Content of Phenolic Acids in Rye Caryopses Determined using DAD-HPLC Method

RYSZARD AMAROWICZ[1] and STANISŁAW WEIDNER[2]

[1]Institute of Animal Reproduction and Food Research – Department of Food Science, Polish Academy of Sciences, Olsztyn, [2]Warmia-Masuria University in Olsztyn – Department of Plant Physiology and Biochemistry, Olsztyn, Poland

Abstract

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Phenolic compounds were extracted from rye caryopses with 80% (v/v) methanol. Phenolic acids were determined as free compounds and those liberated from soluble esters and glycosides. The analyses were performed using a Waters HPLC system equipped with a diode array detector (DAD). The following free phenolic acids were found: *p*-coumaric, ferulic and sinapic; the phenolic acids liberated from soluble esters were as follows: vanillic, caffeic, *p*-coumaric, ferulic and sinapic; and those liberated from soluble glycosides were the following: vanillic, *p*-coumaric, ferulic and sinapic. In rye caryopses, phenolic acids were chiefly in the form of soluble esters. A diode array detector was especially useful for the determination of vanillic acid: the UV spectrum of this compound showed a maximum at 260 nm whereas UV spectra of other phenolic acids were characterised by maxima at longer wavelengths.

Keywords: phenolic acids; rye; HPLC; diode array detector (DAD)

Phenolic acids are the predominant phenolic compounds found in cereals. Several experiments showed that phenolic acids can act as plant germination inhibitors (SREERAMULU 1974; KHAN & UNGAR 1986; WEIDNER *et al*. 1993). A positive correlation between the period of dormancy and the content of phenolic acids was found for developing and ripening barley caryopses (WEIDNER *et al*. 1993). For wheat, rye and triticale, the levels of phenolic acids liberated from soluble esters and the total phenolic acids contents in caryopses showing shallow dormancy were lower than in those showing deeper dormancy. A decreased content of phenolic acids was recorded for rye, triticale, barley and oat during several months of storage in a dry state (WEIDNER *et al.*1996). In the model system employed, ferulic and sinapic acids retarded germination of cereal embryos (WEIDNER *et al.* 1993).

To analyse for phenolic acids in cereal grains, gas chromatography and high performance liquid chromatography are commonly used. In an HPLC a diode array detector (DAD) can acquire spectra for each peak and calculate the absorbance maximum within a specified wavelength range (HUBER 1989). The DAD is especially useful for analysis when separated compounds are characterised by a different wavelength maximum in their spectra. Phenolic acids in cereal extracts are a typical example.

MATERIAL AND METHODS

Plant Material: Rye (cv. Dańkowskie Złote) caryopses were collected 57 days after flowering from an experimental field at the Warmia and Masuria University in Olsztyn.

Extraction: Phenolic compounds were extracted from caryopses according to AMAROWICZ *et al.* (1995). To a 1000ml dark glass bottle, 35 g of ground caryopses were weighed and suspended in 300 ml of 80% (v/v) methanol. The tightly capped bottle was placed in water bath with shaking at 80°C. After 15 min the extract was cooled

and filtered under partial vacuum. The material left on the filter was re-extracted with 300 ml of the fresh solvent. This procedure was triplicated. Following the evaporation of methanol in a rotary evaporator at 45°C, the remaining water solutions were lyophilised .

Separation of Phenolic Acids into Three Classes: Phenolic acids (i.e. free and those liberated from soluble esters and soluble glycosides) were isolated from the extract according to a previously described method (WEIDNER *et al.* 1999). An aqueous suspension of the extract (800 mg in 20 ml) was adjusted to pH 2 (6M HCl), and free phenolic acids were extracted 5 times into 20 ml of diethyl ether using a separatory funnel. The extract was evaporated to dryness under vacuum at room temperature. The aqueous solution was neutralized to pH 7 with NaOH and then lyophilised. The residue was dissolved in 20 ml of 2M NaOH and hydrolysed for 4 h at room temperature under a nitrogen atmosphere. After acidification to pH 2 using 6M HCl, phenolic acids released from soluble esters were extracted from the hydrolysate 5 times into 30 ml of diethyl ether using a separatory funnel. A 15 ml aliquot of 6M HCl was added to the aqueous phase and the solution obtained in this way was placed under a nitrogen atmosphere and hydrolysed for 1 h in a boiling water bath. Phenolic acids released from soluble glycosides were separated from the hydrolysate 5 times into 45 ml of diethyl ether. Then ether was evaporated to dryness. The dry residues were dissolved in 5 ml methanol and filtered through a 0.45 mm nylon filter. The samples obtained in this way were injected onto an HPLC column.

HPLC Analysis: A Waters 600 HPLC system was employed and consisted of the following components: Waters 600E pump, Waters 996 diode array detector, and Waters 715 ultrawisp sample processor. Conditions of separation were as follows: prepacked LiChrospher 100 RP-18 column (5 µm, 4 × 250 mm; Merck); mobile phase comprising water-acetonitrile-acetic acid (88:10:2; v/v/v) (AMAROWICZ & SHAHIDI 1994); flow rate 1 ml/min; and injection volume 20 µl. The content of vanillic acid was calculated from chromatograms that were recorded at

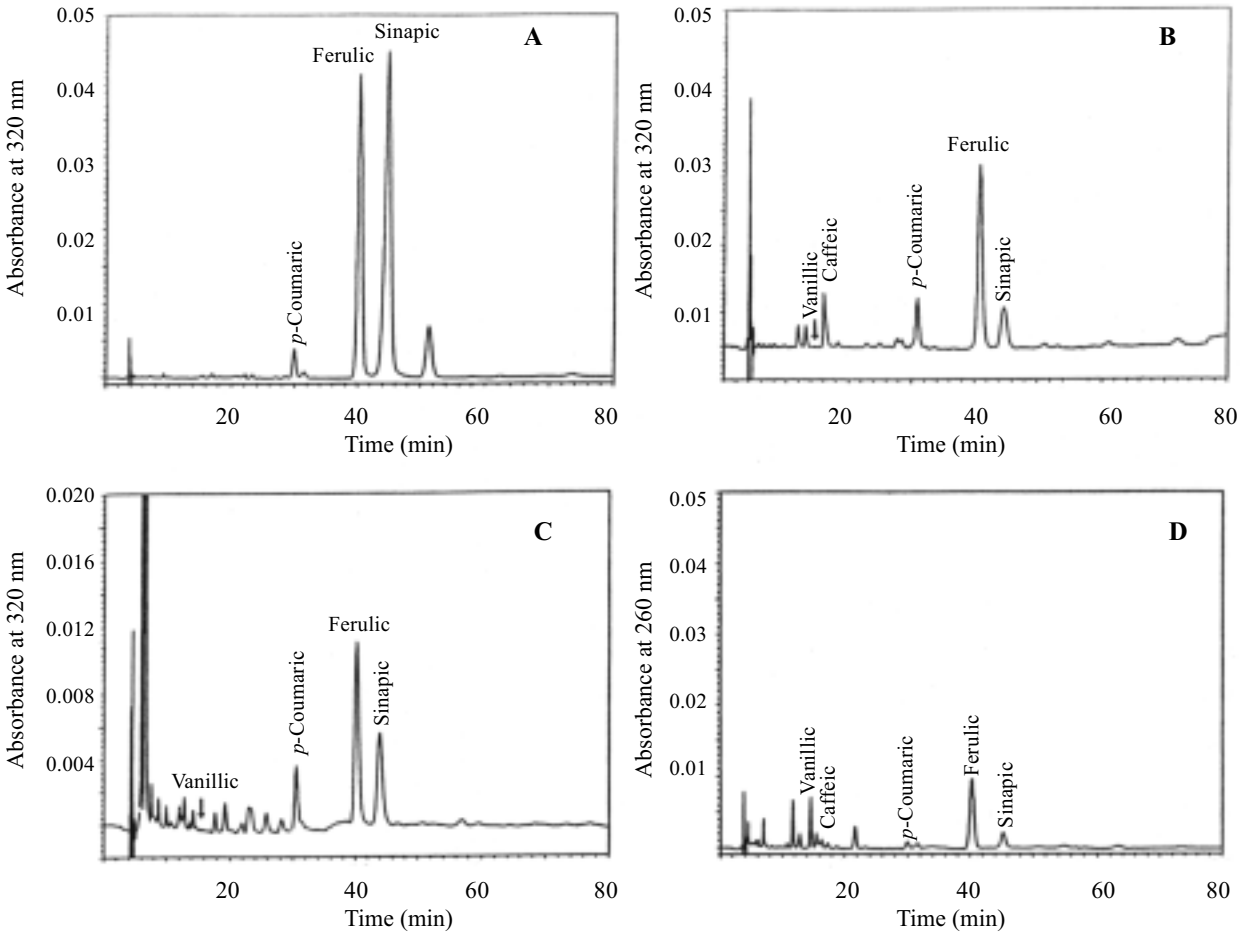

Fig. 1. HPLC separation of phenolic acids extracted from rye caryopses: A – chromatogram of free phenolic acids (detection at 320 nm) , B – chromatogram of phenolic acids liberated from soluble esters (detection at 320 nm), C – chromatogram of phenolic acids liberated from soluble glycosides (detection at 320 nm), D – chromatogram of phenolic acids liberated from soluble esters (detection at 260 nm)

260 nm. For other phenolic acids a wavelength of 320 nm was used.

RESULTS AND DISCUSSION

In rye caryopses, five phenolic acids were detected, namely vanillic, caffeic, *p*-coumaric, ferulic and sinapic. All mentioned acids were present in the caryopses in the form of soluble esters. *p*-Coumaric, ferulic and sinapic acids were present as free compounds. The presence of vanillic, *p*-coumaric, ferulic and sinapic acids in the form of glycosides was observed in this study. The majority of phenolic acids were found in the form of soluble esters (Table 1). Ferulic acid was the main compound among the free and esterified phenolic acids, whereas sinapic acid was the dominant compound in the form of glycosides. In our previous investigations concerning phenolic acids in rye caryopses, a lower content of total phenolic acids was determined by gas chromatography (WEIDNER *et al.* 1996), but a higher content was found by a HPLC method employing typical UV-VIS detection (WEIDNER *et al.* 1999).

Comparison of our results with those from literature is difficult because the concentrations of phenolic acids in cereal grains have not been adequately reported. Variations in the results from those in the literature could have been caused by the different techniques employed for analysis (DĄBROWSKI & SOSULSKI 1984). RYBKA *et al*.

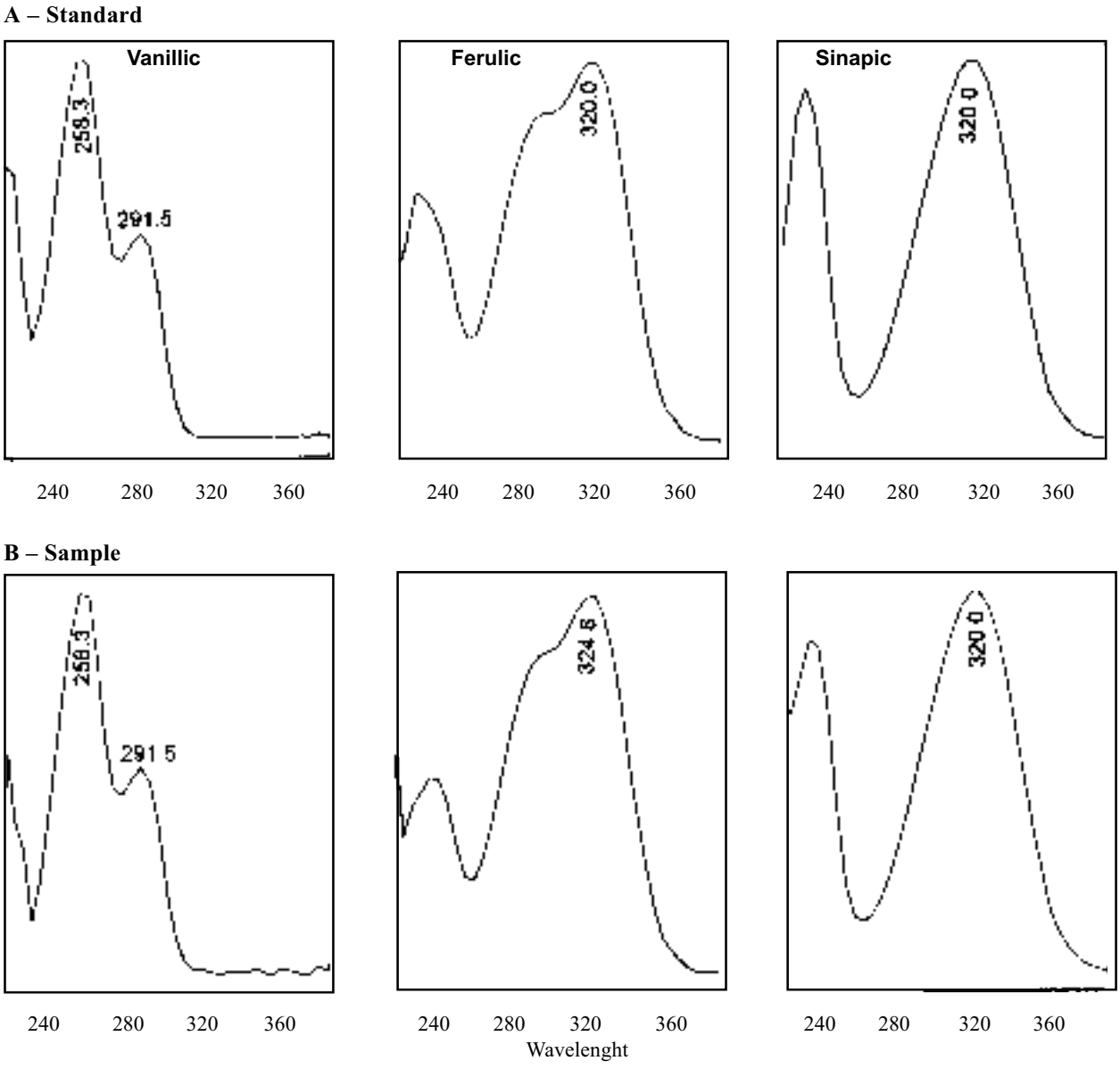

Fig. 2. DAD-UV spectra of vanillic, ferulic and sinapic acids

Table 1. Content of phenolic acids (free, liberated from soluble esters and from soluble glycosides) in rye caryopses (µg/g D.M.)

Phenolic acids	Free phenolic acids	Phenolic acids liberated from soluble		Total
		esters	glycosides	
Vanillic	–	4.18	0.88	5.06
Caffeic	–	3.60	–	3.60
p-Coumaric	0.91	1.85	0.97	3.73
Ferulic	4.59	37.31	7.39	49.29
Sinapic	1.02	27.04	9.24	37.30
Total	6.52	73.98	18.48	98.99

(1993) reported that ferulic acid was a dominant phenolic acid in rye grain although isoferulic, *p*-coumaric, syringic, *p*-hydroxybenzoic and caffeic acids were also detected in trace amounts by TLC. SOSULSKI *et al.* (1982) reported a ferulic acid at a concentration of 64 µg/g in freshly milled wheat flour.

Fig. 1 depicts the chromatograms of phenolic acids separated from rye caryopses. Peaks originating from caffeic, *p*-coumaric, ferulic and sinapic acids were sharp, well separated/resolved and characterised by retention times of 14.35, 16,09, 31.85, 42.06, and 45.12 min, respectively. One peak from an unknown compound was observed in the chromatogram from free phenolic acids at a retention time of 46.67 min (Fig. 1A). A number of unknown compounds were detected in the fraction of phenolic acids liberated from soluble glycosides (Fig. 1C), their retention times were between those of vanillic and *p*-coumaric acids. Chromatogram C of Fig. 1 was also characterised by high peaks close to the solvent front. Those peaks originated most probably from sugars. Chromatogram C of Fig. 1 shows that vanillic acid was detected only at 260 nm. Detection of this phenolic acid could be difficult using a typical UV-VIS detector set at 320 nm.

The spectra presented in Fig. 2 are an evidence of the high quality of chromatographic analysis. Maxima from the standard and samples of vanillic and sinapic acids were recorded at the same wavelengths of 258.3, 291.5, and 320 nm, respectively. For ferulic acid, the maximum from the sample was recorded at a slightly longer wave-

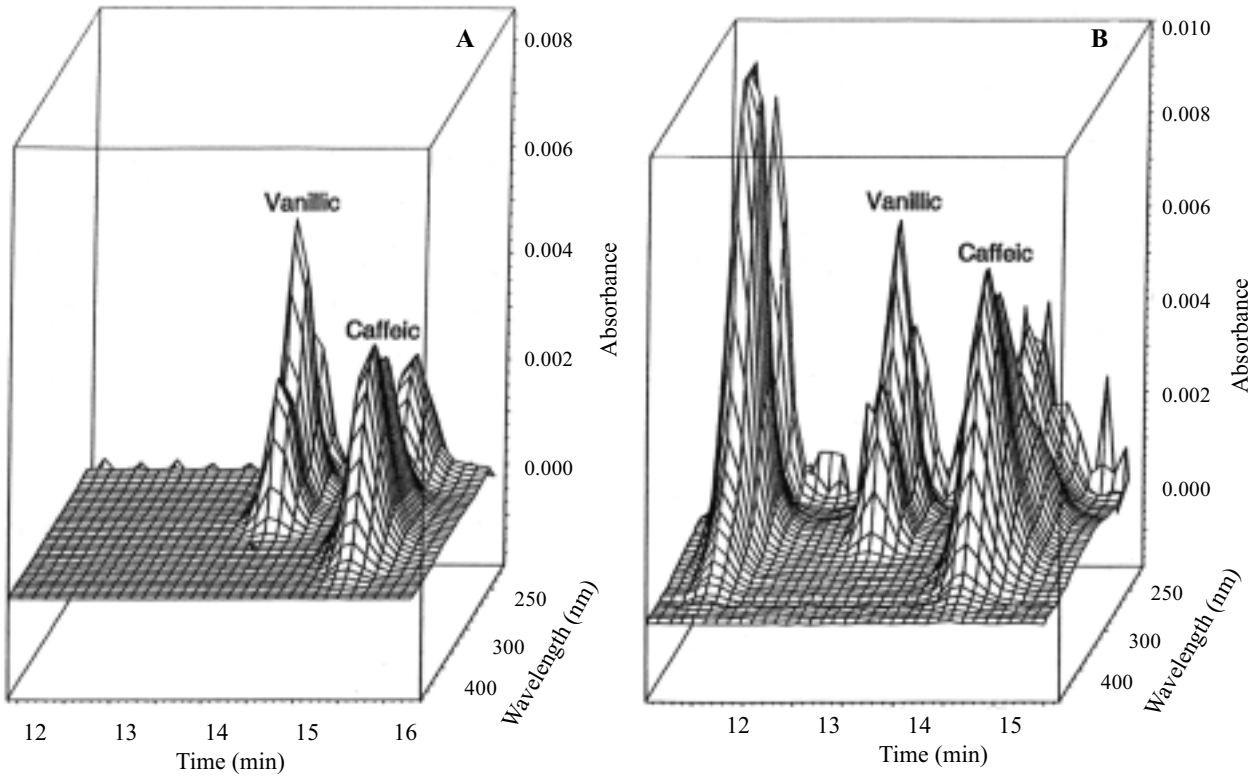

Fig. 3. 3-D chromatogram of vanillic and caffeic acids: A – standard; B – sample

length. Good separation of vanillic and *p*-coumaric acids, based on the conditions of employed chromatography, is evident in the 3-D chromatogram (Fig. 3).

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Souhrn

AMAROWICZ R., WEIDNER S. (2001): **Obsah fenolových kyselin v žitných obilkách stanovený pomocí metody DAD-HPCL**. Czech J. Food Sci., **19**: 201–205.

Ze žitných obilek jsme 80% (obj.) metanolem extrahovali fenolové látky. Fenolové kyseliny jsme stanovili buď čisté, nebo jako látky uvolněné z rozpustných esterů a glycosidů. Analýzu jsme prováděli HPCL – systém Waters s diodovým detektorem DAD. Zjistili jsme volné fenolové kyseliny *p*-kumarovou, ferulovou a sinapovou; z rozpustných esterů jsme izolovali kyselinu vanilovou, kávovou, *p*-kumarovou, ferulovou a sinapovou a kyseliny uvolněné z rozpustných glykosidů zahrnovaly kyselinu vanilovou, *p*-kumarovou, ferulovou a sinapovou. Fenolové kyseliny byly v žitných obilkách obsaženy hlavně ve formě rozpustných esterů. Použití detektoru DAD bylo vhodné zejména pro stanovení kyseliny vanilové; spektrum této látky dosahovalo maxima při 260 nm, zatímco pro spektra ostatních fenolových kyselin byla typická maxima při delších vlnových délkách.

Klíčová slova: fenolové kyseliny; žito; HPLC; diodový detektor DAD

Corresponding author:

Dr. RYSZARD AMAROWICZ, Department of Food Science, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, ul. Tuwima 10, P.O. Box 55, 10-747 Olsztyn 5, Poland

tel.: + 48 89 523 26 75, fax: + 48 89 524 01 24, e-mail: amaro@pan.olsztyn.pl