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Antioxidant Activity of the Main Phenolic Compounds Isolated from Hot Pepper Fruit (*Capsicum annuum* L.)

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Four cultivars (Bronowicka Ostra, Cyklon, Tornado, and Tajfun) of pepper fruit *Capsicum annuum* L. were studied for phenolics contents and antioxidant activity. Two fractions of phenolics, flavonoids (with phenolic acids) and capsaicinoids, were isolated from the pericarp of pepper fruit at two growth stages (green and red) and were studied for their antioxidant capacity. Both fractions from red fruits had higher activities than those from green fruits. A comparison of the capsaicinoid fraction with the flavonoid and phenolic acid fraction from red fruit with respect to their antioxidant activity gave similar results. Phenolic compounds were separated and quantified by LC and HPLC. Contents of nine compounds were determined in the flavonoid and phenolic acid fraction: *trans-p*-feruloyl-β-D-glucopyranoside, *trans-p*-sinapoyl-β-D-glucopyranoside, quercetin 3-*O*-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside, *trans-p*-ferulyl alcohol-4-*O*-[6-(2-methyl-3-hydroxypropionyl] glucopyranoside, luteolin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside, apigenin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside, lutoeolin 7-*O*-[2-(β-D-apiofuranosyl)-β-D-glucopyranoside], quercetin 3-*O*-α-L-rhamnopyranoside, and luteolin 7-*O*-[2-(β-D-apiofuranosyl)-4-(β-D-glucopyranosyl)-6-malonyl]-β-D-glucopyranoside. The main compounds of this fraction isolated from red pepper were sinapoyl and feruloyl glycosides, and the main compound from green pepper was quercetin-3-*O*-L-rhamnoside. Capsaicin and dihydrocapsaicin were the main components of the capsaicinoid fraction. A high correlation was found between the content of these compounds and the antioxidant activity of both fractions. Their antioxidant activities were elucidated by heat-induced oxidation in the β-carotene−linoleic acid system and the antiradical activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) decoloration test. The highest antioxidant activity in the β-carotene−linoleic acid system was found for *trans-p*-sinapoyl-β-D-glucopyranoside, which was lower than the activity of free sinapic acid. Quercetin 3-*O*-α-L-rhamnopyranoside had the highest antiradical activity in the DPPH system, which was comparable to the activity of quercetin. The activities of capsaicin and dihydrocapsaicin were similar to that of *trans-p*-feruloyl-β-D-glucopyranoside in the DPPH model system.

KEYWORDS: ***Capsicum annuum* L.; phenolic compounds; antioxidant activity**

INTRODUCTION

Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants as a result of plant adaptation to biotic and abiotic stress conditions (infection, wounding, water stress, cold stress, high visible light). Protective phenylpropanoid metabolism in plants has been well documented (*1*−*4*). In recent years phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the normal natural metabolism of aerobic cells (*5*, *6*). The antiradical activity of flavonoids and phenolics is principally based on the redox properties of their hydroxy groups and the structural relationships between different parts of their chemical structure (*7*−*9*). Epidemiological data have indicated beneficial effects of antioxidant compounds in the prevention of a multitude of disease states, including cancer, cardiovascular disease, and neurodegenerative disorders (*2*, *10*, *11*). The latest investigations on phenolic compounds suggested that cellular effects of flavonoids may be mediated by their interactions with specific proteins central to intracellular signaling cascades (*12*, *13*). Thus, flavonoids may act as signaling molecules. Phenolic compounds cannot be produced by the human body and thus must be taken in mainly through the daily diet. Knowledge about the nutritional and therapeutic role of dietary phenolic antioxidants is essential for the development of functional foods, which refers to the improvement of conventional foods with added health benefits (*10*). On the other hand, detailed chemical composition of foods considered to be functional is needed, and the main goal of the chemistry of natural compounds is screening for promising biologically active substances of plant origin.

Pepper fruits (*Capsicum annuum* L.) are important vegetables used as vegetable foods and as the spice. Peppers are a good source of vitamins C and E (*14*, *15*) as well as provitamin A

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and carotenoids—compounds with well-known antioxidant properties (*16*−*18*). Hot cultivars are rich in capsaicinoids—alkaloids with pharmacological properties giving the specific taste to pepper fruit (*15*, *19*). They are also present flavonoids and phenolic compounds, but the phenolics composition determined in pepper fruits is incomplete. The presence of derivatives of cinnamic acid and flavonoids has been found in pepper fruit by Sukrasno and Yeoman (*20*). They determined the contents of *p*-coumaryl, caffeoyl, and 3,4-dimethoxycinnamoyl glucoside and of four flavonoid compounds, but identification was given for only two: 3-*O*-rhamnosylquercetin and 7-*O*-glucosylluteolin. Other authors determined the content of two flavonoids (quercetin and luteolin) after acidic hydrolysis of the flavonoid fraction (*21*, *22*). A more detailed analysis of pepper phenolics was made by Iorizzi et al. (*23*), who identified 10 compounds in pepper fruit, 3 of which were based on a new structure; they were capsioside A, capsioside B, and capsianoside VII. However, the analysis of phenolic compounds in pepper focused mainly on qualitative not quantitative determination. Additionally, often flavonoids and phenolic acids were considered separately with capsaicinoids (*22*, *23*), whereas it was found that the phenylpropanoid and capsaicinoid biosynthetic pathways may converge during pepper fruit maturation (*20*, *24*). In our previous studies we defined the structure of nine compounds (without a prior hydrolysis) by MS and ^{13}C and ^{1}H NMR and quantitatively determined them by HPLC methods in hot pepper fruit of one cultivar (Bronowicka Ostra) at the red stage (*25*, *26*). Among them there are two compounds that had not been previously found in plants, and five compounds were identified for the first time in *Capsicum* species.

The aim of the present work was to complete the knowledge on *Capsicum* phenolics. We have determined the antioxidant activity of the flavonoid and capsaicinoid fractions isolated from hot and semihot pepper fruits, at two maturity stages. The primary objective of this study was to determine the concentration of each phenolic compound isolated from the pericarps of four cultivars of pepper fruits at green and red maturity stages. Finally, we compared the antioxidant activity of the main compounds isolated from pepper fruits with the activity of their aglycones.

MATERIALS AND METHODS

Plant Material. Fruits of two hot pepper cultivars, Cyklon and Bronowicka Ostra, and two semihot culvtivars, Tajfun and Tornado, were taken from the field experiment conducted in the years 1997−1999. Fruits were harvested at the stage of technological maturity, that is, completely developed but not colored (green) and at the stage of full ripeness (red). After the fruits had been washed and the seeds removed, fresh pepper fruits were cut and frozen or immediately freeze-dried. The freeze-dried samples were kept at −20 °C until analysis.

Isolation of the Lipophilic Fraction. The lipophilic fraction was isolated according to the method of Lee et al. (*21*), with some modifications. The freeze-dried pericarps of pepper (0.6 g) were homogenized with 80% ethanol (50 cm^3) using the Diax 900 homogenizor. Next, 10 cm^3 of raw extract was taken and evaporated until dryness on a rotary evaporator under reduced pressure at 40 °C. The dry residues were dissolved in 5 cm^3 of water and put on a Sep-Pak C_{18} (Waters) column. Before use, the column was washed with methanol and then with water. After sample loading, the polar compounds were washed out with 10 cm^3 of water and the next two fractions were eluted: the flavonoids—with 40% methanol—and the capsaicinoids—with 70% methanol (v/v). In this system the carotenoid pigments remained on the column.

The obtained methanol fractions were tested for the presence of flavonoids by thin-layer chromatography (TLC) on cellulose in the developing system butanol/acetic acid/water, 4:1:5 (*27*−*29*). Both fractions were evaporated until dryness at 40 °C and dissolved to the volume of 1 cm^3. One part of the obtained solution was analyzed by means of HPLC, and the other was determined with respect to antioxidant properties.

HPLC Characterization of Lipophilic Fractions. The initial qualitative analysis and quantitative determination of particular components of the fractions were made by high-performance liquid chromatography (HPLC) on the WellChrom chromatograph (Knauer) with a UV detector. Separation was done on a column filled with a modified silica gel RP-18 (Vertex Eurosil Bioselect 300 Å, ∅ 5 μm, 4 × 30 mm, endcapped), in gradient solvent systems A (1% H_3PO_4 in water) and B [40% acetonitrile (CH_3CN) in solution A], in such a proportion that the CH_3CN concentration was, to 10 min, 8%; in the 40th min, 20%; and in the 55th min, 40%; the flow speed was 1 cm^3/min, and the detection was at 330 nm. Particular derivatives of flavonoids and of phenolic acids were quantitatively determined with the use as standards phenolic compounds isolated from pepper fruit by means of preparative liquid chromatography, according to the method of Stochmal et al. (*30*), and identified by spectral methods NMR and MS as described in earlier studies (*25*). Quantitative determination of the capsaicinoids fraction was conducted by the HPLC method in an isocratic system with 55% CH_3CN, with the flow speed 1 cm^3/min and detection at 280 nm. The capsaicin and dihydrocapsaicin content was determined on the basis of a standard solution of capsaicin containing also dihydrocapsaicin (Merck).

Determination of Antioxidant Properties. Antioxidant properties on the basis of joint oxidation of β-carotene and linoleic acid were conducted in the way described in the literature (*21*, *31*), with certain modifications. A β-carotene solution in chloroform (0.12 mg/cm^3) was prepared. Next 3 cm^3 was taken and added to a round-bottom flask containing 40 mg of linoleic acid in 400 mg of Tween 20. Chloroform was removed on a vacuum evaporator at 30 °C, and then 100 cm^3 of 30% hydrogen peroxide was added. After thorough mixing, 3 cm^3 of the emulsion was added to 0.5 cm^3 of an extract obtained as described above; the control was 0.5 cm^3 of methanol instead of pepper extract. Oxidation of the β-carotene emulsion was monitored, at 470 nm on the Shimadzu UV-160A spectrophotometer. The measurements were conducted every 20 min for 3 h, starting at the moment of addition of hydrogen peroxide. The tubes between the measurements were thermostated at the temperature of 50 °C. The antioxidant activity (AA) was expressed as percent inhibition of β-carotene emulsion bleaching compared to the control.

Antiradical Activity Assessment. Scavenging free radical potentials were tested in methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described by Burda and Oleszek (*9*). For each compound an 8.6×10^{-3} mol/dm^3 solution in methanol was prepared. Then 0.5 cm^3 of solution was added to the tube containing 3 cm^3 of DPPH (0.05 mol/dm^3) solution. The absorbance was measured at 517 nm every 10 min, starting from the moment of solution mixing. A reference sample was prepared with 0.5 cm^3 of methanol. The antiradical activity was calculated as percentage of DPPH decoloration compared to the control in the time when all samples reached plateau (30 min).

Statistical Analysis. The statistical analysis comprised the results obtained in the three years of the experiments and done in three replications. For each set of data the standard deviation was calculated as a measure of dispersion of the set. To evaluate the significance of the differences between the means, Tuckey's multiple test was applied, assuming a 5% error probability. The calculations were done with Statgraphic v. 3.0 for Windows.

RESULTS AND DISCUSSION

Comparison of Antioxidant Activity of Two Phenolic Fractions of Pepper Fruit. Antioxidant activities of the fractions of capsaicinoids and flavonoid with phenolic acid derivatives isolated from four pepper cultivars were determined as the ability to quench free radicals by the compounds extracted from pepper fruit with 80% ethanol and separated on a microcolumn, SepPak C_{18}, with 40 and 70% methanol. Use of separation on silica gel with the octadecane phase allowed

Table 1. Antioxidant Activity of Flavonoid and Capsaicinoid Fractions from Hot Pepper Fruits at Different Maturity Stages of Various Cultivars of *C. annuum* L.[a]

cultivar	maturity stage	fraction	
		flavonoid	capsaicinoid
Bronowicka Ostra	green	24.5 ± 0.2a	23.3. ± 7.3a
	red	31.6 ± 3.1a	37.7 ± 0.7d
Cyklon	green	37.6 ± 1.4b	26.3 ± 0.9a
	red	40.7 ± 0.1b	30.1 ± 7.4b
Tornado	green	17.5 ± 1.1c	10.0 ± 0.3e
	red	33.4 ± 1.3a	27.9 ± 1.1a
Tajfun	green	16.7 ± 1.1c	5.4 ± 1.8f
	red	29.4 ± 1.9a	27.2 ± 3.2a

[a] Data are expressed as mean ± SD calculated as percent inhibition relative to the control from three values. Means in each column with similar letters are not significantly different ($P = 0.05$, Tuckey's multiple-range test).

strongly polar compounds to be eliminated from the studied fractions, such as L-ascorbic acid, as well as nonpolar compounds such as carotenoids. Both L-ascorbic acid and carotenoids could significantly change the antioxidant properties of pepper extracts (*32*). The results of antioxidant activities of capsaicinoid and flavonoid with phenolic acid derivatives fractions are shown in **Table 1**. Both fractions obtained from red fruits were characterized by higher antioxidant activity than those from green ones. Significant differences in antioxidant activity between flavonoid fractions obtained from red and green fruits were found in cultivars Tornado and Tajfun, for which the activities of the red fruit fraction were higher by 48 and 43%, respectively, than of green. Differences between antioxidant activity of capsaicinoid fractions from green and red fruits were noticed in three cultivars of pepper. In Bronowicka Ostra extracts from red fruits showed activity 38% higher than from green, and in the cultivars Tornado and Tajfun, activities were 64 and 80% higher, respectively. Comparison of antioxidant activities of fractions of capsaicinoids with flavonoid and phenolic acid derivatives showed that both fractions from red pepper had similar values, and the flavonoid fraction from hot pepper exhibited a higher antioxidant activity than that from semihot pepper (cv. Tajfun and Tornado) (**Table 1**). These data are in accordance with the results of our preliminary studies of two cultivars (*33*) and of studies of four cultivars but at one maturity stage (*34*), showing similar differences between antioxidant activities of capsaicinoid and flavonoid fractions.

The values of the percent inhibition after 120 min (**Table 1**) were lower than those obtained by other authors (*21*, *22*). This follows from differences in the composition of the analyzed extracts. Lee et al. (*21*) studied the antioxidant activity of one methanolic fraction obtained from the SepPak C_{18} microcolumn that, besides flavonoids, contained lipophilic phenolics and other nonpolar compounds, including capsaicinoids. Pepper extracts studied by Howard et al. (*22*) contained additionally L-ascorbic acid that positively influenced the extracts antioxidant activity.

Phenolics Contents in Flavonoid with Phenolic Acid Fractions. The quantitative HPLC analysis showed considerable changes in flavonoid fraction composition during fruit maturation. The major compounds of those fractions from red pepper fruit were esters of phenolic acids (**Figure 1**). The highest amounts of *trans*-ferulic acid and *trans*-sinapic acid esters were found in cv. Cyklon (**Table 2**). Very small amounts of ferulic and sinapic acid esters in unripe fruit found in the present study may be explained by the fact that the fruit was harvested when its growth was complete but the fruit was not yet colored. The

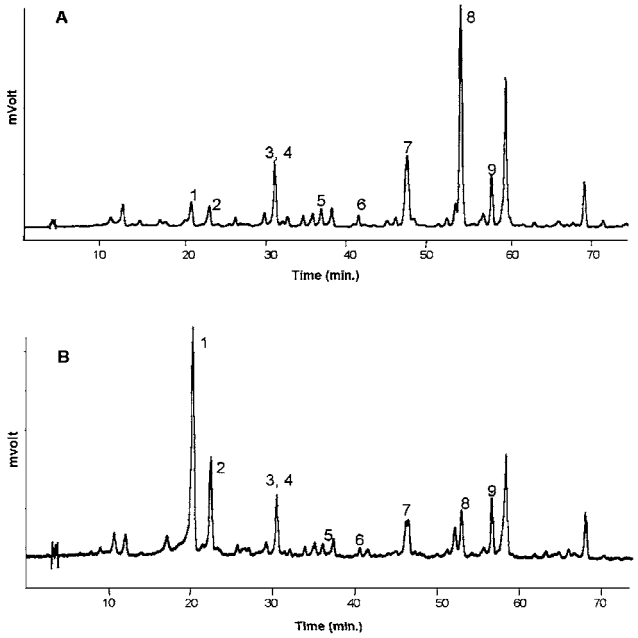


Figure 1. HPLC-UV chromatogram of phenolic compounds isolated from green (**A**) and red (**B**) pepper fruits of cv. Cyklon. Peaks: 1, *trans-p*-feruloyl-β-D-glucopyranoside; 2, *trans-p*-sinapoyl-β-D-glucopyranoside; 3, quercetin 3-*O*-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside; 4, *trans-p*-ferulyl alcohol-4-*O*-[6-(2-methyl-3-hydroxypropionyl] glucopyranoside; 5, luteolin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside; 6, apigenin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside; 7, luteolin 7-*O*-[2-(β-D-apiofuranosyl)-β-D-glucopyranoside]; 8, quercetin 3-*O*-α-L-rhamnopyranoside; 9, luteolin 7-*O*-[2-(β-D-apiofuranosyl)-4-(β-D-glucopyranosyl)-6-malonyl]-β-D-glucopyranoside.

rapid fall in the amount of phenolic acid glycosides may be caused by their inclusion in the structures of cell walls; an increase of these compounds in fully ripe fruit may be due to the completion of the process of fruit maturation (*20*, *35*).

Quercetin occurred as quercetin 3-*O*-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside and quercetin 3-*O*-α-L-rhamnopyranoside. The former compound was isolated from the pepper extract in a mixture with *trans-p*-ferulyl alcohol-4-*O*-[6-(2-methyl-3-hydroxypropionyl] glucopyranoside. The highest amounts of these compounds were also found in red fruits of all cultivars except cv. Cyklon. On the other hand, quercetin 3-*O*-α-L-rhamnopyranoside was the main compound in the flavonoid fraction from green fruits, and the highest amounts were noticed in cv. Cyklon. During fruit maturation its level decreased in all cultivars. Changes in quercetin 3-*O*-rhamnoside content occurred in a way similar to the one described by Sukrasno and Yeoman (*20*), who found that the level of this flavonoid was high in green fruit and decreased during ripening. Similar dependencies were shown by Howard et al. (*22*), who found that during pepper maturation the flavonoid aglycon contents determined after acid hydrolysis decreased.

Luteolin was first detected in the flavonoid fraction as luteolin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside, lutoeolin 7-*O*-[2-(β-D-apiofuranosyl)-β-D-glucopyranoside], and luteolin 7-*O*-[2-(β-D-apiofuranosyl)-4-(β-D-glucopyranosyl)-6-malonyl]-β-D-glucopyranoside, which had never been detected in plants up to now (**Table 2**). The highest amounts of these compounds, as in the case of quercetin 3-*O*-rhamnoside, were noticed in green fruit of cv. Cyklon. During fruit maturation their contents decreased in all cultivars. According to Sukrasno and Yeoman (*20*), 3-*O*-glucosylluteolin accumulated in pepper fruits to 20 days from anthesis and then fell sharply.

Table 2. Contents of the Phenolic Derivatives in Flavonoid Fractions from Hot Pepper Fruit at Different Maturity Stages of Various Cultivars of *C. annuum* L.[a]

compound	maturity stage	cultivar			
		Bronowicka Ostra	Cyklon	Tornado	Tajfun
trans-p-feruloyl-β-D-glucopyranoside	green	0.097 ± 0.013	0.236 ± 0.019	0.084 ± 0.013	0.126 ± 0.006
	red	0.266 ± 0.012	0.359 ± 0.027	0.229 ± 0.012	0.150 ± 0.040
trans-p-sinapoyl-β-D-glucopyranoside	green	0.091 ± 0.014	0.256 ± 0.026	0.074 ± 0.008	0.258 ± 0.014
	red	0.382 ± 0.047	0.324 ± 0.054	0.352 ± 0.021	0.419 ± 0.047
quercetin 3-*O*-α-L-rhamnopyranoside-7-*O*-β-D-glucopyranoside + *trans-p*-ferulyl-alcohol-4-*O*-[6-(2-methyl-3-hydroxypropionyl)] glucopyranoside	green	0.130 ± 0.013	0.361 ± 0.015	0.259 ± 0.001	0.250 ± 0.012
	red	0.208 ± 0.029	0.220 ± 0.012	0.365 ± 0.022	0.365 ± 0.022
quercetin 3-*O*-α-L-rhamnopyranoside	green	0.434 ± 0.085	0.993 ± 0.037	0.346 ± 0.007	0.327 ± 0.036
	red	0.137 ± 0.020	0.113 ± 0.017	0.125 ± 0.005	0.125 ± 0.026
luteolin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside	green	0.128 ± 0.012	0.299 ± 0.009	0.127 ± 0.005	0.170 ± 0.010
	red	0.089 ± 0.028	0.059 ± 0.009	0.084 ± 0.003	0.092 ± 0.007
lutoeolin 7-*O*-[2-(β-D-apiofuranosyl)-β-D-glucopyranoside]	green	0.642 ± 0.030	1.367 ± 0.100	0.470 ± 0.012	0.554 ± 0.008
	red	0.231 ± 0.026	0.160 ± 0.021	0.171 ± 0.010	0.209 ± 0.022
luteolin 7-*O*-[2-(β-D-apiofuranosyl)-4-(β-D-glucopyranosyl)-6-malonyl]-β-D-glucopyranoside	green	0.136 ± 0.009	0.412 ± 0.011	0.366 ± 0.010	0.213 ± 0.005
	red	0.069 ± 0.016	0.126 ± 0.006	0.136 ± 0.006	0.047 ± 0.006
apigenin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside	green	0.057 ± 0.006	0.075 ± 0.010	0.109 ± 0.006	0.048 ± 0.001
	red	0.018 ± 0.003	0.025 ± 0.003	0.041 ± 0.009	0.033 ± 0.005

[a] Data are expressed as mg/g of dry matter (mean ± SD for $n = 3$).

Table 3. Dihydrocapsaicin and Capsaicin Contents in Capsaicinoid Fractions from Hot Pepper Fruit at Different Maturity Stages of Various Cultivars of *C. annuum* L.[a]

compound	maturity stage	cultivar			
		Bronowicka Ostra	Cyklon	Tornado	Tajfun
capsaicin	green	0.442 ± 0.003	0.296 ± 0.001	0.043 ± 0.004	0.029 ± 0.001
	red	0.530 ± 0.006	0.343 ± 0.003	0.035 ± 0.005	0.047 ± 0.004
dihydrocapsaicin	green	0.317 ± 0.006	0.124 ± 0.006	0.028 ± 0.001	0.028 ± 0.005
	red	0.350 ± 0.001	0.142 ± 0.011	0.015 ± 0.002	0.030 ± 0.004

[a] Data are expressed as mg/g of dry matter (mean ± SD for $n = 3$).

The next compound found in the flavonoid fraction was apigenin 6-*C*-β-D-glucopyranoside-8-*C*-α-L-arabinopyranoside. It occurred in the smallest amounts in the flavonoid fraction from both green and red fruits (**Table 2**).

Higher amounts of quercetin 3-*O*-rhamnoside and other flavonoid glycosides in green fruit may be connected with their protective function for the photosynthetic apparatus. In earlier studies it was shown that flavonoids, strongly absorbing radiation within the range of 280−315 nm, that is UVB, may act as filters of UV radiation and in this way may protect photosynthesizing cells that are situated deeper from the surface (*2*, *36*). Quercetin 3-*O*-rhamnoside exhibits absorption maxima at 210, 257, and 347.1 nm, but in the whole UVB range its absorbance is high; because in green fruit the processes of photosynthesis occur as in other green parts of plants, proving a protective function of this compound is justified. A fall in content of that compound in red fruit may be explained by chlorophyll disintegration and inhibition of photosynthesis. The compounds that earlier acted as protectors for the photosynthetic apparatus become transitional compounds in further transformations of secondary metabolism (*2*).

Main Components of Capsaicinoid Fraction. The level of capsaicin concentrations varied greatly among the analyzed cultivars of pepper fruits. Cultivars Bronowicka Ostra and Cyklon belong to hot peppers because capsaicin contents were from 0.3 to 0.5 mg/g of dry matter (dm); cultivars Tornado and Tajfun may be counted among semihot peppers, as the levels were 0.03−0.05 mg/g of dm. The highest amount of dihydrocapsaicin, like capsaicin, was found in the fruit of cv. Bronowicka Ostra (**Table 3**). Cultivar Cyklon contained less dihydrocapsaicin over 60%; cultivars Tornado and Tajfun contained >11 times less than cv. Bronowicka Ostra. The results of HPLC quantitative analysis of the capsaicinoid fraction showed a slight increase of these compounds content in red fruit (**Table 3**).

The results obtained by other authors on changes of capsaicinoids content during maturation of pepper fruit showed that their accumulation starts at an early stage of fruit development, reaching the maximum at the final growth stage of fruit, but the full maturation stage was accompanied by a decrease in their contents (*20*, *37*, *38*). Moreover, on the basis of changes in activity of phenylalanine ammonia-lyase—the enzyme linked to the biosynthesis of capsaicinoids—and of a higher accumulation of these compounds in placenta, it was found that placenta is the only place of capsaicinoids synthesis (*20*, *39*−*41*). The research material for the present study were pericarps without placenta, where we noticed a slight increase in capsaicinoid contents in red fruits, which should be explained by migration of these compounds from placenta to pericarps and not by further processes of their synthesis.

The synthesis of flavonoids may converge with the capsaicinoids pathway during pepper maturation (*24*). According to a study of Sukrasno and Yeoman (*20*) capsaicinoid accumulation

Table 4. Correlation Coefficients of the Phenolics Contents and Antioxidant Activity of the Flavonoid and Capsaicinoid Fractions from Pepper Fruits at Different Maturity Stages of Various Cultivars of *C. annuum* L.

	maturity stage	
	green	red
flavonoid fraction		
main phenolics[a]	0.766	0.723
sum of phenolics[b]	0.836	0.589
capsaicinoid fraction		
main capsaicinoids[c]	0.720	0.894

[a] *trans-p*-Feruloyl-β-D-glucopyranoside, *trans-p*-sinapoyl-β-D-glucopyranoside, and quercetin 3-*O*-α-L-rhamnopyranoside. [b] Identified phenolics in flavonoid fraction (**Figure 1**). [c] Capsaicin and dihydrocapsaicin.

is parallel to the disappearance of flavonoids together with an accumulation of lignin-like substances. Lee et al. (*21*), studying 12 pepper cultivars, found that the heat index, which represented total capsaicinoid content, was not inversely associated with total flavonoid content. Moreover, mild peppers did not have greater flavonoid concentration than hot ones. They said that biosynthesis of flavonoids may be completed with capsaicinoids synthesis in phenylpropanoid metabolism, and each pepper type may regulate flavonoid synthesis differently. Studies on the changes of antioxidant activity of the flavonoid and capsaicinoid fractions in four pepper cultivars conducted in the present study confirm only to a certain degree the thesis formulated by Lee et al. (*21*). Although in the flavonoid fraction the highest activity was exhibited by extracts obtained from a hot pepper cultivar (Cyklon), in the case of another hot cultivar (Bronowicka Ostra) these activities were on the same level as in the extracts from semihot peppers; hence, in the discussed fraction antioxidant activity cannot be linked to the pungency of the fruit. On the other hand, a relatively low activity of the flavonoid fraction from red fruit of the cultivar Bronowicka Ostra and the highest activity of the capsaicinoid fraction of this cultivar seem to confirm Sukrasno and Yeoman's (*20*) thesis. These arguments incline one to state that in hot cultivars the process of capsaicinoid synthesis may occur at the cost of flavonoids.

Antioxidant Activity of the Main Phenolics of Pepper Fruit. To define the influence of compounds isolated from pepper fruits on antioxidant activity of the extracts, the antioxidant activity assay of three compounds from the flavonoid fraction, *trans-p*-feruloyl-β-D-glucopyranoside, *trans-p*-sinapoyl-β-D-glucopyranoside, and quercetin 3-*O*-α-L-rhamnopyranoside, and from the capsaicinoid fraction, capsaicin and dihydrocapsaicin in equimolar amounts, was performed. These compound contents correlated moderately (at $P \leq 0.05$) with antioxidant activity of both fractions from red and green fruits (**Table 4**). Thus, unmeasured phenolic compounds appear to make a considerable contribution to the total antioxidant capacity of flavonoid and capsaicinoid fractions of pepper fruit. Next, the activity of glycosides was compared to the activity of their aglycons. The use of β-carotene−linoleic acid as the antioxidant activity test model and DPPH as a test for scavenging free radical potentials allowed some dependencies between the structure of the investigated compounds and their activity to be evaluated (**Table 5**). The inhibition of bleaching by β-carotene emulsion indicates a strong antioxidant activity of the sinapic acid ester that was 25.8% lower than the activity of sinapic acid, whereas the highest antiradical activity based on the DPPH model system was found for quercetin 3-*O*-α-L-rhamnopyranoside, which was comparable to the activity of quercetin.

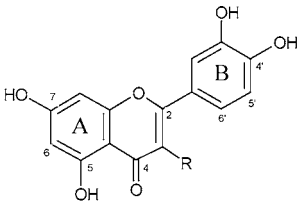
Table 5. Comparison of Antioxidant Activity of Equimolar Amounts of Main Phenolic Compounds Isolated from Pepper Fruit with Quercetin, *trans-p*-Ferulic Acid, and Sinapic Acid

	antioxidant activity	
compound	β-carotene emulsion[a]	DPPH[b]
trans-p-feruloyl-β-D-glucopyranoside	33.5 ± 4.1	10.0 ± 1.8
trans-p-sinapoyl-β-D-glucopyranoside	63.2 ± 1,1	13.3 ± 1.6
quercetin 3-*O*-α-L-rhamnopyranoside	12.6 ± 4.3	74.8 ± 0.6
capsaicin	27.9 ± 1.0	10.9 ± 1.1
dihydrocapsaicin	14.9 ± 2.1	9.3 ± 1.0
trans-p-ferulic acid	60.2 ± 4.9	37.7 ± 3.6
trans-p-sinapic acid	85.2 ± 1.3	49.0 ± 2.1
quercetin	70.3 ± 0.4	76.5 ± 1.7

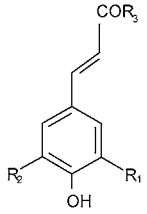
[a] Data are expressed as mean ± SD calculated from the three values of percent inhibition relative to control. Concentration of compounds for this method was 1.2×10^{-3} mol/dm^3. [b] Data are expressed as mean ± SD calculated from the three values, at phenolic concentration of 1.2×10^{-5} mol/dm^3 and DPPH (4.3×10^{-5} mol/dm^3) calculated on the basis of total volume in the tube (3.5 cm^3).

In the β-carotene and linoleic acid model system, the activity of ferulic and sinapic acid esters was lower than the activity of free phenolic acids. On this basis it may be said that ester linkage significantly decreased the antioxidant activity of both compounds (**Table 5**). The antioxidant activity of phenolic acid derivatives is connected with (i) the effect of −CH═CH−COOH group, (ii) the relationship between the number and positions of hydroxyl groups in the aromatic ring, and (iii) the methoxy substituents in the ortho position to the OH (*7*, *8*). Cuvelier et al. (*43*) suggested a decreasing effect in antioxidant potential of esterification; on the other hand, Rice-Evans et al. (*7*) revealed no differences between caffeic acid and its quinic acid ester—chlorogenic acid—in their inhibitory effects on low-density lipoprotein oxidation. They explained this by increased hydrophilicity of the compounds. Our investigations confirmed the results of Cuvelier et al. (*42*), and we can say that the hydrogen of the carboxyl group in −CH═CH−COOH plays an important role in the antioxidant activity of ferulic and sinapic acids. On the other hand, the results of Rice-Evans et al. (*7*) indicate that the hydroxyl substituents in the phenyl ring play the major role in the antioxidant efficiency of polyhydroxycinnamates. Thus, the antioxidant activity of caffeic acid is more connected with *o*-dihydroxy substitution in the aromatic ring than with the presence of the −CH═CH−COOH group. In our investigations we noticed loss of antioxidant activity of phenolic acid esters with respect to free acids; these losses were 44 and 26% when we compared ferulic acid to ferulic acid ester and sinapic acid to sinapic acid ester, respectively. This suggests that the methoxy substituents in the aromatic ring of sinapic acid have a considerable influence on antioxidant potential of this compound and its derivatives. Additionally, we found a bigger loss of antioxidant activity in the pair of sinapic acid and ferulic acid esters (47%) than in free sinapic and ferulic acids (29%). Thus, sinapic acid and its ester are more protective than ferulic acid because of more methoxy groups (**Figure 2**). On this basis we can say that the H-donating ability and subsequent radical stabilization of phenolic acid derivatives are a result of antioxidant capacity of all components in the molecule and structural relationships between them.

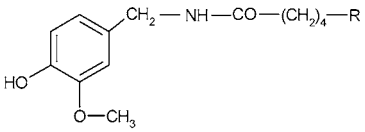
The decrease in antioxidant activity of quercetin 3-rhamnoside in comparison with quercetin showed that the antioxidant activity of polyphenols is connected with the presence of free hydroxyl groups (*7*−*9*, *43*). All flavonols with a free hydroxyl in the C-3 position and the double bond between C-2 and C-3



	R
quercetin	OH
quercetin 3-*O*-α-L-rhamnopyranoside	O-Rha



	R_1	R_2	R_3
trans-p-ferulic acid	H	OCH_3	OH
trans-p-sinapic acid	OCH_3	OCH_3	OH
trans-p-feruloyl-β-D-glucopyranoside	H	OCH_3	O-Glc
trans-p-sinapoyl-β-D-glucopyranoside	OCH_3	OCH_3	O-Glc



	R
capsaicin	CH = CH – CH – $(CH_3)_2$
dihydrocapsaicin	CH_2 – CH_2 – CH – $(CH_3)_2$

Figure 2. Structures of the main phenolic compounds isolated from pepper fruit of *C. annuum* L.

and with the second hydroxyl in the ortho position in the B ring had a high antioxidant activity. The blockade of the C-3 hydroxyl by glycosidic linkage with rhamnose caused a loss of activity (**Figure 2**).

There are few reports on the antioxidant activity of capsaicin and dihydrocapsaicin depending on their structure. The antioxidant activities of these compounds measured by the β-carotene and linoleic acid model system were comparable with the activity of ferulic acid ester (**Table 5**). It may be the result of the presence of the same groups in the phenolic ring (a methoxy group in ortho position to OH) of capsaicinoids and ferulic acid ester, which influenced the antioxidant properties. This is in agreement with Henderson et al. (*44*), who showed that the amide group present in capsaicin does not play a major role in its antioxidant activity under free radical oxidation conditions, that the antioxidant behavior for capsaicin was due primarily to the phenolic moiety in the molecule, and that the main product of capsaicin oxidation is its dimer—dicapsaicin. A comparison of the results of antioxidant activities of capsaicin and dihydrocapsaicin obtained in the present studies showed a lower antioxidant activity of dihydrocapsaicin than of capsaicin (**Table 5**). This may suggest that the double bond in the lipid chain influenced also the antioxidant activity of capsaicin (**Figure 2**).

The radical scavenging activity based on the DPPH model system was thought to be due to the hydrogen-donating ability of antioxidants (*43*). The changes in antiradical activity of the five investigated compounds were generally similar to their antioxidant activity in β-carotene and linoleic acid emulsion, with the exception of quercetin 3-*O*-rhamnoside, the antiradical activity of which was comparable to the activity of its aglycon. On the basis of the present study and literature data (*7*−*9*), it can be said that in DPPH model system the antiradical activity is more connected with the presence of *o*-dihydroxy groups in the B ring than with C-3 OH and also the double bond between C-2 and C-3, because of blocking of C-3 OH in quercetin rhamnoside by glycosidic linkage, which lowered the antiradical activity of that compound to only a small degree.

The antiradical activities of capsaicin and dihydrocapsaicin in the DPPH model system were comparable (**Table 5**). This suggests that in this model system, the double bond in the lipid chain plays a minor role in the antiradical activity of capsaicin. Kogure et al. (*45*) have found that the C-7 benzyl carbon, but not the phenolic OH group of capsaicin, is responsible for the scavenging site. Additionally, they found vanillin and 8-methyl-6-noneamide as products of capsaicin oxidative decomposition.

On the basis of the data in this study and in the literature, there is a likelihood that pepper fruits may provide the types of nutritional and health benefits associated with the consumption of fresh pepper fruits in general. Pepper fruits contain complex phenolic compounds, which occur with sugars as glycosides. Nevertheless, glycosides are extensively metabolized in vivo and the bioactive forms are not those found in plants (*12*), although knowledge about the activity of glycosides is very important, because ground pepper is used as a spice and simultaneously as a food protector (*46*, *47*). Further studies into the activity of minor compounds of peppers are needed to evaluate their potential health and food-protecting benefits.

LITERATURE CITED

(1) Shetty, K. Role of proline-linked pentoze phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochem.* **2004**, *39*, 789−803.

(2) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481−504.

(3) Pitchersky, E.; Gang, D. R. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends Plant Sci.* **2000**, *5*, 459−445.

(4) Douglas, C. J. Phenylpropanoid metabolism and lignin biosynthesis: from weeds to trees. *Trends Plant Sci.* **1996**, *1*, 171−178.

(5) Bors, W.; Heller, W.; Michel, C.; Stettmaier, K. Flavonoids and polyphenols: Chemistry and biology. *Handb. Antioxid.* **1996**, 409−466.

(6) Halliwell, B. Antioxidants in human health and disease. *Annu. Rev. Nutr*. **1996**, *16*, 39−50.

(7) Rice-Evans, C.; Miller, N. J.; Paganga, G. Structure−antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med*. **1996**, *20*, 933−956.

(8) Rice-Evans, C. A.; Miller, J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152−159.

(9) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem*. **2001**, *49*, 2774−2779.

(10) Ferrari, C. K. B.; Torres, E. A. F. S. Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. *Biomed. Pharmacother*. **2003**, *57*, 251−260.

(11) Hollman, P. C. H.; Katan, M. B. Dietary flavonoids: intake, health effects and bioaviability. *Food Chem. Toxicol*. **1999**, *37*, 937−942.

(12) Williams, R. J.; Spencer, J. P. E.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radical Biol. Med.* **2004**, *36*, 838−849.
(13) Schroeter, H.; Boyd, C.; Spencer, J. P. E.; Williams, R. J.; Cadenas, E.; Rice-Evans, C. MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. *Neurobiol. Aging* **2002**, *23*, 861−880.
(14) Palevitch, D.; Craker, L. E. Nutritional and medicinal importance of red pepper (*Capsicum* spp.). *J. Herbs Spices Med. Plants* **1995**, *3*, 55−83.
(15) Daood, H. G.; Vinkler, M.; Markus, F.; Hebshi, E. A.; Biacs, P. A. Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors. *Food Chem*. **1996**, *55*, 365−372.
(16) Krinsky, N. I. The biological properties of carotenoids. *Pure Appl. Chem*. **1994**, *66*, 1003−1010.
(17) Krinsky, N. I. Carotenoids as antioxidants. *Nutrition* **2001**, *17*, 815−817.
(18) Matsufuji, H.; Nakamura, H.; Chino, M.; Takeda, M. Antioxidant activity of capsantin and the fatty acid esters in paprika (*Capsicum annuum*). *J. Agric. Food Chem*. **1998**, *46*, 3468−3472.
(19) Wachtel, R. E. Capsaicin. *Regist. Anest. Pain Med*. **1999**, *24*, 361−363.
(20) Sukrasno, N.; Yeoman, M. M. Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. *Phytochemistry* **1993**, *32*, 839−844.
(21) Lee, Y.; Howard, L. R.; Villalon, B. Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *J. Food Sci*. **1995**, *60*, 473−476.
(22) Howard, L. R.; Talcott, S. T.; Brenes, C. H.; Villalon, B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as infuenced by maturity. *J. Agric. Food Chem*. **2000**, *48*, 1713−1720.
(23) Iorizzi, M.; Lanzotti, V.; DeMario, S.; Zollo, F.; Blanco-Molina, M.; Macho, A.; Munoz, E. New glycosides from *Capsicum annuum* L. var. *acuminatum*. Isolation, structure determination and biological activity. *J. Agric. Food Chem*. **2001**, *49*, 2022−2029.
(24) Diaz, J.; Bernal, A.; Merino, F.; Ros Barcelo, A. Phenolic metabolism in *Capsicum annuum* L. *Recent Res. Dev. Phytochem*. **1998**, *2*, 155−169.
(25) Materska, M.; Piacente, S.; Stochmal, A.; Pizza, C.; Oleszek, W.; Perucka, I. Isolation and structure elucidation of flavonoid and phenolic acid glycosides from pericarp of hot pepper fruit *Capsicum annuum* L. *Phytochemistry* **2003**, *63*, 893−898.
(26) Materska, M.; Perucka, I.; Stochmal, A.; Piacente, S.; Oleszek, W. Quantitative and qualitative determination of flavonoids and phenolic acid derivatives from pericarp of hot pepper fruit cv. Bronowicka Ostra. *Pol. J. Food Nutr. Sci.* **2003**, *12/53*, SI 2, 72−76.
(27) Terencio, M. C.; Sanz, M. J.; Fonseca, M. L.; Manez, S.; Rios, J. L. Phenolic compounds from *Lactuca viminea* L. *Z. Naturforsch.* **1992**, *47C*, 17−20.
(28) Hostettmann, K.; Lederer, M.; Marston, A.; Leipzig-Pagani, E. A study of the cyclodextrin complexes of flavonoids by thin-layer chromatography. *Phytochem. Anal.* **1997**, *8*, 173−175.
(29) Sherma, J. Thin-layer chromatography in food and agricultural analysis. *J. Chromatogr. A* **2000**, *880*, 129−147.
(30) Stochmal, A.; Piacente, S.; Pizza, C.; De Riccardis, F.; Leitz, R.; Oleszek, W. Alfalfa (*Medicago sativa* L.) flavonoids. 1. Apigenin and luteolin glycosides from aerial parts. *J. Agric. Food Chem.* **2001**, *49*, 753−758.
(31) Wanasundara, U. N.; Amarowicz, R.; Shahidi, F. Partial characterization of natural antioxidants in canola meal. *Food Res. Int.* **1996**, *28*, 525−530.
(32) Kaur, Ch.; Kapoor, H. C. Antioxidants in fruits and vegetables—the millennium's health. *Int. J. Food Sci. Technol.* **2001**, *36*, 703−725.
(33) Perucka, I.; Materska, M. Phenylalanine ammonia lyase and antioxidant activities of lipophilic fraction of fresh pepper fruits *Capsicum annuum* L. *Innovative Food Sci. Emerging Technol.* **2001**, *2*, 189−192.
(34) Perucka, I.; Materska, M. Antioxidant activity and contents of capsaicinoids isolated from paprika fruits. *Pol. J. Food Nutr. Sci.* **2003**, *12/53*, 2, 15−18.
(35) Chen, M.; Gitz, D. C.; McClure, J. W. Soluble sinapoyl esters are converted to wall-bound esters in phenylalanine ammonia lyase inhibited radish seedlings. *Phytochemistry* **1998**, *49*, 333−340.
(36) Harborne, J. B. Part of plant pigments. In *The Flavonoids: Recent Advances*; Goodwin T. W., Ed.; Academic Press: New York, 1988.
(37) Contreras-Padilla, M.; Yahia, E. M. Changes in capsaicinoids during development, maturation and sesescence of chile peppers and relation with peroxidase activity. *J. Agric. Food Chem.* **1998**, *46*, 2075−2079.
(38) Minami, M.; Toyota, M.; Inoue, T.; Nemoto, K.; Ujihara, A. Changes of capsaicinoid contents during maturing stage in chili pepper (*Capsicum* spp.). *J. Fac. Agric. Shinshu Univ*. **1998**, *35* (1), 45−49.
(39) Fujiwake, H.; Suzuki, T.; Iwai, K. Capsaicinoid formation in the protoplast from the placenta of *Capsicum* fruits. *Agric. Biol. Chem*. **1982**, *46*, 2591−2592.
(40) Hall, R. D.; Holden, M. A.; Yeoman, M. M. The accumulation of phenylpropanoid acid and capsaicinoid compounds in cell cultures and whole fruit of the chilli pepper. *Plant Cell Tissue Organ Cult.* **1987**, *8*, 163−176.
(41) Perucka, I. Effect of 2-chloroethylphosphonic acid on phenylalanine ammonia-lyase activity and formation of capsaicinoids in placenta of hot pepper fruits. *Acta Physiol. Plant.* **1996**, *18*, 7−12.
(42) Cuvelier, M. E.; Richard, H.; Berset, C. Comparison of the antioxidative activity of some acid-phenols. Structure−activity relationships. *Biosci., Biotechnol., Biochem*. **1992**, *56*, 324−325.
(43) Wang, M.; Li, J.; Rangarajan, M.; Shao, Y.; LaVoie, E. J.; Huang, T.; Ho, Ch. Antioxidative phenolic compounds from sage (*Salvia officinalis*). *J. Agric. Food Chem.* **1998**, *46*, 4869−4873.
(44) Henderson, D. E.; Slickman, A. M.; Henderson, S. K. Quantitative HPLC determination of the antioxidant activity of capsaicin on the formation of lipid hydroperoxides of linoleic acid: a comparative study against BHT and melatonin. *J. Agric. Food Chem.* **1999**, *47*, 2563−2570.
(45) Kogure, K.; Goto, S.; Nishimura, M.; Yasumoto, M.; Abe, K.; Ohiwa, Ch.; Sassa, H.; Kusumi, T.; Terada, H. Mechanism of potent antiperoxidative effect of capsaicin. *Biochim. Biophys. Acta* **2002**, *1573*, 84−92.
(46) Aguirrezabal, M. M.; Mateo, J.; Dominguez, M. C.; Zumalacarregui, J. M. The effect of paprika, garlic and salt on rancidity in dry sausages. *Meat Sci*. **2000**, *54*, 77−81.
(47) Aymerich, T.; Artigas, M. G.; Garriga, M.; Monfort, J. M.; Hugas, M. Effect of sausage ingredients and additives on the production of enterocin A and B by *Enterococcus faecium* CTC492. Optimization of *in vitro* production and anti-listerial effect in dry fermented sausages. *J. Appl. Microbiol.* **2000**, *88*, 686.

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