Antioxidant and Antiradical Activity of Ferulates

Magdalena KARAMAĆ[1], Adam BUCIŃSKI[1], Ronald B. PEGG[2] and Ryszard AMAROWICZ[1]

[1]Division of Food Science, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland; [2]Department of Applied Microbiology and Food Science, College of Agriculture, University of Saskatchewan, Saskatoon, Canada

Abstract

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Antioxidant and antiradical activities of ferulates (i.e., ferulic acid, isoferulic acid, coniferyl aldehyde, and methyl ferulate) were investigated using a β-carotene-linoleate model system and a DPPH radical scavenging assay, respectively. Compounds so tested exhibited antioxidant and antiradical properties to varying degrees. Methyl ferulate showed the strongest antioxidant activity, whereas the parent phenolic acid was the most active ferulate to scavenge the DPPH radical (DPPH$^{\bullet}$). Isoferulic acid at concentrations ranging from 10 to 100 nmol/assay did not impart an antiradical efficacy; this may be attributed to the location of the hydroxyl group in the *meta* position on the aromatic ring.

Keywords: antioxidant and antiradical activity; DPPH; ferulates; ferulic acid; isoferulic acid; coniferyl aldehyde; methyl ferulate

Hydroxycinnamic acid derivatives are ubiquitous phenolics found in cereals, grains (Weidner *et al.* 1996, 1999, 2000), rapeseed, roasted coffee, asparagus, peas, vegetables and many other plants (Zadernowski 1987; Naczk *et al.* 1998). Of these, ferulic acid and its derivatives have been the subject of a number of research investigations. In cereals, for example, much of the ferulic acid was found to exist as ester derivatives of the stanol and sterol types (Herrmann 1989). The highest concentration of steroyl ferulates (i.e., oryzanol) has been reported in rice bran oil: its constituents comprised a variety of ferulic acid esters called α-, β-, and γ-oryzanol (Fukushi 1966). Apparently acting on the brain's control of digestion, and possibly through a direct effect on the stomach, γ-oryzanol may help to ease heartburn and other digestive-system discomforts. γ-Oryzanol supplements have also relieved pain, nausea, vomiting, and other post-meal gastrointestinal symptoms in a study of participants suffering from gastritis (http://wholehealthmd.com).

The antioxidant activity of ferulic acid and derivatives was reported in several *in vitro* studies reviewed by Graf (1992). This phenolic acid provided a strong inhibition of lipid peroxidation in rat brain homogenates (Sharma 1976). The antioxidant effect of ferulic acid on the peroxidation of ghee during storage for 30 days at 37°C was observed by Gupta *et al.* (1979). Toda *et al.* (1991) reported that ferulic acid scavenged the superoxide anion radical and inhibited lipid peroxidation induced by superoxide. Structure-activity relationships of such phenolics have been mainly related to the

type and number of functional groups attached to the aromatic ring (Brand-Williams *et al.* 1995). The antioxidant activity of γ-oryzanol has been noted in several studies (Fukushi 1966; Yagi & Ohishi 1979).

The aim of the present work is to characterise the antioxidant and antiradical performance of four ferulates (i.e., ferulic acid, isoferulic acid, coniferyl aldehyde, and methyl ferulate) using β-carotene-linoleate and DPPH radical model systems, respectively.

MATERIALS AND METHODS

Chemicals. Methanol of analytical grade was acquired from the P.O.Ch. Company (Gliwice, Poland). Polyoxyethylenesorbitan monopalmitate (Tween 40), β-carotene, linoleic acid, 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), butylated hydroxyanisole (BHA), ferulic acid (4-hydroxy-3-methoxycinnamic acid), isoferulic acid (3-hydroxy-4-methoxycinnamic acid) and coniferyl aldehyde (4-hydroxy-3-methoxycinnamaldehyde) were obtained from Sigma Chemical Co. Ltd. (Poznań, Poland), while methyl ferulate (methyl *trans*-4-hydroxy-3-methoxy cinnamate) was acquired from Apin Chemicals Ltd. (Milton Park, Abingdon, UK).

Evaluation of antioxidant activity. Antioxidant activities of the extracts were evaluated using a β-carotene-linoleate model system (Miller 1971). Methanolic solutions (0.2 ml) containing 12.5mM concentrations of ferulic acid, isoferulic acid, coniferyl aldehyde, and methyl ferulate were added to a series of tubes containing 5 ml of prepared emulsion of linoleic acid and β-carotene. Samples were incubated in water bath at 50°C for 120 min, the absorbance being read at 470 nm every 15 min.

Scavenging of DPPH radical. The scavenging capacity of ferulates for the DPPH radical was monitored according to the method of Hatano *et al.* (1988), with slight modifications. In test tubes, a 0.1-ml methanolic solution containing either 10 to 100 nmol of ferulic and isoferulic acids, or 10 to 500 nmol of coniferyl aldehyde and methyl ferulate was diluted with 2 ml of methanol to which 0.25 ml of a 1mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl• (DPPH•) was added. The content of each tube was vortexed for 15 s, then left to stand at room temperature for 30 min after which the absorbance measurement of the solution was taken at 517 nm. A methanolic solution of DPPH• that had decayed and hence no longer exhibited a purple colour (i.e., 2 mg of BHA dissolved in 2 ml of methanol with 0.25 ml of the DPPH• solution added) was chosen for the background correction, instead of pure methanol.

Physico-chemical parameters. The physico-chemical parameters, including heat of formation (ΔH_f), dipole, hydration of molecule, and atoms' charge, were calculated using HyperChem molecular modeling software (Anonym 2003).

Statistics. All results in this study are reported as mean values of three independent analyses. The

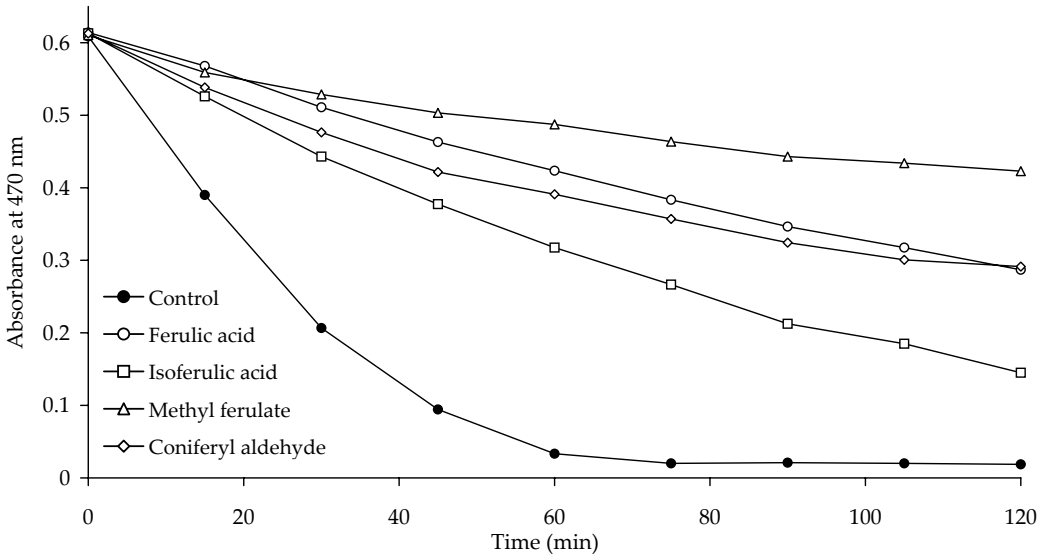

Figure 1. Antioxidant activity of ferulate derivatives in a β-carotene-linoleate model system, as measured by changes in the absorbance at 470 nm

method used for antioxidant activity was characterised by a CV of less than 4% and for scavenging of DPPH radical – less than 2%.

RESULTS AND DISCUSSION

The antioxidant performance of ferulates in a β-carotene-linoleate model system is depicted in Figure 1. Compounds so tested exhibited antioxidant properties to varying degrees which were in the order of methyl ferulate > ferulic acid > coniferyl aldehyde > isoferulic acid. Antioxidant activity of ferulic acid is stronger than that of coniferyl aldehyde even in the course of 75–100 min. It was distinctly stronger after 45, 60 and 75 min while only after the latter point it was nearly similar. After the latter point, the antioxidant properties of both phenolics were similar. The antioxidant activities of methyl ferulate, ferulic acid and coniferyl aldehyde at the mmol/assay concentrations used in this study were similar to those of the extracts of phenolic compounds from rapeseed oil cake (Amarowicz *et al.* 1995, 2001), wheat and rye caryopses as well as their embryos (Amarowicz *et al.* 2002; Karamać *et al.* 2002). In the studies cited, the extracts from rapeseed were tested at a level of 2 mg/assay whereas the extracts from cereals were employed at a level of 4 mg/assay.

Seeing that the chief mechanism of the action of phenolic antioxidants is considered to be the scavenging of free radicals, the reactivity of ferulic acid and its derivatives was examined toward the

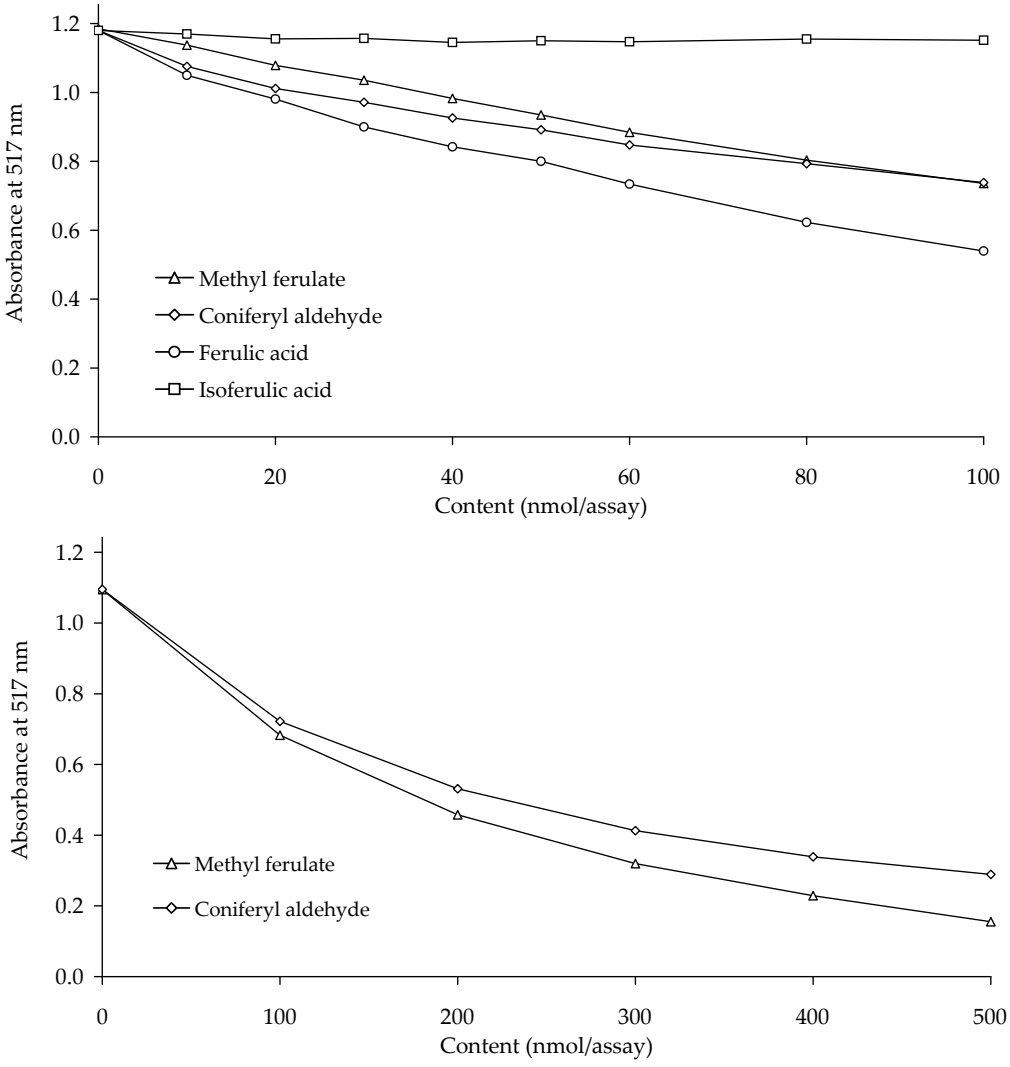

Figure 2. Scavenging effect of ferulate derivatives on 2,2-diphenyl-1-picrylhydrazyl• (DPPH•), as measured by changes in absorbance at 517 nm

Table 1. Calculated physico-chemical parameters of ferulates using HyperChem software

Physico-chemical parameter	Ferulic acid	Isoferulic acid	Coniferyl aldehyde	Methyl ferulate
Heat of formation (kJ/mol)	–686.32	–675.85	–433.25	–658.67
Hydration energy of molecule (kJ/mol)	–58.24	–50.32	–17.30	–39.76
Dipole (Debyes)	3.126	2.644	3.781	3.426
Atom charge*	0.2357	0.2196	0.2356	0.2355

*Hydrogen of –OH group

stable radical, DPPH$^{\bullet}$: the results showed that ferulic acid was more efficient than any of the other compounds investigated (Figure 2). Isoferulic acid was found to impart no antiradical action at the 10 to 100 nmol/assay concentrations used in this study. Methyl ferulate and coniferyl aldehyde demonstrated similar scavenging capacities at concentrations ranging from 10 to 100 mmol/assay; however, at higher concentrations of 100 to 500 nmol/assay, methyl ferulate was markedly more effective.

According to Nenadis *et al.* (2003), ferulates in solution are expected to be fully planar or to deviate only marginally from the plane. Any differences in the activity amongst them should, therefore, be ascribed to electronic phenomena rather than to steric hindrance effects. In other words, the presence of electron-donating groups attached to the aromatic ring such as $–CH_3$ and –OH ought to increase the ease of hydrogen atom abstraction and, consequently, antiradical performance, whereas groups with electron-withdrawing properties such as –COOH, –CHO and –COOR should have the opposite effect. Although electron-donating –OH groups are attached to the aromatic ring in both ferulic and isoferulic acids, the hydrogen atom is more easily abstracted from the –OH of ferulic acid than that of isoferulic acid. In ferulic acid, the –OH moiety is in the *para* or 4-position of the ring, not the *meta* or 3-position as in the case of isoferulic acid. A greater number of canonical resonance forms exist for the generated ferulic acid radical than that of its isoferulic acid counterpart. This may be the reason why isoferulic acid showed no antiradical activity at the concentrations employed in the present study. The effect of the groups with electron-withdrawing properties on the antiradical reactivity of the ferulates was found to be in the order of –COOH > –CHO ≈ $–COOCH_3$. A decrease in the antioxidant/antiradical performance of ferulic acid was observed by Fukushi (1966) after its acetylation. The results of our study dealing with ferulic acid and its derivatives are similar to the data presented by Nenadis *et al.* (2003). In the latter study, the scavenging activity of ethyl ferulate toward DPPH$^{\bullet}$ was more pronounced than that of ferulic acid.

Some physico-chemical parameters of the ferulates under investigation were calculated using the HyperChem molecular modeling software (Table 1). Lien *et al.* (1999) reported that a model could be derived to calculate the redox potential (i.e., a direct measure of the antioxidant property) of phenolic compounds using calculated parameters such as the heat of formation (ΔH_f) of phenoxyl radicals and their corresponding parent phenols. A combination of ΔH_f and other calculated parameters were found to be quite satisfactory for predicting the antioxidant activities, or redox potentials, of new phenolic antioxidants. A model to predict the antioxidant activity from the data on ferulic acid and its derivatives is presently being developed.

References

Amarowicz R., Fornal J., Karamać M. (1995): Effect of seed moisture on phenolic acids in rapeseed oil cake. Grasas Aceites, **46**: 354–356.

Amarowicz R., Fornal J., Karamać M., Shahidi F. (2001): Antioxidant activity of extracts of phenolic compounds from repeseed oil cake. Journal of Food Lipids, **8**: 65–74.

Amarowicz R., Karamać M., Weidner S., Abe S., Shahidi F. (2002): Antioxidant activity of wheat caryopses and embryos extracts. Journal of Food Lipids, **9**: 201–210.

Anonym (2003): Hypercube, Inc., HyperChem for Microsoft® Windows, Version 7.5. Gainesville, FL, USA.

Brand-Williams W., Cuvelier M.E., Berset C. (1995): Use of a free radical method to evaluate antioxidant

activity. Lebensmittel-Wissenschaft und -Technologie, **28**: 25–30.

Fukushi T. (1966): Studies on the edible rice bran oils. Part 3. Antioxidant effects of oryzanol. Report of the Hokkaido Institute of Public Health, **16**: 111–114.

Graf E. (1992): Antioxidant potential of ferulic acid. Free Radical Biology & Medicine, **13**: 435–448.

Gupta S., Sukhija P.S., Bhatia I.S. (1979): Role of phenolics and phospholipids as antioxidants for ghee. Milchwissenschaft, **34**: 205–206.

Hatano T., Kagawa H., Yasuhara T., Okuda T. (1988): Two new flavonoids and other counstituents in licorice root: their relative astringency and radical scavenging effects. Chemical & Pharmaceutical Bulletin, **36**: 2090–2097.

Herrmann K. (1989): Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Critical Reviews in Food Science and Nutrition, **28**: 315–347.

Karamać M., Amarowicz R., Weidner S., Abe S., Shahidi F. (2002): Antioxidant activity of rye caryopses and embryos extracts. Czech Journal of Food Sciences, **20**: 209–214.

Lien E.J., Ren S., Bui H.-H., Wang R. (1999): Quantitative structure-activity relationship analysis of phenolic antioxidants. Free Radical Biology & Medicine, **26**: 285–294.

Miller H.E. (1971): A simplified method for the evaluation of antioxidants. Journal of the American Oil Chemists' Society, **45**: 91.

Naczk M., Amarowicz R., Sullivan A., Shahidi F. (1998): Current research developments on polyphenolics of rapeseed/canola: a review. Food Chemistry, **62**: 489–502.

Nenadis N., Zhang H-Y., Tsimidou M.Z. (2003): Structure-antioxidant activity relationship of ferulic acid derivatives: Effect of carbon side chain characteristic groups. Journal of Agricultural and Food Chemistry, **51**: 1874–1879.

Sharma O.P. (1976): Antioxidant activity of curcumin and related compounds. Biochemical Pharmacology, **25**: 1811–1812.

Toda S., Kumura M., Ohnishi M. (1991): Effects of phenolcarboxylic acids on superoxide anion and lipid peroxidation induced by superoxide anion. Planta Medica, **57**: 8–10.

Weidner S., Paprocka J., Łukaszewicz D. (1996): Changes in free, esterified and glycosidic phenolic acids in cereal grains during the after-ripening. Seed Science and Technology, **24**: 107–114.

Weidner S., Amarowicz R., Karamać M., Dąbrowski G. (1999): Phenolic acids in caryopses of two cultivars of wheat, rye and triticale that display different resistance to pre-harvest sprouting. European Food Research and Technology, **210**: 109–113.

Weidner S., Amarowicz R., Karamać M., Frączek E. (2000): Changes in endogenous phenolic acids during development of *Secale cereale* caryopses and after dehydration treatment of unripe rye grains. Plant Physiology and Biochemistry, **38**: 595–602.

Yagi K., Ohishi N. (1979): Action of ferulic acid and its derivatives as antioxidants. Journal of Nutritional Science and Vitaminology, **25**: 127–130.

Zadernowski R. (1987): Studies of phenolic compounds of rapeseeed flours. Acta Academiae Agriculturae Technicae Olstenensis, Technologia Alimentarum, **21F**: 3–55.

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Corresponding author:

Doc. Dr. Ryszard Amarowicz, Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, ul. Tuwima 10, P.O. Box 55, 10-747 Olsztyn, Poland
tel.: + 48 89 523 46 27, fax: + 48 89 524 01 24, e-mail: amaro@pan.olsztyn.pl