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ANTIOXIDANT ACTIVITY AND CONTENT OF CAPSAICINOIDS ISOLATED FROM PAPRIKA FRUITS

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Key words: paprika, capsaicinoids, antioxidant activity, antioxidants

Antioxidant activity of capsaicinoid fractions as well as the concentration of capsaicinoids, total flavonoids, ascorbic acid, β-carotene and tocopherols in red pepper fruits (*Capsicum annuum* L.) varieties Bronowicka Ostra, Cyklon, Tornado, and Tajfun were investigated. The obtained results showed that the fractions of capsaicinoids had similar antioxidant activity as the flavonoid fractions depending on the level of capsaicinoids in pepper fruits. A comparison of the concentration of antioxidant compounds in different varieties has shown that the fruits with lower level of capsaicinoids were also characterized by lower antioxidant activities, reduced vitamin E and provitamin A content, but higher vitamin C content as compared to the fruits of hot varieties.

INTRODUCTION

A distinct relation has been found between increased fruit and vegetables intake and reduction of the risk of cancer and heart diseases. Attention of researchers has been focused on application of organic microcomponents contained in fruit and vegetables, such as carotenoids (β-carotene, β-cryptoxanthin, lycopene), flavonoids and derivatives of folic acid, in nutrition [Bartnikowska, 1995; Duthie & Dobson, 1999].

It has been shown that dietary carotenoids protect against oxidative damage and stimulate the DNA repair of single damages in both *in vitro* and *in vivo* conditions. Protection from oxidative damage is most probable due to their considerable antioxidant ability. However, the control repair activity of these compounds is something new, and the mechanisms are being studied on the level of transcriptional and post-transcriptional regulation [Astley *et al.*, 1999].

Paprika is a vegetable that is particularly rich in organic microcomponents with antioxidant properties. Red fruits are rich in carotenoids (β-carotene and β-cryptoxanthin). They are also a good source of phenolic compounds with antioxidant properties [Daood *et al.*, 1996; Lee *et al.*, 1995; Perucka & Bubicz, 1990; Perucka, 1996; Perucka & Materska, 2001].

Hot and semi-hot varieties of paprika contain a group of compounds that give the fruits a spicy taste – they are capsaicinoids. Capsaicin makes up about 60% and dihydrocapsaicin about 30% of total capsaicinoids content. The remaining minor compounds include nordihydrocapsaicin and homocapsaicin. Pharmacological properties of capsaicin have been known for a long time. This compound stimulates the tips of sensory nerves of the skin and mucous membranes. In farmacotherapy it is used in peripheral pain conditions such as rheumatism, arthritis and in diabetic neurotherapy. It is an inhibitor of lamella aggregation and displays anti-inflammatory properties. Recent studies show that capsaicin and dihydrocapsaicin have the ability to inhibit metabolism and mutagenity of chemical cancerogens [Surh *et al.*, 1998]. Until now there has been no information about their antioxidant properties. Studies were only limited to the model capsaicin solution [Lee *et al.*, 1995].

The objective of the presented paper was to determine quantitative composition of compounds with antioxidant properties including L-ascorbic acid, β-carotene, tocopherols, capsaicinoids and flavonoids, in several paprika varieties as well as to determine and compare antioxidant activity of isolated capsaicinoid and flavonoid fractions.

MATERIAL AND METHODS

Fruits of four varieties of paprika were taken for the study. These included the hot varieties Bronowicka Ostra and Cyklon and semi-hot ones Tornado and Tajfun. All collected fruits were at the phase of full ripeness.

The vitamin C content in the paprika fruits was determined by the Tillmans' method [Roe, 1967] and β-carotene level by the method described by Bubicz [1965]. Carotenoids were extracted from fresh plant material with acetone and petroleum ether mixture (1:1) until the tissues were completely decoloured. After removing the acetone with water, the ether extract was dried with Na_2SO_4, and after separation in separatory funnel it was concentrated under reduced pressure at 35°C. Then β-carotene was isolated from the remaining carotenoids by the column chromatography method. Chromatography column (20 cm × 1 cm) was filled with $Ca(OH)_2$. To eluate β-carotene, gradient

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solution of petroleum ether and acetone was used (0 to 2% of acetone). β-Carotene isolated this way was spectrophotometrically determined on a Shimadzu spectrophotometer UV-260 at 450 nm.

Tocopherols were separated from carotenoids by means of thin-layer chromatography using the Müller-Mulot's [1968] method. The extracts were put on the plates covered with silica gel (Merck) and then were developed in the petroleum ether-diethyl ether (9:1 v/v) system. Chromatograms of the extracts were made visible with the solution of phosphomolybdic acid in ethanol. Tocopherols formed a blue band, which allowed defining the R_f values. The bands of the adsorbent with tocopherols, but without phosphomolybdic acid, were scrapped off, ethyl alcohol, acetate buffer, α,α-dipirydyl and iron chloride were added, and the obtained coloured solution was measured by the spectrophotometry at 525 nm.

Flavonoids were extracted with ethanol. The extract was evaporated, suspended in water and loaded on the C18 column (Solid Phase Extraction). The column was successively washed with water (carbohydrate fraction – rejected), 40% MeOH (flavonoids) and 70% MeOH (capsaicinoids) [Perucka & Materska, 2001]. Separation of capsaicin and dihydrocapsaicin as well as quantitative determinations were done by means of HPLC on Knauer-Well chromatograph with a spectrophotometric detector (λ 280 nm) and C18 column (VertexEurosil-Bioselect). A mixture of acetonitrile and water (55:45 v/v) [Perucka & Oleszek, 2000] was used as the developing system.

Heat (50°C, 3 h) induced oxidation of aqueous emulsion system of β-carotene and linoleic acid was used as the antioxidant activity test model [Lee *et al.*, 1995]. The free radicals formed *in vitro* oxidized the β-carotene and the speed of oxidation that depended on the added flavonoids or capsaicinoids was measured with the method of absorption spectrophotometry every 15 min on Shimadzu UV-VIS spectrophotometer at 470 nm. The obtained results were presented in the form of charts.

The obtained results were analyzed statistically using the Statgraphic v. 3.1 for Windows program. Data were subjected to one way analysis of variance and means were compared using the least significant difference (P=0.05).

RESULTS AND DISCUSSION

Content of vitamins with antioxidant properties

On the basis of the obtained results clear differences were noticed between particular varieties in the vitamin level (Table 1). Among the studied varieties Tajfun fruits were characterized by the highest content of vitamin C, Bronowicka Ostra fruits – of vitamin E, and Cyklon fruits – of provitamin A. It was found that the vitamin E content in the fruits of the Bronowicka Ostra and Cyklon varieties was 2 to 1.5 times higher, and content of provitamin A 2–3 times higher than these in the fruits of the Tornado and Tajfun varieties. The results of studies by other authors showed that the vitamin E content in the fruits of sweet paprika ranged from 3 to 8 mg/100 g fresh mass [Horbowicz, 1989], whereas vitamin C content – from 100 to 200 mg of fresh mass [Perucka & Bubicz, 1990]. Studying the L-ascorbic acid content in the fruit of 12 paprika varieties Lee *et al.*, [1995] found that the level of this compound ranged between 147 and 168 g/100 g of fresh mass. In the present studies the results of the L-ascorbic acid content are expressed in the values of dry mass that for sweet varieties were lower than for hot varieties.

TABLE 1. Tocopherols, L-ascorbic acid and β-carotene content in red fruits of paprika [mg/100 g dry mass].

Variety	Compounds with antioxidant properties*		
	L-ascorbic acid	Tocopherols	β-Carotene
Bronowicka Ostra	1320^{a}±209	68.3^{a}±3.34	27.91^{a} ±1.49
Cyklon	1320^{a}±64	58.7^{a}±2.62	39.65^{b}±1.57
Tornado	1550^{a}±116	36.5^{b}±0.95	14.06^{c}±1.85
Tajfun	1980^{b}±182	36.0^{b}±3.48	19.73^{c}±0.94

*The results are means (n=4) ± standard deviation. Different letters in the same column indicate significant differences between means (p=0.05).

Level of flavonoids and capsaicinoids in the fruits of selected paprika varieties

The content of flavonoids ranged from 90 mg/100 g of dry mass in the fruits of the Bronowicka Ostra and Cyklon varieties to 84–81 mg/100 g of dry mass in the fruits of the Tornado and Tajfun varieties (Table 2). The differences between the varieties were not bigger than 10 mg/100 g of dry mass.

TABLE 2. Flavonoids, capsaicin and dihydrocapsaicin content in red fruits of paprika [mg/100 g of dry mass].

Variety	Compounds with antioxidant properties*	
	Flavonoids	Capsaicin and dihydrocapsaicin
Bronowicka Ostra	91.0^{a}±6.2	88.0^{a}±0.49
Cyklon	90.3^{a}±6.2	48.5^{b}±0.78
Tornado	84.1^{a}±7.4	5.0^{c}±0.14
Tajfun	81.2^{a}±9.3	7.7^{cd}±0.85

*The results are means (n=4) ± standard deviation. Different letters in the same column indicate significant differences between means (p=0.05).

The studied varieties differed in the level of the two main capsaicinoids – capsaicin and dihydrocapsaicin (Table 2). Bronowicka Ostra and Cyklon are hot varieties and the capsaicinoid content in their fruits was 88 and 48.5 mg/100 g of dry mass, respectively. The capsaicinoid content in fruits in Tornado is more than 17 times, and in Tajfun more than 11 times lower than that in Bronowicka Ostra.

Antioxidant activity of flavonoid and capsaicinoid fraction

Antioxidant activity of flavonoids expressed in per cent of inhibition of β-carotene oxidation by free radicals formed *in vitro* with H_2O_2 and linoleic acid is presented in Figure 1. The highest activity was shown by the fraction obtained from fruits of the hot Cyklon variety and the lowest by one of the semi-hot Tajfun variety.

Studies by Lee *et al.* [1995] did not show any relations between flavonoids activity and spiciness of the paprika fruits. This discrepancy between the results obtained by those authors and the ones obtained in the present paper may be due to differences in the method of isolation and quantitative determination of flavonoid and capsaicinoid fraction.

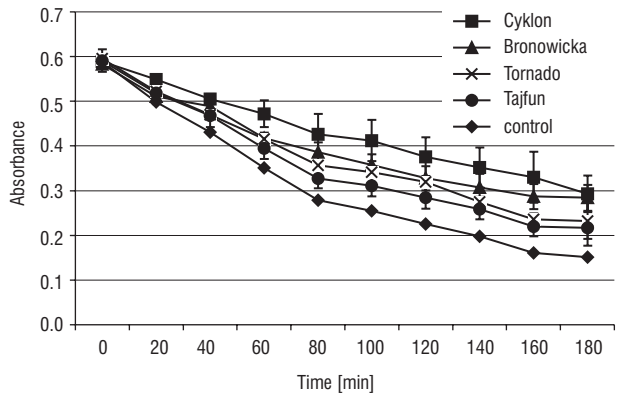

FIGURE 1. Antioxidant activity of flavonoid fraction of red fruits, measured as the speed of cooxidation of β-carotene and linoleic acid emulsion.

Capsaicinoid fractions obtained from the studied fruits showed, similar to flavonoid fraction, a strong antioxidant activity different for different varieties (Figure 2). The strongest antioxidant properties were shown by the fraction of capsaicinoids obtained from the fruits of Bronowicka Ostra and the weakest by the ones from Tornado and Tajfun. This dependency was connected with a low capsaicin and dihydrocapsaicin content in fruits of the Tornado and Tajfun varieties (Table 2).

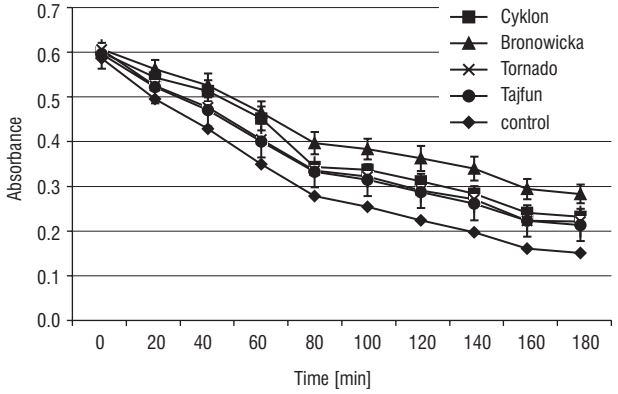

FIGURE 2. Antioxidant activity of capsaicinoid fraction from red fruits, measured as the speed of cooxidation of β-carotene and linoleic acid emulsion.

CONCLUSIONS

1. The fractions of capsaicinoids isolated from paprika fruits showed similar antioxidant activity as the flavonoid fraction.

2. The fruits of the varieties with a lower level of capsaicinoids were also characterized by a lower vitamin E and provitamin A content, but a higher vitamin C content than the fruits of hot varieties.

3. Because of the relatively high vitamin C, vitamin E, provitamin A as well as flavonoids and capsaicinoids content, paprika fruits may be a valuable food additive.

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AKTYWNOŚĆ ANTYOKSYDACYJNA I ZAWARTOŚĆ KAPSAICYNOIDÓW WYIZOLOWANYCH Z OWOCÓW PAPRYKI

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W pracy przedstawiono wyniki badań aktywności antyoksydacyjnej frakcji kapsaicynoidów oraz ich zawartości, całkowitego poziomu flawonoidów, kwasu askorbinowego, β-karotenu i tokoferoli w czerwonych owocach papryki (*Capsicum annuum* L.) odmiany Bronowicka Ostra, Cyklon, Tornado i Tajfun (tab. 1). Otrzymane wyniki wykazały, że frakcje kapsaicynoidów miały podobną aktywność antyoksydacyjną jak frakcje flawonoidów, zależną od poziomu kapsaicynoidów w owocach papryki (tab. 2). Owoce o niższym poziomie kapsaicynoidów charakteryzowały się niższą aktywnością antyoksydacyjną (rys. 1), niższą zawartością witaminy E i prowitaminy A, ale wyższą koncentracją witaminy C niż owoce odmian ostrych.