CHROMATOGRAPHIC SEPARATION OF PHENOLIC COMPOUNDS FROM RAPESEED BY SE-HPLC – A SHORT REPORT

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Phenolic compounds from rapeseed were extracted into 80% (v/v) methanol. The crude extract was then separated using size exclusion high-performance liquid chromatography (SE-HPLC) on a TSK G2000SWXL column with a mobile phase of 45% acetonitrile and 0.1% TFA (v/v). Six peaks were identified on the chromatogram: the main peak originated from sinapin. Calibration of the assay was characterised by an excellent linearity of the sinapin standard (r²=1.0). The method so described can be used for screening phenolic constituents in rapeseed or canola. It is also useful for the purification of the sinapin as a standard from the crude extract.

INTRODUCTION

Phenolic acids are the major phenolic compounds found in rapeseed/canola. They are derivatives of benzoic and cinnamic acids and are present in the free, esterified, glycosylated and insoluble-bound forms [Amarowicz & Formal, 1995; Naczk et al., 1998]. Many studies have reported the antioxidative properties of crude rapeseed extracts and their fractions, which have resulted from the presence of phenolic constituents [Amarowicz et al., 1995a, 2000a,b, 2001]. Besides antioxidant activity, other characteristics of phenolic compounds from rapeseed have been investigated. For example, Nowak et al. [1992] reported bactericidal properties of rapeseed phenolics, while Kurowska et al. [2001] showed that the hydrolysed extract from defatted rapeseed seeds, which contained 24.5% free phenolic acids, effectively inhibited melanoma cells, and to a lesser extent breast, colon, lung, and prostate cancer cells.

Chromatography on a stationary column filled with Sephadex LH-20 and using methanol or ethanol as the mobile phase has been the chief means for separation and collection of fractions of phenolic compounds from rapeseed/canola extracts [Amarowicz et al., 1992]. Furthermore, this separation technique has been helpful to isolate pure phenolics from rapeseed/canola [Amarowicz & Shahidi, 1994; Wanasinghara et al., 1994; Amarowicz et al., 1995c]. RP-HPLC is the routine method for quantitative analysis of phenolic acids from rapeseed/canola. The same technique can also be used for the separation of individual phenolic constituents on a semi-preparative scale. The analytical problem of RP-HPLC is the occurrence of a broad tailing peak originating from sinapin. Application of SE-HPLC offers the advantage of separating/analysing rape-seed phenolic constituents in their native form without the need for hydrolysis. Therefore, the aim of the present study was to separate phenolic compounds from rapeseed/canola using SE-HPLC on a TSK G2000SWXL column.

MATERIALS AND METHODS

Preparation of crude extract. Commercial rapeseed of the double-low Polish 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 >6h. Phenolic constituents were extracted from the defatted meal with 80% (v/v) methanol at a material-to-solvent ratio of 10:100 (w/v) at 50°C for 30 min [Amarowicz et al., 1995b]. Extraction was carried out in dark-coloured flasks using a constant-temperature shaking water bath. The extraction was repeated two more times; supernatants were combined and methanol was evaporated off under vacuum at <40°C using a Büchi Rotavapor. Residual water in the product was removed by lyophilization. The prepared extract was stored at 4°C until further investigated.

Purification of sinapin. Free phenolic acids from an aqueous suspension of the crude extract (100 mg in 10 mL) after acidification to pH 2 using 6 mol/L HCl were extracted into ethyl ether. The water phase was collected and lyophilized. Sinapin was separated from the ether residue using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCL 10A system controller, SPD-M 10A photodiode array detector, and a semi-preparative prepacked LUNA C18 column (10 × 250 mm, 5 µm; Phenomenex, Torrance, CA, USA). The mobile phase comprised...
Separation of phenolic compounds of rapeseed via the SE-HPLC method. Phenolic compounds from the rapeseed crude extract were analysed using a Waters HPLC system (San Ramon, CA, USA) consisting of a pump and system controller (Model 600), sample processor (715 Ultra WISP), and TSK G2000SWXL column (7.8 × 300 mm, 5 μm; Hamilton, Reno, NV, USA). The mobile phase, consisting of 45% acetonitrile (v/v) and 0.1% TFA, was delivered at a rate of 0.2 mL/min. Samples (20 μL) were introduced onto the column using an autosampler. Detection was monitored at 320 nm.
RESULTS AND DISCUSSION

The chromatogram of the rapeseed crude extract shows the presence of six peaks (1–6) (Figure 1A). The peaks were recorded at retention times of 48.2 (1), 50.5 (2), 55.5 (3), 56.8 (4), 58.5 (5), and 60.5 (6) min. The dominant signal occurred at 50.5 min. Peaks 1, 2, 5, and 6 were base-line resolved, but the separation of compounds giving peaks 3 and 4 was not complete. UV-DAD spectra of compounds recorded as peaks 1–6 were characterized by maxima at 323 (1), 332 (2), 332 (3), 328 (4), 328 (5), and 342 nm (Figure 2). The UV-DAD spectrum indicated that the compounds separated on the TSK G2000SWXL column belong to the family of phenolic acids [Naczk et al., 1992]. Based on the retention times for the sinapin and sinapic acid standards (Fig. 1B, C) as well as the spectra of both phenolic constituents (Figure 3), peaks 2 and 5 in the chromatogram of the rapeseed crude extract can be tentatively identified as sinapin and sinapic acid, respectively. Other peaks are likely esters or glycosides of sinapic acid. The presence of ferulic and caffeic acids or their derivatives was absent from the chromatograms. In rapeseed/canola, the content of these phenolic acids is much lower than that of sinapin and those of other sinapic acid derivatives [Zadernowski, 1987].

Figure 4 illustrates that a complete linearity of the sinapin standard (r²=1) with the detector response is achieved by SE-HPLC. The conditions of separation for sinapin on TSK G2000SWXL are identical to or better than those afforded by C₁₈ columns or columns for RP-HPLC fabricated with a polymer matrix [Olkowski et al., 2003; Karamać et al., 2005]. The retention times of the separated compounds observed in this study are similar to those reported using C₁₈ columns. Faster analysis of rapeseed/canola phenolics can, however, be achieved by means of capillary electrophoresis or RP-HPLC with columns made with a special polymer matrix [Amarowicz & Kołodzieczyk, 2001; Olkowski et al., 2003; Karamać et al., 2005].

CONCLUSIONS

SE-HPLC with a TSK G2000SWXL column gives a good separation of sinapin and sinapic acid, and seems to be a useful tool for the analysis of crude extracts from rapeseed/canola. Moreover, it can be applied for easy separation of a sinapin standard from crude extracts.

REFERENCES


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Związki fenolowe zawarte w nasionach rzepaku ekstrahowano 80% metanolem. Uzyskany ekstrakt poddano analizie metodą SE-HPLC na kolumnie TSK G2000SWXL. Fazą ruchomą był 45% acetonitrylu (v/v) z dodatkiem 0.1% TFA. Na chromatogramie zanotowano obecność sześciu pików pochodzących od fenolokwasów i ich pochodnych. Głównym związkiem była sinapina. Kalibracja metody charakteryzowała się doskonałą liniowością wzorca sinapiny ($r^2 = 1$). Opisana metoda może znaleźć zastosowanie w analizie przesiewowej składników fenolowych nasion rzepaku lub kanoli oraz przy oczyszczaniu standardu sinapiny z surowego ekstraktu.