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CHANGES IN FERULIC AND SINAPIC ACID ESTERS AND QUERCETIN RHAMNOSIDE CONTENTS IN THE SELECTED HOT PEPPER CULTIVARS AS INFLUENCED BY MATURITY

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Abstract. The effect of fruit maturation on changes in the three phenolic compounds: ferulic and sinapic acid esters as well as quercetin rhamnoside in different pepper cultivars ('Bronowicka Ostra', 'Cyklon', 'Tornado and Tajfun') was determined. The concentration of phenolic acid esters increased during maturity, contributing 10.31-53.42 mg/kg of f.w. and 9.01-56.94 mg/kg of f.w. for ferulic and sinapic acid ester, respectively. Level of quercetin rhamnoside decreased during fruit maturation and its content was between 11.33-110.81 mg/kg of f.w. The concentration of phenolic compounds varied greatly among the pepper cultivars. The richest in these compounds were hot cultivars 'Bronowicka Ostra' and 'Cyklon'.

Key words: Phenolic compounds, *Capsicum annuum* L.

INTRODUCTION

Pepper fruit is a perfect source of natural antioxidants (vitamins C and E as well as carotenoids) [Palevitch and Craker 1995, Daood et al. 1996]. Fruit of hot cultivars additionally contains capsaicinoids – alkaloids that give them spicy taste. This is why hot pepper has a long history in traditional medicine and up to the present day it has been used in phytotherapy and conventional medicine [Surh and Lee 1996, Chang et al. 1999]. Apart from the mentioned components it was found that pepper fruit contains a lot of phenolic compounds, among them derivatives of quercetin and luteolin [Sukrasno and Yeoman 1993, Lee et al. 1995, Howard et al. 2000], as well as apigenin and phenolic acids, mainly ferulic and sinapic, as was noticed in our own studies [Materska et al. 2003 a, b]. In recent years scientists' interest in this group of compounds, generally called polyphenols, has increased. This results from their promising chemical properties. They are strong antioxidants that may protect people from free radicals attacks

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[Bartnikowska 1995, Bors et al. 1996]. Phenolic compounds cannot be synthesized by man and hence they have to be supplied with food and their quantity in the food has to be precisely determined.

On the basis of our earlier studies it was found that fractions of phenolic compounds isolated from red and green fruit of the 'Bronowicka Ostra' cultivar revealed antioxidant activity in the model system β-carotene – linoleic acid [Perucka and Materska 2001]. On the basis of subsequent studies it was found that in the fruit of this cultivar ferulic and sinapic acid esters and quercetin rhamnoside occurred in greatest quantities [Materska et al. 2003 a]. The aim of the present study was to determine the changes in the contents of these compounds in the course of maturation of the fruit in four cultivars of hot pepper.

MATERIALS AND METHODS

Four hot pepper cultivars were the material for the study; two hot i.e. 'Cyklon' and 'Bronowicka Ostra', and two semi-hot i.e. 'Tornado' and 'Tajfun'. Fruit at the stage of full maturity and of technological maturity, that is completely formed but not colored, were used for the study.

The phenolic compounds fraction was isolated from freeze-dried fruit according to the method described by Lee et al. [1995]. Freeze-dried pepper pericarps (0.6 g) were homogenized with 80% ethanol solution (v/v), next, after separation of the plant tissue, 10 cm^3 of the extract was taken, evaporated till dry under reduced pressure at 40°C, and dissolved in 5 cm^3 of water. The water extract was put on Sep-Pak C_{18} column (Waters) that was washed with methanol and then with water before use. The hydrophilic compounds present in the extract were washed out with water (10 cm^3), and the phenolic compounds fractions with 40% methanol solution, and then they were analyzed by the high performance liquid chromatography method on WellChrom chromatograph (Knauer) with UV detector. Separation was conducted on column filled with modified silica gel RP-18 (Vertex Eurosil Bioselect 300 Å, Ø 5 μm, 4 × 30 cm, to separate the discussed fraction a gradient solvent system was used, from 0 to 40% acetonitrile in 1% solution of H_3PO_4. The speed of the flow was 1ml/min, and the detection was at 330 nm. Particular phenolic compounds were determined qualitatively using standard solutions of phenolic compounds isolated and identified in our earlier studies [Materska et al. 2003 b].

Statistical analysis comprised the results obtained in three years of experiments and in three repetitions. For each series of data standard deviation was calculated as a measure of dispersion of the series. To appraise the significance of the differences between the means, Tuckey's multiple test was used, assuming a 5% probability of making a mistake. On the basis of this test groups of means were distinguished that significantly differed from each other, and the value of the least significant difference was determined. The calculations were done with the Statgraphic v. 3.0 for Windows.

RESULTS AND DISCUSSION

Quantitative analysis of the fractions of phenolic compounds conducted in the present study by means of HPLC showed that during fruit maturation considerable changes occurred in its composition. It was found that red fruit of all the studied pepper cultivars were characterized by a higher content of glycosidic derivatives of ferulic and sinapic acid than green fruit. The highest concentration of ferulic acid ester was found in red fruit of the 'Cyklon' cultivar (53.42 kg^{-1} fresh mass), and the lowest – in fruit of the 'Tajfun' cultivar (13.48 kg^{-1} fresh mass). A comparison between the contents of ferulic acid glycoside in one fruit did not reveal a significant difference between these cultivars (Table 2). An analysis of the content of sinapic acid ester in green fruit showed that the

Table 1. The content of phenolic compounds in pepper fruits, mg·kg^{-1} of fresh weight
Tabela 1. Zawartość związków fenolowych w owocach papryki, mg·kg^{-1} świeżej masy

Cultivar Odmiana	Maturity stage Faza dojrzałości	Dry matter, % Sucha masa, %	Ferulic acid ester Ester kwasu ferulowego	Sinapic acid ester Ester kwasu synapinowego	Quercetin rhamnoside Ramnozyd kwercetyny
Bronowicka	green zielone	14.78	14.34^{a}* ± 1.95	13.50^{a} ± 2.02	64.20^{a} ± 12.50
Ostra	red czerwone	14.92	39.64^{b} ± 1.80	56.94^{bd} ± 7.06	20.44^{b} ± 2.99
Cyklon	green zielone	11.89	28.02^{c} ± 2.31	30.48^{c} ± 3.06	110.81^{c} ± 4.44
	red czerwone	14.88	53.42^{d} ± 4.03	48.21^{d} ± 7.99	16.86^{d} ± 2.53
Tornado	green zielone	12.23	10.31^{e} ± 1.55	9.01^{a} ± 0.98	42.27^{e} ± 0.88
	red czerwone	13.77	31.58^{c} ± 1.66	48.47^{d} ± 2.87	17.21^{d} ±0.73
Tajfun	green zielone	8.30	10.49^{e} ± 0.50	21.42^{e} ± 1.14	27.09^{b} ± 2.99
	red czerwone	9.11	13.63^{a} ± 3.63	38.14^{c} ± 4.30	11.33^{d} ± 2.38

*Means of values in the columns with the same letters are not significantly different at $p \leq 0.05$.
*Średnie wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0,05$.

Table 2. The content of phenolic compounds in pepper fruits, mg/fruit
Tabela 2. Zawartość związków fenolowych w owocach papryki, mg/owoc

Cultivar Odmiana	Maturity stage Faza dojrzałości	Mass of 1 fruit, g Masa 1 owocu, g	Ferulic acid ester Ester kwasu ferulowego	Sinapic acid ester Ester kwasu synapinowego	Quercetin rhamnoside Ramnozyd kwercetyny
Bronowicka	green zielone	14.49	0.208^{a}* ± 0.028	0.196^{a} ± 0.029	0.930^{a} ± 0.181
Ostra	red czerwone	17.68	0.701^{b} ± 0.032	1.007^{b} ± 0.124	0.361^{b} ± 0.053
Cyklon	green zielone	15.99	0.449^{c} ± 0.038	0.487^{c} ± 0.049	1.888^{c} ± 0.071
	red czerwone	21.93	1.171^{d} ± 0.089	1.057^{b} ± 0.175	0.370^{b} ± 0.056
Tornado	green zielone	21.48	0.222^{a} ± 0.033	0.194^{a} ± 0.021	0.908^{a} ± 0.019
	red czerwone	22.90	0.723^{b} ± 0.038	1.110^{b} ± 0.066	0.394^{b} ± 0.016
Tajfun	green zielone	70.95	0.744^{b} ± 0.035	1.520^{d} ± 0.081	1.922^{c} ± 0.212
	red czerwone	80.60	1.098^{d} ± 0.292	3.074^{e} ± 0.346	0.913^{a} ± 0.192

*Means of values in the columns with the same letters are not significantly different at $p \leq 0.05$.
*Średnie wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0,05$.

highest content of this compound was in 'Cyklon' fruit (30.48 kg^{-1} fresh mass), and in red fruit – of 'Bronowicka Ostra' (56.94 kg^{-1} fresh mass). A comparison of the contents of sinapic acid in one fruit did not reveal a significant difference between the cultivars; only the large-fruited 'Tajfun' cultivar contained about three times more of this component than the remaining ones (Table 2).

Changes in quercetin rhamnoside content were contrary to the changes in derivatives of phenolic acids. The greatest amount of this compound was found in green fruit of all the studied cultivars. During fruit maturation its content was considerably decreased. The greatest, sixfold decrease in quercetin rhamnoside content in the course of maturation, was found in 'Cyklon' fruit, a more than triple decrease in 'Bronowicka Ostra' fruit, and a more than double decrease in 'Tornado' and 'Tajfun' (Table 1). Analysis of quercetin rhamnoside in one fruit of the studied cultivars showed that in green fruit its the highest concentration was found in 'Cyklon' and in red fruit – in 'Bronowicka Ostra'.

Of all the compounds discussed in the present paper only the content of quercetin rhamnoside had been earlier determined by Sukrasno and Yeoman [1993], and according to their findings it was 0.654 mg/fruit. In the present study the content of this compound ranged between 0.361 and 1.922 mg/fruit (Table 2), so the present results are close to Sukrasno and Yeoman's ones [1993], and the differences that were found result from cultivar differences and the dry mass content in the fruit. In Sukrasno and Yeoman's fruit [1993] dry mass content was 19%, whereas in the pepper cultivars in the present study it ranged from 8.3 to 14.92% (Table 2). On the basis of studies of radish, Chen et al. [1998] found that soluble sinapic acid esters, as well as esters of other phenolic acids are built into the structure of cell walls. A correlation between the content of these compounds and lignification processes occurring mainly in the seeds of pepper fruit was confirmed by Sukrasno and Yeoman [1993]. The very low amount of ferulic and sinapic acid esters in immature fruit found in the present study may be explained by building these compounds into the structures of cell walls. The increase in the content of these compounds in fully mature fruit may be caused by completing the process of fruit maturation.

Changes in quercetin rhamnoside content also occurred in a way similar to the one described by Sukrasno and Yeoman [1993], where the content of flavonoid compounds was high in green fruit, and then it decreased as the fruit ripened. Similar correlations were also proven by Howard et al. [2000], who determined the total of flavonoid, found that during pepper fruit maturation its content decreased, whereas the amount of other phenolic compounds increased. A high concentration of quercetin rhamnoside in green fruit may be connected with the function of protecting the photosynthetic apparatus. In earlier studies it was shown that flavonoids that strongly absorb radiation within the range 280-315 nm, that is UVB, may act as filters of UV radiation, in this way protecting photosynthesizing cells – that are situated deeper – against being damaged [Harborne and Williams 2000]. The decrease in quercetin rhamnoside in red fruit may be explained by the fact that at the moment when chlorophylls are disintegrated the photosynthetic process stops. The compounds that earlier fulfilled the protective function for the photosynthetic apparatus become intermediate compounds for further transformations of secondary metabolism.

CONCLUSIONS

1. During hot pepper fruit maturation the quantitative composition of the phenolic compounds fraction changed.

2. Along with fruit maturation quercetin rhamnoside content decreased, and the content of glucosidic esters of ferulic and sinapic acids increased.

3. The higher contents of the discussed compounds were found in the fruit of hot cultivars than in semi-hot ones.

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ZMIANY ZAWARTOŚCI ESTRÓW KWASU FERULOWEGO I SYNAPINOWEGO ORAZ RAMNOZYDU KWERCETYNY W OWOCACH WYBRANYCH ODMIAN PAPRYKI W ZALEŻNOŚCI OD FAZY DOJRZAŁOŚCI

Streszczenie. W pracy określono zmiany zawartości trzech związków fenolowych: estrów kwasu ferulowego i synapinowego oraz ramnozydu kwercetyny w trakcie dojrzewania owoców w czterech odmianach papryki: 'Cyklon', 'Boronowicka Ostra', 'Tornado' i 'Tajfun'. Na podstawie otrzymanych wyników badań stwierdzono, że podczas dojrzewania owoców papryki zmieniał się skład ilościowy frakcji fenylopropanoidów. Wraz z dojrzewaniem owoców zmniejszała się zawartość ramnozydu kwercetyny, której największe ilości zanotowano w owocach odmiany 'Cyklon' (110,81 mg/kg św.m.), a wzrastała zawartość estrów glukozydowych kwasów ferulowego i synapinowego, których największą zawartość zanotowano odpowiednio w owocach odmiany 'Cyklon' (53,42 mg/kg św.m.) i 'Bronowicka Ostra' (56,94 mg/kg św. m.).

Słowa kluczowe: związki fenolowe, *Capsicum annuum* L.

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