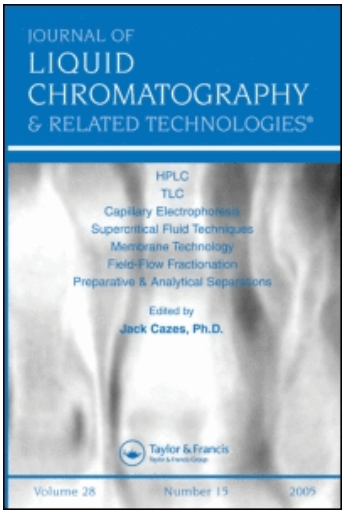

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Chromatographic Separation of Phenolic Compounds from Rapeseed by a Sephadex LH-20 Column with Ethanol as the Mobile Phase

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ABSTRACT

Phenolic compounds from rapeseed were chromatographed using an open column filled with Sephadex LH-20 as the stationary phase and ethanol as the mobile phase. Typically, methanol has been used as the eluent of choice in previous studies, but it was hypothesized that ethanol's lesser polarity would provide better separation of the rapeseed phenolics. In this work, three fractions (I–III) containing phenolic

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compounds were collected; their characteristics were evaluated qualitatively and quantitatively by UV spectrophotometry, a colorimetric assay, and capillary electrophoresis (CE). Although fraction I possessed the greatest relative proportion of the crude extract (62.2%), fraction III contained the richest source of rapeseed phenolics (176 mg *trans*-sinapic acid equivalents g^{-1}). Capillary electrophoresis identified sinapine as the dominant phenolic compound of fraction I and indicated that a number of phenolic compounds were present in fractions II and III. Although free and esterified derivatives of phenolic acids were constituents of fraction II, the electropherogram revealed that sinapic acid was the chief phenolic compound present, based on retention time mapping. Application of ethanol as the eluent, rather than methanol, gave a better baseline separation of the phenolic constituents from rapeseed.

Key Words: Rapeseed phenolics; Column chromatography; Sephadex LH-20; Capillary electrophoresis.

INTRODUCTION

The dominant phenolic compounds found in rapeseed/canola are phenolic acids. These are present in the free, esterified, glycosidic, and insoluble-bound forms, and are derivatives of benzoic and cinnamic acid.[1,2] A number of studies have reported on the antioxidative properties of crude rapeseed extracts and their fractions, which are due in great part to the presence of phenolic constituents.[3–6] Besides antioxidant activity, other characteristics of phenolic compounds from rapeseed have also been investigated. For example, Nowak et al.[7] examined rapeseed for its bactericidal properties, while Kurowska et al.[8] showed that the hydrolysed extract from defatted canola seeds containing 24.5% of free phenolic acids, effectively inhibited melanoma cells, and to a lesser extent breast, colon, lung, and prostate cancer cells.

Chromatography using a stationary column filled with Sephadex LH-20 has been the chief means for separation and collection of fractions of phenolic compounds from rapeseed/canola extracts.[9] This separation technique has been extremely helpful in isolating pure phenolics from rapeseed/canola.[10–12] In the aforementioned studies, methanol was the mobile phase used. Yet, the separation of phenolics might be better resolved using ethanol as the solvent of choice, as it is less polar than methanol. Therefore, the aim of the present work is to separate phenolic compounds from rapeseed on a Sephadex LH-20 column using ethanol, instead of methanol, as the mobile phase.

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EXPERIMENTAL

Preparation of the Rapeseed Extract

Commercial rapeseed of the double-low *Bolko* variety was obtained from a local source and used in this study. Seeds were ground in a commercial coffee mill and then defatted with petroleum ether in a Soxhlet apparatus for >6 h. Phenolic constituents were extracted from the defatted meal with 80% (v/v) methanol at a material-to-solvent ratio of 10 : 100 (w/v).[13] Extraction was carried out at 50°C for 30 min in dark-colored flasks using a shaking Magni Whirl constant-temperature bath (Blue M Electric Company, Model MSG-1122A-1, Blue Island, IL). Afterwards, the slurry was filtered through Whatman No. 1 filter paper and the residue was re-extracted twice more. Supernatants were combined and methanol was evaporated off under vacuum at 40°C using a Büchi Rotavapor/Water bath (Models EL 131 and 461, respectively, Brinkmann Instruments [Canada] Ltd., Mississauga, ON). Residual water in the product was removed by lyophilization. The prepared extract was stored at 4°C in air until further investigated.

Sephadex LH-20 Fractionation of the Extract

A 0.5 g portion of the extract so obtained was dissolved in 8 mL of methanol and applied to a chromatographic column (2 × 60 cm) packed with Sephadex LH-20 (Sigma) and eluted with 95% (v/v) ethanol by gravity. Eluates (4 mL, v) were collected using a fraction collector (Model SC-100, Beckman Instruments [Canada] Ltd., Mississauga, ON) and their absorbance was measured at 330 nm, which is the characteristic wavelength maximum for sinapine, the major phenolic constituent of rapeseed. Eluates were then pooled into major fractions, solvent evaporated off, and residues weighed for mass balance determination.

Determination of the Content of Total Phenolics

The content of total phenolic compounds in each fraction was estimated according to Naczk and Shahidi.[14] 3,5-Dimethoxy-4-hydroxycinnamic acid (*trans*-sinapic acid) was used as a standard in this work. UV spectra of individual fractions were recorded with a Beckman DU 7500 diode array spectrophotometer.

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Capillary Electrophoresis of the Extract Fractions

Capillary electrophoresis (CE) of separated fractions was performed using a Hewlett Packard ^{3D}CE instrument with UV diode array detection (Agilent Technologies, Wilmington, DE). The buffer employed for CE consisted of 150 mM boric acid, 15 mM dimethyl β-cyclodextrin and 75 mM sodium dodecyl sulfate (pH 8.5).[15] An uncoated fused-silica capillary (50 µm i.d. × 72 cm) was used to deliver the sample to the instrument. Separation was carried out at 30 kV and the capillary temperature was maintained at 35°C. The photodiode detector was set at 220 nm.

RESULTS AND DISCUSSION

Sephadex LH-20 is often employed in the fractionation of plant phenolics, as it provides an efficient, high-capacity medium for analytical and preparative works in non-aqueous solvents. This chromatographic material combines adsorption with molecular sieving in the separation of phenolic compounds.[16] In this study three fractions (I–III) containing phenolic compounds were obtained from the chromatography of the methanol extract of *Bolko* rapeseed using Sephadex LH-20 column chromatography with 95% (v/v) ethanol as the mobile phase (Fig. 1). Based on optical density (OD) readings at 330 nm, the chromatogram exhibited one main peak (I) and two smaller ones (II and III). The relative proportion of fraction I in the extract was the greatest at 62.2%, whereas the smallest content at 13.2% was determined for fraction III (Table 1). It was interesting to note, however, that the highest content of total phenolics was determined in fraction III; the content of total phenolics found in fraction I was the lowest.

Amarowicz et al.[9] reported the chromatography of *Bolko* rapeseed phenolics from a methanol extract on a Sephadex LH-20 column (5 × 74 cm) using methanol as the eluent. The chromatogram of the 1992 study was different from that obtained in the present investigation: four main peaks were recorded based on OD readings at 235 nm;[9] this particular wavelength is used to detect the presence of glycosides, aglycones, and free sugars. Shahidi et al.[17] showed the gel filtration chromatogram of phenolic compounds from a methanol extract of defatted canola meal on a Sephadex LH-20 column (1.5 × 77 cm), with methanol as the mobile phase: four main peaks were observed based on OD readings recorded at 280 nm. Monitoring OD values at 280 nm is common when polyphenolics are of concern, because all classes exhibit a maximum at this wavelength. The volume of methanol used for the separation in the Shahidi et al. study was 400 mL.[17] By utilizing a shorter column such as the one in the present work (i.e., 60 cm), only 200 mL

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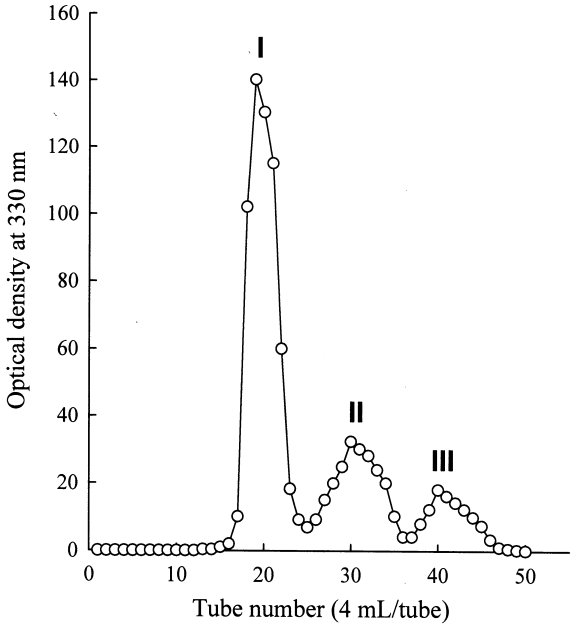

Figure 1. Separation of a crude extract of phenolic compounds from rapeseed into three fractions (I–III) by a Sephadex LH-20 column with ethanol as the mobile phase.

of ethanol were sufficient for complete chromatography. Employment of ethanol as the mobile phase through the Sephadex LH-20 column resulted in a better separation of phenolic compounds.

The content of phenolics found in the three fractions, so obtained, was quite high. Wanasundara et al.[12] examined the phenolic fractions of a defatted

Table 1. Relative content of each fraction from the crude extract and the quantity of total phenolics ($mg\,g^{-1}$).[a]

Fraction	Relative content (%)	Total phenolics ($mg\,g^{-1}$)
I	62.2	78
II	24.6	133
III	13.2	176

[a]Results of total phenolics are expressed as mg of *trans*-sinapic acid equivalents g^{-1} of dried fraction.

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canola seed extract, which were separated by Sephadex LH-20 column chromatography with methanol as the eluent and determined their content to range from 22 to 194 mg of total phenolics g^{-1} extract. The content of phenolic compounds in fractions separated from canola hulls using Sephadex LH-20 column chromatography with ethanol as the mobile phase, ranged from 14 to 112 mg g^{-1} extract.[5] Much lower levels (i.e., from 2.7 to 24.7 mg g^{-1}) were noted in fractions of extracts separated from leguminous seeds, such as pea, faba bean, broad bean, and everlasting bean, using similar column chromatography with methanol as the eluent.[18,19] In the aforementioned references, a low content of phenolic compounds was observed in the first main fraction of all, as was the case in the present study.

The UV spectrum of fraction I exhibited a maximum at 330 nm (Fig. 2). Absorption maxima for fractions II and III were noted at a wavelength of 328 nm. These results suggested that sinapine was the dominant phenolic compound in fraction I, and that free and esterified derivatives of phenolic acids were present in fractions II and III.[20] In general, the absorption band at 330 nm due to sinapine is typical of crude extracts from

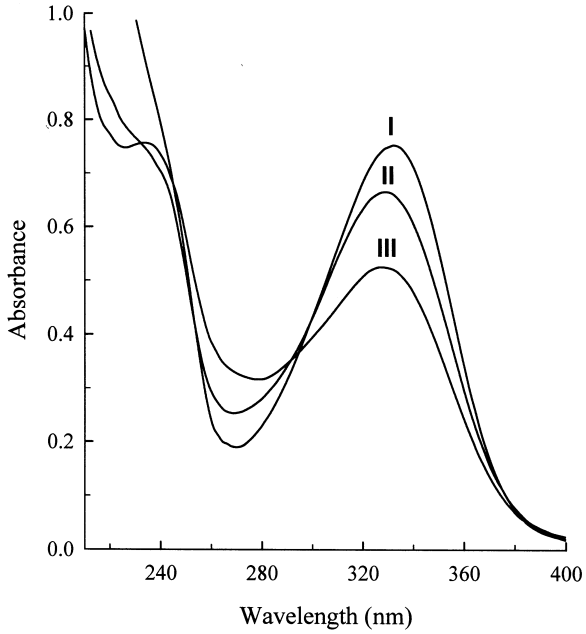

Figure 2. UV spectra of fractions (I–III) separated by a Sephadex LH-20 column.

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rapeseed.[3,6] Glucopyranosyl sinapinate has been characterised at the same UV maximum.[17]

The electropherogram of fraction I (Fig. 3, I) shows only sinapine to be present (migration time of 14.72 min). In fraction II (Fig. 3, II) sinapic acid (migration time of 10.15 min) was the dominant phenolic compound found. Additional phenolic compounds with migration times of 7.14, 7.54, 12.85, and 14.75 min were detected in fraction II. Migration time of the main peak for the electropherogram of fraction III (Fig. 3, III) was 8.27 min. The UV-DAD spectrum (results not shown) indicated that this peak was not phenolic in nature. However, phenolic compounds in fraction III were recorded with migration times of 13.15, 14.85, and 15.29 min.

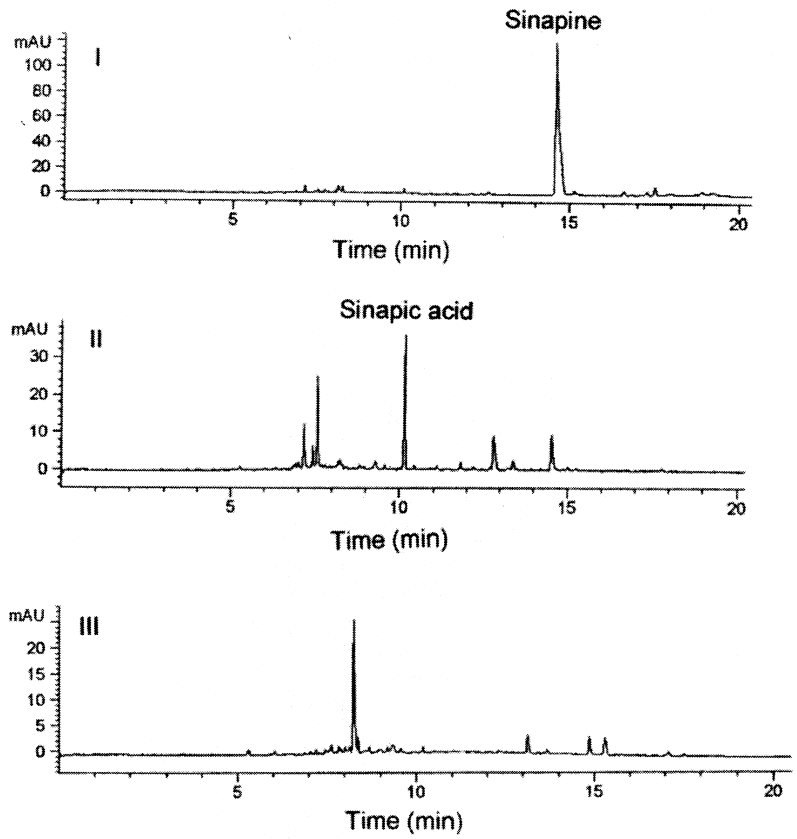

Figure 3. Electropherograms of fractions I–III separated by a Sephadex LH-20 column.

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CONCLUSION

Application of ethanol as the eluent, rather than methanol, through a Sephadex LH-20 column resulted in a better baseline separation of the phenolic constituents from rapeseed. In fraction I, sinapine was the dominant phenolic compound detected by CE, whereas a number of compounds were found in fractions II and III. Although free and esterifed phenolic acids were suspected as being present, sinapic acid was the chief phenolic constituent identified in the second fraction, based on retention time mapping. Capillary electrophoresis proved to be a useful technique at monitoring the results of the Sephadex LH-20 column chromatography.

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