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ANTIOXIDANT ACTIVITIES OF CINNAMIC AND BENZOIC ACID DERIVATIVES

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Abstract. Antioxidant and antiradical activities of cinnamic and benzoic acid derivatives, using different methods, were investigated. The total antioxidant effect was evaluated using method with linoleic acid-water emulsion. The ability to scavenge free radicals was checked using 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet}$) or 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$). The reducing power of the studied compounds solutions was also evaluated. It was stated, that the applied measuring method strongly influenced the obtained result, because in the system with $ABTS^{\bullet}$ free radicals, the highest antioxidant activities possessed vanillic, sinapic and protocatechuic acid, whereas the most effective $DPPH^{\bullet}$ radical scavengers were methyl ferulate, methyl syringate, syringaldehyde and vanillic acid. In another method, linoleic acid peroxidation was effectively inhibited by the caffeic, sinapic and syringic acid. The highest reducing powers possessed protocatechuic acid, caffeic, sinapic and ferulic acid, higher than BHT and comparable to Trolox. Methylated derivatives of coumaric acid, caffeic acid, vanillic acid and syringic acid turned out to be weaker antioxidants and reducing agents than the free forms, except methyl ferulate, which possessed only slightly lower antioxidant activity than free ferulic acid, and in some measuring systems was a more effective antioxidant than ferulic acid.

Key words: cinnamic acids, benzoic acids, antioxidant activity, radical scavenging activity

INTRODUCTION

Cinnamic and benzoic acid derivatives are very widely distributed in plant world. They predominantly exist as esters of organic acids or glycosides, or are bound to a number of cell wall polymers, with only a small part of them being present as free forms [Hermann 1992]. There have been proceeded a number of studies concerning antioxidant actions of phenolic acids *in vitro* [Brand-Williams et al. 1995, Heinonen et al. 1998, Meyer et al. 1998, Natella et al. 1999, Watanabe 1999]. Caffeic acid has been shown to be a potent antioxidant in vitro in different oxidation systems [Chen and Ho 1997, Cuvelier et al. 1992, Moon and Terao 1998]; in *in vivo* studies, dietary supplementation of caffeic acid to rats resulted in α-tocopherol increase in plasma and lipopro-

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tein; caffeic acid supplemented to rats doubled total antioxidant activity of plasma, higher resistance of lipoproteins to Cu^{2+}-catalyzed oxidation was also observed [Nardini et al. 1997]. Ferulic acid is present in considerable amounts in outer layers of cereal grains [Pan et al. 1998]. Ferulate cross-linking of arabinoxylans to lignin restrict the enzymic degradation of the polysaccharides in cell walls of graminaceous plants [Grabber et al. 1998]. Ferulic acid and p-coumaric acid being present in barley and malt in free and insoluble, bound forms, are very potent and interesting antioxidants [Maillard and Berset 1995, Maillard et al. 1996]. Other authors also pointed out cinnamic acid derivatives as very potent antioxidants in wort and beer [Bellmer et al. 1995, Fantozzi et al. 1998], especially ferulic acid, which decreased carbonyl formation, inhibited lipid peroxidation during the storage, and effectively scavenged hydroxyl radicals in beer [Walters et al. 1997 a]. Short chains of feruloylated arabinoxylans exhibited sligthly stronger antioxidant activities than free acid *in vitro*; what is more, metabolism products of this group of compounds in rats plasma were detected, what could suggest their impact on antioxidant status *in vivo* [Ohta et al. 1994, Ohta et al. 1997]. Red wine also contains a range of phenolic acids, with coumaric, caffeic and protocatechuic acids with strong antioxidant activities. The most active phenolic acid turned out to be caffeic acid, which also showed strong sparing activity towards α-tocopherol [Abu-Amsha et al. 1996].

Taking under consideration, that major part of the phenolic acids in foodstuffs is present in ester or ether form, the authors of the presented paper wanted to check if esterification of free acids can modify their antioxidant action. The objective of this work was: comparison of the antioxidant and antiradical activities of a number of cinnamic and benzoic acid derivatives as well as checking if esterification of the free acid can change the total antioxidant activity of the acid.

MATERIALS AND METHODS

Reagents and apparatus. Myoglobin (from horse heart), Tween 20, linoleic acid (min. 990 $g \cdot kg^{-1}$) and butylated hydroxytoluene (BHT, 990 $g \cdot kg^{-1}$) were purchased from Sigma Chemical Co. (St. Louis, MO), ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6--sulphonic acid) diammonium salt; min. 980 $g \cdot kg^{-1}$) and Trolox (6-hydroxy-2,5,7,8--tetrametylchroman-2-carboxylic acid, Hoffman-La Roche, min. 970 $g \cdot kg^{-1}$) were obtained from Aldrich Chemical Co., Gillingham, Dorset SP8 4JL, England. 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) was gained from ICN Biomedicals Inc., Aurora, Ohio. Hydrogen peroxide (300 $g \cdot kg^{-1}$), methanol (spectrophotometric grade), potassium ferricyanide (980 $g \cdot kg^{-1}$), trichloroacetic acid, ferric chloride (980 $g \cdot kg^{-1}$) disodium hydrogen orthophosphate 12-hydrate and sodium dihydrogen orthophosphate dihydrate, were obtained from POCh, Poland.

O-coumaric acid and p-coumaric acid were from Ubichem, Staines, England. Caffeic acid, caffeic acid dimethyl ether, veratric acid, syringic acid and syringaldehyde were purchased from Sigma, St. Louis, USA. Ferulic acid was from Carl Roth, Karlsruhe, Germany. Sinapic acid and vanillic acid were from Fluka AG, Buchs, Switzerland. Protocatechuic acid was from EGA Chemie, Germany. Veratric alcohol and veratric aldehyde were from Aldrich, Germany. Benzoic acid, coumarin and 4-dimethoxyaminobenzoic acid were from POCH, Gliwice, Poland. Cinnamaldehyde was from BDH Chemicals, Poole, England. Methyl ferulate, methyl coumarate, methyl vanillate and methyl syringate were obtained from Apin Chemicals Ltd., England.

All spectrophotometric data were gained using Unicam 5625 UV/VIS Spectrophotometer.

Measurement of Trolox Equivalent Antioxidant Activity (TEAC) was performed as described by Miller et al. [1993] in the following manner: all the reagents at desired concentrations were prepared in 5 mmol·dm^{-3}, pH 7.4 phosphate buffer. Reagents in the measuring cell of the spectrophotometer were at concentrations: 0.15 mmol·dm^{-3} ABTS; 0.0025 mmol·dm^{-3} metmyoglobin and 0.375 mmol·dm^{-3} hydrogen peroxide. 0.03 cm^3 of the phenolic acid solution (1 mmol·dm^{-3}) was transferred to the measuring cell of the spectrophotometer (Unicam 5625 UV/VIS Spectrometer). The sample was then flushed with 0.135 cm^3 of distilled water, then 1.5 cm^3 of the ABTS/metmyoglobin reagent was added and at last 0.125 cm^3 of the hydrogen peroxide solution added started the reaction. The temperature was kept at 20°C. Changes in absorbance at 734 nm as the function of time were recorded every 10 s until the absorbance started to increase. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analogue, was used as an antioxidative standard. Total antioxidant activity was expressed in molar concentration of Trolox solution (TEAC value), that inhibited the increase in absorbance equally to the reaction mixture with phenolic acid. System was standardized using 23 sequential and separately prepared stock standards of Trolox in the range from 0.02 mmol·dm^{-3} to 2 mmol·dm^{-3}. These stock standards replaced 0.030 cm^3 of the investigated sample during the standardization of the assay.

Measurement of inhibition of linoleic acid peroxidation was performed as described by Lingnert et al. [1979], with the following modifications: linoleic acid and Tween 20 (equal volume to the acid) were added to 50 mmol·dm^{-3} , pH 7.6 phosphate buffer, so that the final concentration of the acid was 30 mmol·dm^{-3}. Emulsions were prepared daily fresh for experiments. Directly before use emulsion was sonicated in ultrasonic bath for 10 minutes. Solutions of the phenolic compounds (0.2 cm^3) were added to 1 cm^3 of the linoleic acid emulsion and placed in tubes with stoppers. Controls without studied solutions and samples with addition of 0.2 cm^3 of 2 mmol·dm^{-3} Trolox or BHT solution were run simultaneously. The tubes were then placed in darkness at 40^{o}C and prior the incubation and every 4 hours during the incubation (until 28 hours) an aliquot (0.1 cm^3) of the mixture was withdrawn. Then 1 cm^3 of absolute methanol and 3 cm^3 of 60% methanol-in-water solution were added, and absorbance at 234 nm (Unicam 5625 UV/VIS Spectrometer) was read against the methanol solution as a reference. The Antioxidant Effect was calculated according to the following equation:

$$A.E. = \Delta A_{234}(C) - \Delta A_{234} / \Delta A_{234}(C) \quad (1)$$

where: ΔA_{234} – increase of absorption at 234 nm in assayed mixture during the incubation,

$\Delta A_{234}\,(C)$ – the corresponding increase of absorption in control.

The antiradical activities of studied compounds against DPPH$^\bullet$ were measured using method developed by Brand-Williams et al. [1995]. A volume of 1 cm^3 of antioxidant solution (1 mmol·dm^{-3}) was added to 2 cm^3 of a 0.06 mmol·dm^{-3} methanol DPPH$^\bullet$ solution. The decrease in absorbance was measured spectrophotometrically at 515 nm every 1 minute until the absorbance reached a plateau. The exact DPPH$^\bullet$ concentration was calculated from a calibraton curve, using the equation:

$$\text{Abs. at } 515 \text{ nm} = 6.3257\,[\text{DPPH}^\bullet] + 00218$$

For every compound studied, the reaction kinetics was plotted. The concentration of residual DPPH $^{\bullet}$ at steady state was taken as a value of antiradical action of the investigated compounds.

Reducing power measurement. Reducing power was determined according to the method of Oyaizu [1986] in the following manner: 1 mmol·dm^{-3} solution of the phenolic acid (0.2-1 cm^3) was added to 2.5 cm^3 of phosphate buffer (0.2 mol·dm^{-3}; pH 6.6) and 2.5 cm^3 of 10 g·kg^{-1} potassium ferricyanide [$K_3Fe(CN)_6$] solution; the mixture was then incubated at 50^{o}C for 20 minutes. After incubation, 2.5 cm^3 (10 g·kg^{-1}) of trichloroacetic acid solution was added, then to 2.5 cm^3 of the resulting solution 2.5 cm^3 of distilled water and 0.5 cm^3 of 0.1 g·kg^{-1} $FeCl_3$ solution was added, and the absorbance was read at 700 nm. The increased absorbance indicated increased reducing power of the solution under investigation.

RESULTS

The Trolox Equivalent Antioxidant Activity values of cinnamic and benzoic acid derivatives are shown in Table 1.

The highest antiradical activity, equal to the activity of BHT, showed vanillic, protocatechuic and sinapic acid. In the case of coumaric acid, caffeic acid, ferulic acid, vanillic acid and syringic acid, the antioxidant activities of these phenolic acids methyl derivatives were lower in comparison to the activities of their corresponding free forms. Methyl ferulate showed only slightly lower TEAC value than free ferulic acid, whereas vanillic, coumaric and syringic acid methyl esters didn't exhibited antioxidant activity. Similarly, caffeic acid after double etherification with methanol showed very low TEAC value in comparison to free acid. It must be emphasised, that although ferulic acid showed much lower antioxidant activity (1.36 mmol·dm^{-3}) than a number of cinnamic and benzoic acid derivatives, its esterification did not cause such a fall in TEAC value as in the case of other methyl esters. The studied solutions of the aldehydes showed poor or no antiradical activity, and in the case of veratric derivatives the activities were: veratric acid > veratric alcohol > veratric aldehyde. There is a difference between TEAC values for both forms of coumaric acid, indicating the role of OH–group position attached to the phenol ring, and no activity recorded for methyl coumarate showed the importance of the CH=CH-COOH group in the molecule.

Tables 2 and 3 show the Antioxidant Effect of cinnamic and benzoic acid derivatives. In the reaction medium with linoleic acid, the highest AE value after 2-hour incubation was recorded for ferulic acid, and methyl ferulate had only slightly lower antioxidant activity than the free form. Also in the case of other methylated phenolic acids, their free forms showed higher AE values, what confirms the results obtained using the previously presented method. Caffeic acid dimethyl ether acted as a prooxidant in this medium. P-coumaric acid possessed 2-fold higher AE value than o-coumaric acid, what underlines the importance of the OH-group position. In 6-hour trial, different schemes of antioxidant activity evolution can be recorded. The general tendency for aldehydes was prooxidant action after 2 hours of incubation (AE value is below zero), then low antioxidant activity after 4 hours of incubation and then again prooxidant action after 6 hours of incubation, although p-dimethylaminobenzoic aldehyde and syringaldehyde maintained the antioxidant activity during the whole time of incubation.

Table 1. Trolox Equivalent Antioxidant Activity (TEAC values) of cinnamic and benzoic acids derivatives
Tabela 1. Wartości TEAC (aktywności przeciwutleniającej wyrażonej w równoważnikach Trolox-u) pochodnych kwasu cynamonowego i benzoesowego

Compound Badany związek chemiczny	TAEC value Wartość TEAC mmol/L
o-coumaric acid	0.034
p-coumaric acid	0.134
methyl coumarate	0
caffeic acid	1.410
caffeic acid dimethyl ether	0.034
ferulic acid	1.360
methyl ferulate	0.952
sinapic acid	2.023
cinnamic aldehyde	0
protocatechuic acid	1.920
coumarin	0
vanillic acid	2.066
methyl vanillate	0
veratric acid	0.034
veratric alcohol	0.017
veratric aldehyde	0
syringic acid	1.853
methyl syringate	0.051
syringaldehyde	0.017
benzoic acid	0
p-dimethylaminobenzoic aldehyde	0
BHT	2.040

BHT and Trolox suppressed linoleic acid peroxidation very effectively, reaching complete inhibition of the unwanted phenomena in the case of BHT. The AE of both antioxidant compounds constantly increased during the 6-hour incubation.

Table 4 presents scavenging of DPPH$^{\bullet}$ radical by cinnamic and benzoic acid derivatives. The strongest DPPH$^{\bullet}$ radical scavenger turned out to be methyl ferulate, which possessed antiradical activity equal to Trolox and was superior to BHT. Free ferulic acid was not so efficient radical scavenger, and similarly in the case of syringic acid, also the methylated form was better antioxidant. On the other hand, free vanillic, caffeic and p-coumaric acid lowered the DPPH$^{\bullet}$ concentration at steady state more effectively than

Table 2. Antioxidant Effect (AE) of cinnamic and benzoic acid derivatives after 2 hours of incubation with linoleic acid
Tabela 2. Aktywność przeciwutleniająca (AE) pochodnych kwasu cynamonowego i benzoesowego po 2 godzinach inkubacji z kwasem linolowym

Compound Badany związek chemiczny	AE value Wartość AE
o-coumaric acid	0.11
p-coumaric acid	0.24
methyl coumarate	0.09
caffeic acid	0.47
caffeic acid dimethyl ether	–0.11
ferulic acid	0.61
methyl ferulate	0.52
sinapic acid	0.38
protocatechuic acid	0.36
syringic acid	0.38
vanillic acid	0.36
methyl vanillate	0.17
veratric acid	0.16
veratric alcohol	0.27

Table 3. Antioxidant Effect (AE) of cinnamic and benzoic acid derivatives after 2, 4 and 6 hours of incubation with linoleic acid
Tabela 3. Aktywność przeciwutleniająca (AE) pochodnych kwasu cynamonowego i benzoesowego po 2, 4 i 6 godzinach inkubacji z kwasem linolowym

Compound Badany związek chemiczny	AE value measured after Wartość AE mierzona po		
	2 hours of incubation 2 godzinach inkubacji	4 hours of incubation 4 godzinach inkubacji	6 hours of incubation 6 godzinach inkubacji
cinnamic aldehyde	–0.08	0.12	–0.14
veratric aldehyde	–0.08	0.24	–0.33
methyl syringate	0.22	0.36	0.28
syringaldehyde	0.12	0.65	0.24
benzoic acid	0.24	0.24	0.14
coumarin	–0.08	0	0.14
p-dimethylaminobenzoic aldehyde	0.68	0.88	1.00
BHT	0.56	0.77	0.9

their methylated derivatives; in the case of caffeic acid dimethyl ether there was no suppression of DPPH$^{\bullet}$ formation at steady state. There can be also seen the difference in antioxidant capacity between o-coumaric acid p-coumaric acid that was mentioned already before, and in this medium o-coumaric acid showed practically no radical scavenging activity. The aldehydes studied were no antioxidants with the exception of syringaldehyde, which turned out to be quite a good and radical scavenger, what agrees with the results of the previously presented systems with ABTS and linoleic acid.

Table 4. Scavenging of DPPH$^{\bullet}$ radicals measured as decrease of free radical concentration gained at steady state.The initial concentration of DPPH $^{\bullet}$ = 0.06 mmol·dm^{-3}
Tabela 4. Zdolność „zmiatania" rodników DPPH$^{\bullet}$ wyrażona jako obniżenie stężenia wolnego rodnika w stanie równowagowym układu. Stężenie początkowe rodnika = 0,06 mmol·dm^{-3}

Compound Badany związek chemiczny	DPPH$^{\bullet}$ concentration measured at steady state Stężenie DPPH$^{\bullet}$ mierzone w stanie równowagowym mmol/L
o-coumaric acid	0.056
p-coumaric acid	0.039
methyl coumarate	0.047
caffeic acid	0.044
caffeic acid dimethyl ether	0.060
ferulic acid	0.035
methyl ferulate	0.007
sinapic acid	0.034
cinnamic aldehyde	0.060
protocatechuic acid	0.036
vanillic acid	0.028
methyl vanillate	0.046
veratric alcohol	0.058
veratric aldehyde	0.060
syringic acid	0.034
methyl syringate	0.011
syringaldehyde	0.028
benzoic acid	0.060
coumarin	0.060
p-dimethylaminobenzoic- aldehyde	0.060
veratric acid	0.060
BHT	0.030
Trolox	0.008

The reducing power is a measure of the ability of the compound to convert free radicals to more stable forms. The compound having the reducing power is an electron donor which terminates the radical chain reactions. Figure 1 shows, that reducing powers of 1 mmol·dm^{-3} solution of ferulic acid or p-coumaric acid were higher than reducing powers of their corresponding methyl esters; moreover, reducing powers of ferulic acid and its methyl ester were also higher than reducing powers of both forms of p-coumaric acid.

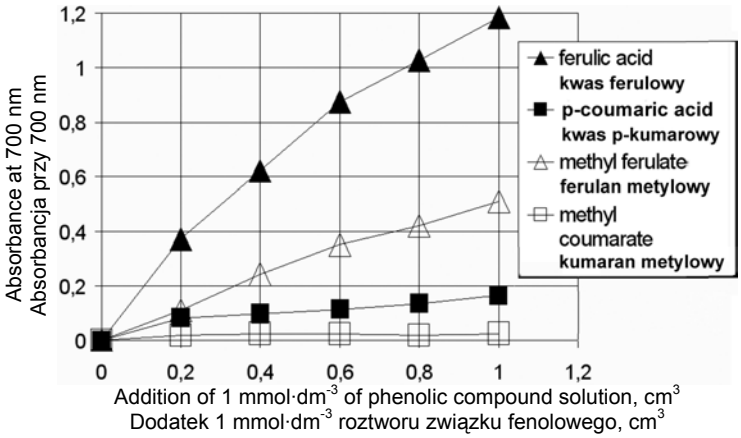

Fig. 1. Reducing powers of 1 mmol·dm^{-3} solution of ferulic and p-coumaric acid and their corresponding methyl esters
Rys. 1. Siły redukcyjne 1 mmol·dm^{-3} roztworów kwasu ferulowego, kumarowego oraz ich estrów metylowych

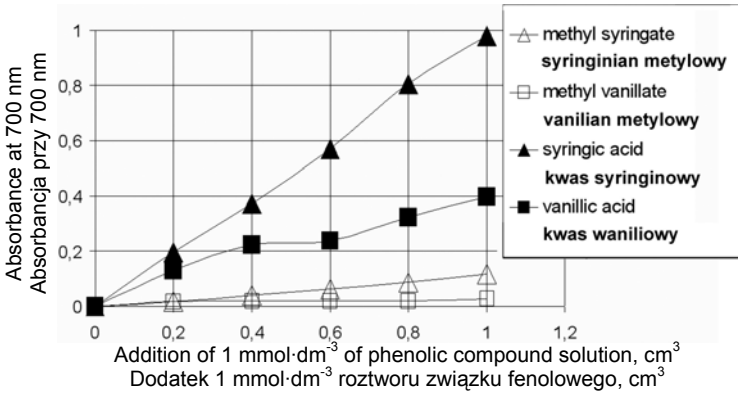

Fig. 2. Reducing powers of 1 mmol·dm^{-3} solution of syringic and vanillic acid and their corresponding methyl esters
Rys. 2. Siły redukcyjne 1 mmol·dm^{-3} roztworów kwasu syringowego, waniliowego oraz ich estrów metylowych

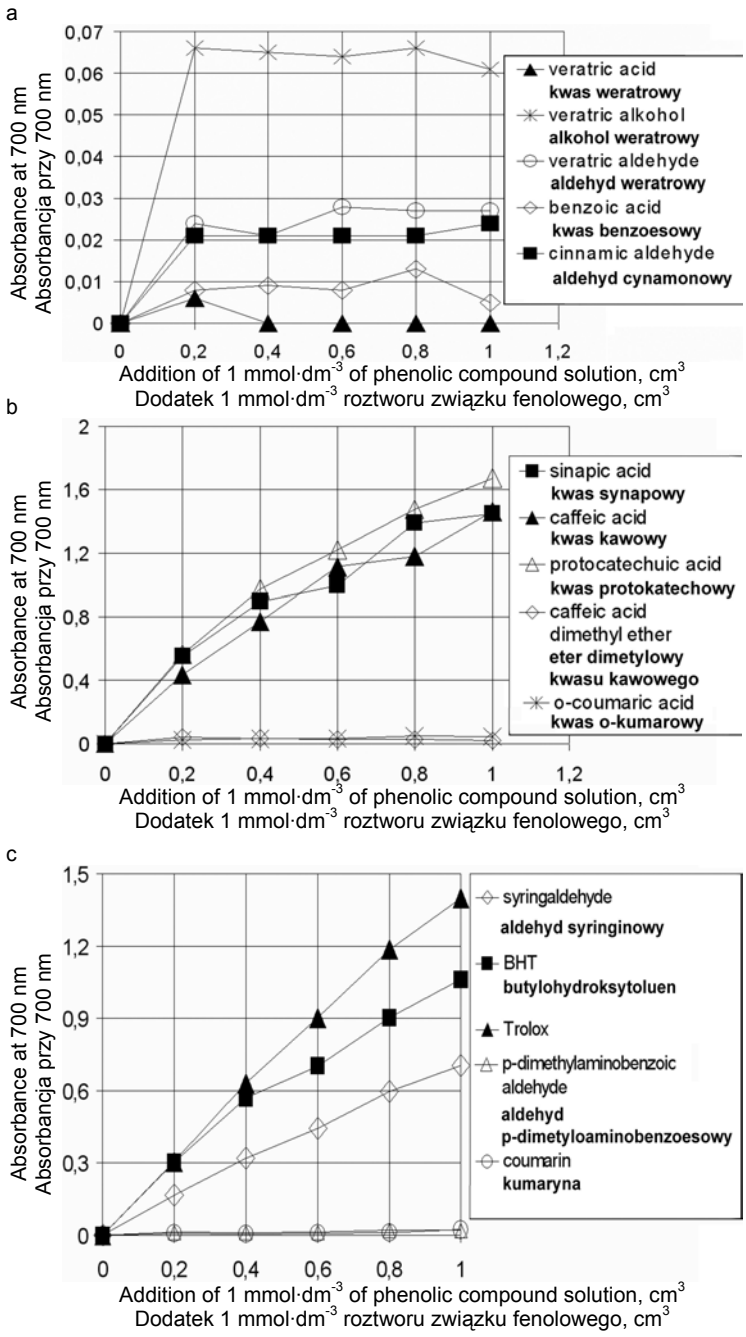

Fig. 3. Reducing powers of 1 mmol·dm^{-3} solutions of selected cinnamic acid and benzoic acid derivatives

Rys. 3. Siły redukcyjne 1 mmol·dm^{-3} roztworów wybranych pochodnych kwasu cynamonowego i benzoesowego

Figure 2 presents reducing powers of standard solutions of syringic and vanillic and their methyl esters; it can be noticed, that as in the case of cinnamic acid derivatives (Fig. 1) reducing powers of both free phenolic acids were higher than their ester forms. High reducing power of methyl ferulate among other methylated derivatives of phenolic acids must be emphasised.

Data presented in illustrations a-c gathered in Figure 3 present the reducing powers of 1 mmol·dm^{-3} solutions of a number of selected cinnamic acid and benzoic acid derivatives. Among phenolic acids which were studied, the highest reducing power was recorded in the case of protocatechuic acid (Fig. 3 b), but caffeic, sinapic (Fig. 3 b) and ferulic acid (Fig. 1) also possessed high reducing power, higher than BHT and comparable to Trolox (Fig. 3 c). The high reducing power of syringaldehyde (Fig. 3 c) must be emphasised, when compared to reducing power estimated for syringic acid and methyl syringate standard experimental solutions (Fig. 2). Although free caffeic acid had a high reducing power, caffeic acid dimethyl ether showed a very poor reducing power (Fig. 3 a). O- coumaric acid showed a very low reducing power (Fig. 3 b), much lower than the reducing power of free p-coumaric acid and comparable to the reducing power of methyl coumarate (Fig. 1). A number of the studied compounds, namely benzoic acid, veratric acid, coumarin and p-dimethyloaminobenzoic aldehyde exhibited a very low reducing power at experimental concentrations applied in this study.

DISCUSSION

A number of investigations conducted in order to evaluate the antioxidant activities of pure cinnamic and benzoic acid derivatives indicated a structure- activity relationship in model systems; Cuvelier et al. [1992] showed, that the antioxidant potential depends on substitution of the phenol ring with hydroxyl groups in *ortho* or *para* position. The methylation of the phenol ring in *ortho* position relative to the hydroxyl group also increased the activity. These authors also pointed out, that higher antioxidant activity of cinnamic acid derivatives in comparison to their respective benzoic acids can be ascribed to the presence of CH=CH-COOH group in these molecules. Neverthless, in our study, benzoic acids, like protocatechuic and vanillic showed in some measuring systems higher antioxidant activities, than cinnamic derivatives- caffeic and ferulic acid, respectively. The activity strongly depended on the applied medium and reflected polar or apolar nature of the compound. It was stated, that polar antioxidant acts more effectively in apolar medium and apolar antioxidant in medium with polar solvent. Most of the acids exist in plants in forms of sugar ethers and esters, with only a small part as free forms. Chen and Ho [1997] compared antioxidant activities of caffeic and ferulic acids and their phenethyl esters; in this study, the antioxidant and antiradical potencies differed between pure cinnamic acids and their phenethyl esters, also depending on the method used. In general, phenolic acid esters turned out to be equally or less effective antioxidants than the corresponding pure acids. Moreover, chlorogenic acid which is ester of caffeic and quinic acid showed in this study lower activity than caffeic acid. Meyer et al. [1998] compared antioxidant activities of pure caffeic, p-coumaric, ferulic acid with their tartaric acid esters. The differences in antioxidant potencies were related to the degree of hydroxylation and methoxylation of the molecules; esterification to the tartaric acid sligtly enhanced the activity of p-coumaric and ferulic acid, whereas esteri-

fication of caffeic acid to quinic acid decreased the activity. Esterification of caffeic acid with tartaric acid had no significant influence on the activity of this cinnamic acid derivative. Our results agree with observations made by above mentioned authors, and in all applied measuring systems free phenolic acids had higher antiradical activities than their corresponding ether or ester forms, except methyl ferulate and methyl syringate, which very effectively "scavenged" DPPH$^{\bullet}$ radical.

Moon and Terao [1998] investigated the role of the 2,3-double bond in the structure of caffeic acid and compared the antioxidant potency of caffeic and dihydrocaffeic acid in different systems. The authors concluded, that double bond increased the antioxidant efficiency of caffeic acid in some reaction mixtures, whereas in other measuring systems dihydrocaffeic turned out to be stronger antioxidant. This suggests, that double 2,3 bond cannot be considered as responsible for the inherent antioxidant activity of the caffeic acid. This conclusion agrees with our observations, because protocatechuic acid, which lacks a double 2,3 bond in molecule showed higher antioxidant activities than caffeic acid in two measuring systems (Tables 1 and 4). In another study, performed by Natella et al. [1999] the antioxidant activities of four benzoic and four homologous cinnamic acid derivatives were compared. The latter group of compounds: p-coumaric, caffeic, ferulic and sinapic acid showed higher antioxidant activities than the corresponding: p-hydroxybenzoic, protocatechuic, vanillic and syringic acids. This confirms the role of propenoic side chain as an active group in this context. Presence of the second hydroxy group strongly enhanced the antioxidant activities and the substitution of methoxy groups in ortho- position to the hydroxy group also increased this activity. Neverthless, in our study, protocatechuic and vanillic were better antioxidants than caffeic and ferulic acid.

Ferulic acid forms the cross-links in arabinoxylans and lignins and may restrict enzymatic degradation of structural polysaccharides from aleurone cell walls. Maillard et al. [1996] investigated the total antioxidant activity of extracts from malt at different stages of malting; the authors confirmed strong influence of bound ferulic and p-coumaric acids on this activity. Ferulic acid is most abundand cinnamic acid derivative in barley endosperm cell walls and can influence some technological processes during malting and brewing like releasing of cell wall structural polysaccharides into wort, e.g. arabinoxylans. These pentosans together with β-glucans may cause some filtration problems, as well as beer non-microbial hazes. Ohta et al. [1994] enzymatically isolated hemicellulose fragments from insoluble corn bran dietary fiber, mainly ferulic acid arabinoxylane esters, having a range of molecular weight. It was stated, that cell wall hemicellulose fragments from corn had useful antioxidant activities; high molecular fractions of arabinoxylans esterified by diferulic acid were stronger antioxidants than free ferulic acid, 5-O-feruloyl-L-arabinofuranose and low molecular arabinoxylans. The antioxidant activities of these high molecular fractions of arabinoxylans can depend on diferulic acid concentration, because they contained much more diferulic moieties than other fractions. In another study, Ohta et al. [1997] investigated differences in antioxidant activities of 5-O-feruloyl-L-arabinofuranose and free ferulic acid. Free acid showed less suppressive effect on low density lipoproteins oxidation than ferulic acid sugar esters, probably due to the better affinity of the LDL molecules to the ferulic acid esters, which has hydrophilic sugar moiety. We must emphasise, that in our study free ferulic acid in model solution was a better antioxidant, but methyl ferulate was a very efficient DPPH$^{\bullet}$ radical scavenger, 5-fold stronger than the free acid. What is also important, the

portant, the authors have revealed, that feeding rats with 5-O-feruloyl-L-arabinofuranose resulted in presence of more polar derivatives of this ester in plasma, probably conjugated form, in contrary to the control group of rats, where no ferulic acid derivatives were detected. This verifies the absorption of 5-O-feruloyl-L-arabinofuranose from digestive tract to the plasma and can be an important step in studying the role and metabolism of ferulic acid sugar esters in human organism.

Fantozzi et al. [1998] evaluated the contents of phenolic compounds at every stage of the brewing process. It was concluded, that more than 80% of total phenolic compounds in beer after bottling and pasteurization are phenolic acids, responsible for relatively high *in vitro* antioxidant activity of beer. Other authors [Walters et al. 1997 a, b] studied the influence of ferulic acid on formation of carbonyl compounds during the forced-aging of beer. Ferulic acid increased the formation of some carbonyl compounds which are markers of flavour deterioration, neverthless this acid was very effective hydroxyl radical quencher and inhibited lipid peroxidation.

Some food matrix components like proteins can influence the antioxidant activities of phenolic acids, for example Heinonen et al. [1998] studied effect of bovine serum albumin (BSA) on the activity of ferulic and caffeic acids and certain flavonoids in lecithin-liposome oxidation system. The presence of protein in reaction medium enhanced antioxidant activity of ferulic acid; caffeic acid being poor antioxidant in applied system without protein, turned out to be prooxidant or inactive in the presence of BSA.

CONCLUSIONS

The study was undertaken in order to study the antioxidant and antiradical activities of a number of cinnamic and benzoic acid derivatives as well as checking if esterification of the free acid can change the total antioxidant activity of the acid. It was stated, that the applied measuring method strongly influences the obtained result. In the system containing 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), the antioxidant activities of the studied acids were as follows: vanillic acid > sinapic acid > protocatechuic acid > syringic acid > caffeic acid > ferulic acid. The antiradical activities of methyl derivatives were much lower than the activities of free forms, except methyl ferulate, which scavenged ABTS radical only slightly less effectively. This compound was the most efficient $DPPH^{\bullet}$ scavenger from all the compounds studied, equal to Trolox at the same concentration. The compunds tested scavenged the $DPPH^{\bullet}$ radical in decreasing order: methyl ferulate > methyl syringate > syringaldehyde = vanillic acid > syringic acid = sinapic acid = protocatechuic acid = ferulic acid > p-coumaic acid > caffeic acid > methyl vanillate = methyl coumarate. Also in the linoleic acid oxidation medium, ferulic acid and methyl ferulate were strong antioxidants. In 2-hour-incubation trials the two compounds were followed by: caffeic acid > sinapic acid = syringic acid > protocatechuic acid = vanillic acid > veratric alcohol > p-coumaric acid. In 6-hour incubation trial, the most antioxidant compounds were in following order: p-dimethylaminobenzoic aldehyde > syringaldehyde > methyl syringate. The highest reducing powers owned protocatechuic acid, caffeic, sinapic and ferulic acid, higher than BHT and comparable to Trolox. Free ferulic, vanillic, syringic, p-coumaric and caffeic acid had stronger reducing powers than their methyl esters, although quite high reducing power of methyl ferulate among other methylated derivatives must be emphasised.

REFERENCES

Abu-Amsha R., Croft K.D., Puddley I.B., Proudfoot J.M., Beilin L.J., 1996. Phenolic content of various beverages determines the extent of inhibition of human serum and low-density lipoprotein oxidation *in vitro*: identification and mechanism of action of some cinnamic acid derivatives from red wine. Clin. Sci. 91, 449-458.

Bellmer H.-G., Galensa R., Gromus J., 1995. Bedeutung der Polyphenole für die Bierherstellung. Brauwelt 30, 1477-1495.

Brand-Williams W., Cuvelier M.E., Berset C., 1995. Use of free radical method to evaluate antioxidant activity. Lebensm.-Wiss. Technol. 28, 25-30.

Chen J.H., Ho C.T., 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. J. Agric. Food Chem. 45, 2374-2378.

Cuvelier M.-E., Richard H., Berset C., 1992. Comparison of the antioxidative activity of some acid-phenols: structure- activity relationship. Biosci. Biotech. Biochem. 56 (2), 324-325.

Fantozzi P., Montanari L., Mancini F., Gasbarrini, A., Addolorato, G., Simoncini, M., 1998. *In vitro* antioxidant capacity from wort to beer. Lebensm.-Wiss. Technol. 31, 221-227.

Grabber J.H., Ralph J., Hatfield R.D., 1998. Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. J. Agric. Food Chem. 46, 2609-2614.

Heinonen M., Rein D., Satue-Gracia M.T., Huang S.-W., German J.B., Frankel E.N., 1998. Effect of protein on the antioxidant activity of phenolic compounds in a lecithin- liposome oxidation system. J. Agric. Food Chem. 46, 917-922.

Hermann H., 1992. Contents of principle plant phenols in fruits. Fluess. Obst. 59, 66-70.

Lingnert H., Vallentin K., Eriksson C.E., 1979. Measurement of antioxidative effect in model system. J. Food Process. Preserv. 3, 87-103.

Maillard M.-N., Berset C., 1995. Evolution of antioxidant activity during kilning: role of insoluble bound phenolic acids of barley and malt. J. Agric. Food Chem. 43, 1789-1793.

Maillard M.-N., Soum M.-H., Boivin P., Berset C., 1996. Antioxidant activity of barley and malt: relationship with phenolic content. Lebensm.-Wiss. Technol. 29, 238-244.

Meyer A.S., Donovan J.L., Pearson D.A., Waterhouse A.L., Frankel E.N., 1998. Fruit hydroxycinnamic acids inhibit human low- density lipoprotein oxidation *in vitro*. J. Agric. Food Chem. 46, 1783-1787.

Miller N.J., Rice-Evans C., Davies M.J., Gopinathan V., Milner A., 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Sci. 84, 407-412.

Moon J.-H., Terao J., 1998. Antioxidative activity of caffeic acid and dihydrocaffeic acid in lard and human low- density lipoprotein. J. Agric. Food Chem. 46, 5062-5065.

Nardini M., Natella F., Gentili V., Di Felice M., Scaccini C., 1997. Effect of caffeic acid dietary supplementation on the antioxidant defense system in rat: an *in vivo* study. Arch. Biochem. Biophys. 342 (1), 157-160.

Natella F., Nardini M., Di Felice M., Scaccini C., 1999. Benzoic and cinnamic acid derivatives as antioxidants: structure-activity relation. J. Agric. Food Chem. 47, 1453-1459.

Ohta T., Semboku N., Kuchii A., Egashira Y., Sanada, H., 1997. Antioxidant activity of corn bran cell-wall fragments in the LDL oxidation system. J. Agric. Food Chem. 45, 1644-1648.

Ohta T., Yamasaki S., Egashira Y., Sanada, H., 1994. Antioxidant activity of corn bran hemicellulose fragments. J. Agric. Food Chem. 42, 653-656.

Oyaizu M., 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307-315.

Pan G.X., Bolton J.L., Leary G.J., 1998. Determination of ferulic and p-coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. J. Agric. Food Chem. 46, 5283-5288.

Walters M.T., Heasman A.P., Hughes P.S., 1997 a. Comparison of (+)- catechin and ferulic acid as natural antioxidants and their impact on beer flavor stability. Part 1: Forced-Aging. J. Am. Soc. Brew. Chem. 55 (2), 83-89.
Walters M.T., Heasman A.P., Hughes P.S., 1997 b. Comparison of (+)- catechin and ferulic acid as natural antioxidants and their impact on beer flavor stability. Part 2: Extended Storage Trials. J. Am. Soc. Brew. Chem. 55 (3), 91-98.
Watanabe M., 1999. Antioxidative phenolic compounds from Japanese Barnyard Millet (*Echinochloa utilis*) grains. J. Agric. Food Chem. 47, 4500-4505.

AKTYWNOŚĆ PRZECIWUTLENIAJĄCA POCHODNYCH KWASU CYNAMONOWEGO I BENZOESOWEGO

Streszczenie. W pracy badano aktywność przeciwutleniającą pochodnych kwasu cynamonowego i benzoesowego mierzoną wybranymi metodami. Całkowita aktywność przeciwutleniająca została zbadana za pomocą metody z użyciem emulsji kwasu linolowego w wodzie. Zdolność „zmiatania" wolnych rodników zbadano używając kwasu 2,2'-azinobis-3-etylobenzotiazolino-6-sulfonowego (ABTS$^{\bullet}$) i w układzie badawczym zawierającym rodnik DPPH$^{\bullet}$. Badano także siłę redukcyjną związków fenolowych. Stwierdzono, że wynik oznaczania aktywności przeciwutleniającej był uzależniony od zastosowanej metody badawczej. W układzie zawierającym rodnik ABTS$^{\bullet}$, aktywność przeciwutleniającą związków fenolowych uporządkowano w szeregu malejącym: kwas waniliowy > kwas synapowy > kwas protokatechowy > kwas syringowy > kwas kawowy > kwas ferulowy. Wolne rodniki DPPH$^{\bullet}$ ulegały „zmiataniu" z następującą malejącą siłą: ferulan metylowy > syringinian metylowy > aldehyd syringinowy = kwas waniliowy > kwas syringowy = kwas synapowy = kwas protokatechowy = kwas ferulowy > kwas kumarowy > kwas kawowy > wanilian metylowy = kumaran metylowy. Również w układzie badawczym zawierającym kwas linolowy wykazano istotne zróżnicowanie całkowitej aktywności przeciwutleniającej. Utlenianie kwasu linolowego po 2-godzinnej inkubacji było hamowane w następującym porządku malejącym: kwas kawowy > kwas synapowy = kwas syringowy > kwas protokatechowy = kwas waniliowy > kwas weratrowy > kwas p-kumarowy. Największą siłą redukcyjną, większą niż BHT i porównywalną z Trolox-em, charakteryzowały się kwasy: protokatechowy, synapowy i ferulowy. Metylowe estry kwasów fenolowych wykazały mniejszą aktywność przeciwutleniającą niż wolne kwasy fenolowe, z wyjątkiem ferulanu metylowego, którego aktywność była zbliżona do aktywności wolnego kwasu ferulowego.

Słowa kluczowe: pochodne kwasu cynamonowego, pochodne kwasu benzoesowego, aktywność przeciwutleniająca, aktywność „zmiatania" wolnych rodników

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