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GEL FILTRATION OF CONDENSED TANNINS AND PHENOLIC ACIDS OF CANOLA HULLS ON SEPHADEX G-25 AND G-50

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Condensed tannins and phenolic acids were extracted from canola hulls into acetone-water (70:30; v/v) and fractionated using Sephadex G-25 and G-50 column chromatography with 50% (v/v) acetone as the mobile phase. Three fractions containing phenolic compounds were obtained from the extract using Sephadex G-25 and four using Sephadex G-50. The latter fractions eluted from the columns, packed with either gel, both gels were free of condensed tannins. The contents of total phenolics and condensed tannins were determined in the range of 52–280 mg/g, 0.198–1.153 A_{500}/mg (vanillin method), and 0.333–1.330 A_{550}/mg (after *n*-butanol/HCl hydrolysis). The method described can be used as a step in the purification of condensed tannins of canola hulls before semi-preparative RP-18 HPLC column chromatography.

INTRODUCTION

A number of liquid chromatographic methodologies have been described in the literature for fractionation of polyphenols using Sephadex G-25 [McMurrough & McDowell, 1978; Michaud & Margail, 1977; Somers, 1977], Sephadex LH-20 [Asquith *et al*., 1983; Boukharta *et al.*, 1988; Davis & Hoseney, 1979; Lea & Timberlake, 1974; Strumeyer &Malin, 1975; Thompson *et al*., 1972]; Sepharose CL-4B [Hoff & Singleton, 1977], Fractogel (Toyopearl TSK-HW 40(s) gel) [Derdelinckx & Jerumanis, 1984; Mateus *et al*., 2001; Ricardo da Silva *et al*., 1991], Fractogel (Toyopearl) TSK 50(f) [Labarbe *et al*., 1999; Meirelles *et al*., 1992], and inert glass microparticles [Labarbe *et al*., 1999].

The presence of condensed tannins in rapeseed hulls was first reported by Bate-Smith & Ribereau-Gayon [1959]. The contents of soluble, SDS-extractable, and insoluble condensed tannins were determined by Naczk *et al.* [2000]. The antioxidant activity of the tannin extracts of canola/rapeseed hulls was reported by Amarowicz *et al.* [2000a, b, c] and Naczk *et al.* [2005]. The protein precipitating capacity of canola tannins was investigated by Naczk *et al*. [2001a]. For separation of canola/rapeseed phenolic compounds, column chromatography using Sephadex LH-20 was employed [Amarowicz *et al.,* 2000b; Naczk *et al.,* 2001b].

In the present study we present in detail the application of Sephadex G-25 and G-50 for chromatography of condensed tannins and phenolic acids isolated from canola hulls.

MATERIALS AND METHODS

Preparation of crude extract. Cyclone canola hulls were prepared according to Sosulski & Zadernowski [1981]. Hulls were extracted with hexane for 12 h using a Soxhlet apparatus and then dried at room temperature. Soluble polyphenols were extracted from the hulls with acetone-water (70:30; v/v) twice at room temperature using a Waring Blender for 2 min at the maximum speed. Extracts were combined, evaporated to near dryness at 40° C, and then lyophilized.

Column chromatography. A 200 mg portion of the so-obtained crude extract was dissolved in 4 mL of 50% (v/v) acetone and then applied onto a column (2.5 × 60 cm) packed with Sephadex G-25 or G-50 (Sigma-Aldrich Chemical Co.) and eluted with 50% (v/v) acetone. Fractions (4 mL) were collected using a fraction collector. Analysis of eluates from each tube was performed by measuring their absorbance at 725 nm and 500 nm after a colour development reaction for total phenolics [Naczk & Shahidi, 1989] and condensed tannins [Price *et al.,* 1978], respectively. Acetone-water eluates were pooled into main fractions. Following this, organic solvent was evaporated and samples were lyophilised.

Total phenolics. The content of total phenolic compounds in each fraction was estimated according to Naczk & Shahidi [1989]. 3,5-Dimethoxy-4-hydroxycinnamic acid (*trans*-sinapic acid) was used as a standard in this work. The total content of phenolics in fractions was expressed as mg of sinapic acid equivalents/g.

Condensed tannins. The content of condensed tannins in each fraction was determined using the modified vanillin method assay [Price *et al.,* 1978] and the proanthocyanidin assay described by Porter *et al.* [1986]. Results were expressed as absorbance units at 500 nm per 1 mg extract (A_{500}/mg) for the vanillin method and as absorbance units at 550 nm per 1 mg extract (A_{550}/mg) for the proanthocyanidin assay.

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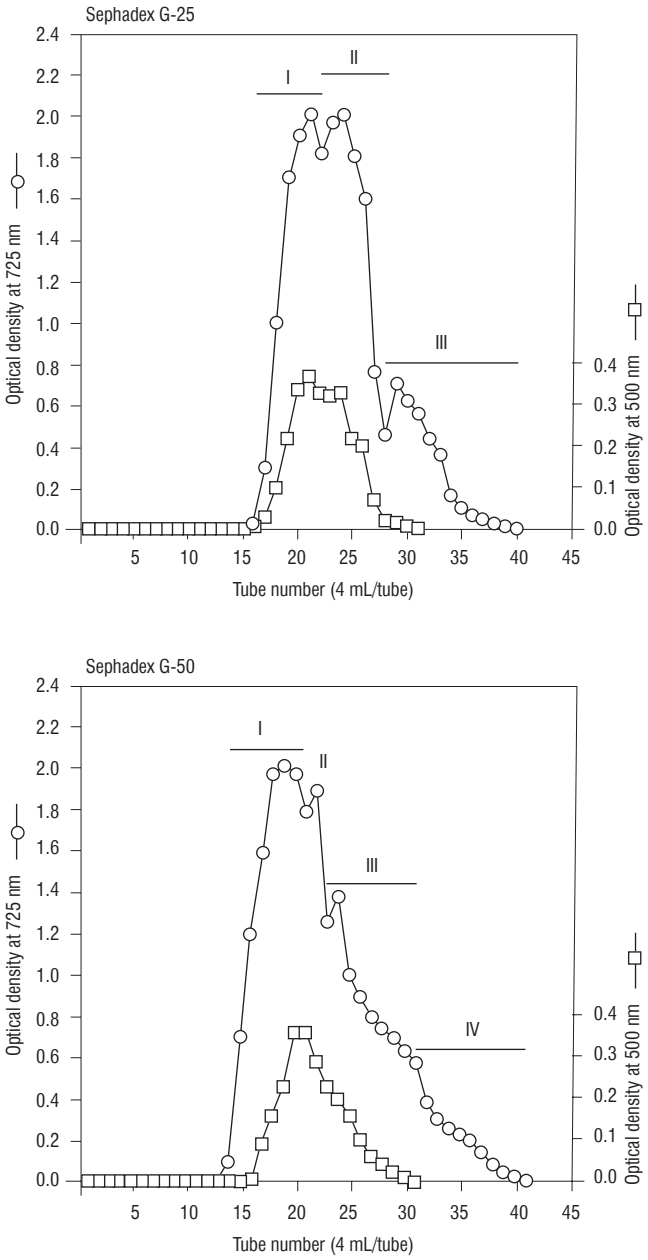

FIGURE 1. Separation a crude tannins from an acetonic canola extract of phenolic compounds from canola hulls on Sephadex G-25 and G-50 with 50% (v/v) acetone as the mobile phase.

UV spectra. UV spectra of individual fractions dissolved in pure methanol were recorded with a Beckman DU 7500 diode array spectrophotometer.

Statistical analysis. The results presented in the tables and figures are mean values (n=6 for tables and n=3 for figures) ±SD (standard deviation).

RESULTS AND DISCUSSION

Three fractions (I–III) containing phenolic compounds were obtained from the chromatography of the crude extract of Cyclone hulls using Sephadex G-25 column chromatography with 50% (v/v) acetone as the mobile phase (Figure 1). The chromatogram, based on optical density readings at 725 nm after colour reaction for total phenolic compounds, exhibited two main peaks (I and II) and a smaller one (III). The contents of fractions I and II were alike. The highest content was noted for fraction III (Table 1). It was interesting to note, however, that the content of total phenolics in fractions I and II was much higher than that found in fraction III. Condensed tannins were detected only in fractions I and II. Of these, fraction I contained a slightly higher content of this class of compounds (Table 1).

TABLE 1. Characterization of the separation of crude tannins from an acetonic canola hulls' extract using Sepahdex G-25 column chromatography.

Fraction	Content (mg)	Total phenolics (mg/g)	Tannins (A_{500}/mg)	Tannins (A_{550}/mg)
I	30	214 ± 6	0.829 ± 0.048	1.116 ± 0.089
II	33	181 ± 5	0.602 ± 0.036	0.736 ± 0.060
III	54	52 ± 2	-	-

The chromatographic profile of the crude hulls extract fractionated using the column packed with Sephadex G-50 was characterized by the presence of four fractions (I–IV). On the chromatogram of Sephadex G-50, the peak II observed in the chromatogram of Sephadex G-25 was split in two peaks (II and III). Condensed tannins were not detected only in fraction IV (Table 2).

TABLE 2. Characterization of the separation of crude tannins from an acetonic canola hulls' extract using Sepahdex G-50 column chromatography.

Fraction	Content (mg)	Total phenolics (mg/g)	Tannins (A_{500}/mg)	Tannins (A_{550}/mg)
I	27	235 ± 7	0.933 ± 0.056	1.254 ± 0.100
II	15	280 ± 8	1.153 ± 0.069	1.330 ± 0.106
III	12	146 ± 4	0.198 ±0.012	0.333 ± 0.027
IV	47	63 ± 2	-	-

The content of total phenolics in fractions I and II (Sephadex G-25), and I, II, III (Sephadex G-50) was several times greater than that reported for the crude phenolic extract of rapeseed [Amarowicz *et al.*, 2001]. Also the total phenolics content of the non-tannin phenolics' fractions of canola hulls separated on a Sephadex LH-20 column with ethanol as the mobile phase, ranged only from 14 to 112 mg/g [Amarowicz *et al*., 2000b]. Furthermore, the contents of condensed tannins reported here (Tables 1 and 2) are much higher than those found in tannin-rich extracts of leguminous seeds [Amarowicz *et al*., 2004].

The UV spectrum of fractions I and II (Sephadex G-25), and I, II, III (Sephadex G-50) exhibited a maximum at 282 nm (Figure 2). Additional maxima at 320 to 324 nm were observed for fractions II and III (Sephadex G-25) and II, III, IV (Sephadex G-50). A maximum at 282 nm is typical of condensed tannins [Amarowicz & Troszyńska, 2003; Amarowicz *et al.*, 2005]. On the other hand, maxima recorded here at the longer wavelength are typical of sinapic acid derivatives [Amarowicz *et al.*, 2001].

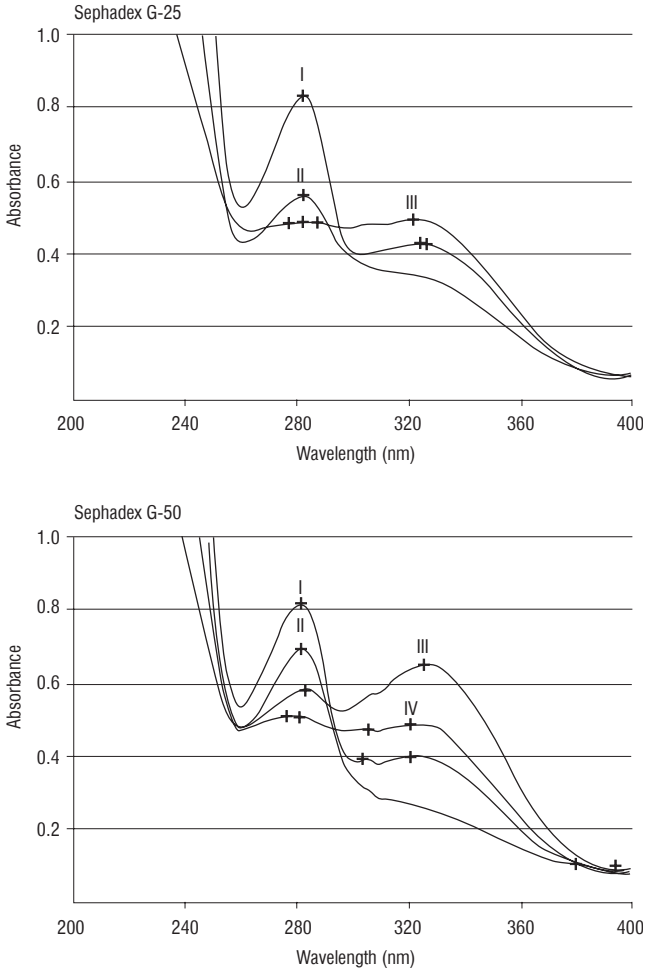

FIGURE 2. UV spectra of fractions separated using chromatography on Sephadex G-25 and G-50.

The formation of a coloured solution during *n*-butanol/HCl hydrolysis (the proanthocyanidin assay) confirmed the presence of proanthocyanidins in the fractions separated, and corresponds to literature data. Durkee [1971] identified cyanidin, pelargonidin, and an artifactual *n*-butyl derivative of cyanidin in the hydrolytic products of rapeseed hulls. Leung *et al.* [1979] reported that condensed tannins of rapeseed hulls contained leucocyanidin as their basic unit. Cyanidin was the only compound liberated during *n*-butanol/HCl hydrolysis from insoluble condensed tannins of canola hulls [Naczk *et al.*, 2000].

CONCLUSION

Gel filtration using Sephadex G-25 and G-50 with 50% (v/v) acetone as the mobile phase offers a fast and economical partial separation of condensed tannins from canola hulls. It can be used as a workup step in the purification of condensed tannins of canola hulls before semi-preparative RP-18 HPLC column chromatography.

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FILTRACJA ŻELOWA SKONDENSOWANYCH TANIN I FENOLOKWASÓW Z ŁUSEK NASION KANOLI NA ŻELACH SEPHADEX G-25 I G-50

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Ekstrakt skondensowanych tanin i fenolokwasów uzyskany z łusek z nasion kanoli układem rozpuszczalników aceton-woda (70:30; v/v) frakcjonowano na kolumnie wypełnionej żelem Sephadex G-25 i G-50 stosują układ aceton-woda (1:1; v/v) jako fazę ruchomą. W wyniku chromatografii na żelu Sephadex G-25 otrzymano 3 frakcje zaś na żelu Sephadex G-50 4 frakcje zawierające związki fenolowe. W obydwóch przypadkach ostatnia wymywana z kolumny frakcja zawierała tylko fenolokwasy. Zawartość fenoli ogółem w frakcjach wynosiła wahała się od 52 do 280 mg/g, W przypadku skondensowanych tanin ich zawartości w frakcjach wynosiła 0,198–1,153 A_{500}/mg (wyniki uzyskane metodą wanilinową) oraz 0,333–1,330 A_{550}/mg (po hydrolizie *n*-butanol/HCl). Opisana w pracy metoda chromatograficzna może być stosowana w procesie wyodrębniania skondensowanych tanin z łusek z kanoli/rzepaku przed ostatecznym oczyszczeniem ich na kolumnie semipreparatywnej RP-18 metodą HPLC.