka Ostra'). W owocach wszystkich ocenianych odmian relacje ilościowe kapsaicyny do dihydrokapsaicyny były bardzo wyrównane i kształtowały się średnio w zakresie od 11,9 do 12,9. Zawartość kapsaicynoidów w owocach badanych odmian była zróżnicowana w latach badań: 2005 (302) i 2006 (243 mg·kg⁻¹).

Słowa kluczowe: *Capsicum annuum* L., kapsaicyna, dihydrokapsaicyna, odmiana

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