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APPLICATION OF HPLC-DAD FOR DETECTION OF PHENOLIC COMPOUNDS BOUND TO RAPESEED 12S GLOBULIN – A SHORT REPORT

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Cruciferin (12S globulin) was extracted and purified from rapeseed and analysed using the HPLC method with a diode array UV detector. The reversed phase column and a gradient of acetonitrile in water were applied for the separation of protein and phenolic constituents. The chromatogram was characterised with a broad peak of globulin with retention time of 24.65 min. Additional peaks showed that phenolic compounds can occur as a complex with rapeseed 12S globulin. Two compounds were identified as sinapic acid derivatives. Their UV-DAD spectra possessed maxima at 328 and 336 nm.

INTRODUCTION

Cruciferin (12S globulin) is the major storage protein of the total proteins in mature rapeseeds [Höglund *et al.*, 1992]. This globulin is built of six subunits, each of them composed of a large and a smaller polypeptide chain [Gerbanowski *et al.*, 1999]. Phenolic compounds are the major cause of dark colour and undesirable taste of rapeseed protein preparations [Sosulski, 1979; Xu & Diosady, 2002]. These constituents bind to rapeseed proteins through a variety of mechanisms such as a hydrogen bonding, hydrophobic interactions, and ionic bonding [Mason, 1955; Loomis & Battaile, 1966; Hagerman & Butler, 1978; Rubino *et al.*, 1996]. The presence of phenolic compounds in rapeseed albumin fraction was previously confirmed using HPLC with a Mono Q column [Amarowicz *et al.*, 1993] and capillary electrophoresis [Amarowicz *et al.*, 2003]. In this study phenolic compounds present in rapeseed 12S globulin were detected using RP-HPLC method with a diode array detector.

The aim of this work was to apply the HPLC method with a diode array UV detector for detection of phenolic compounds bound to rapeseed 12S globulin.

MATERIALS AND METHODS

Rapeseeds of the double improved Bolko variety defatted with hexane were used in this study. For extraction and salting out of 12S globulin with ammonium sulphate use was made of the method described by Raab and Schwenke [1984]. For the final separation, a preparative Sephadex G-200 gel filtration with standard phosphate buffer, pH 8 (0.05 mol/L phosphate, sodium chloride, I=0.5), as a mobile phase was used. Briefly, 2 g of the lyophilisate of globulin fraction was dissolved in 20 mL of buffer and applied onto a column (95 × 3.5 cm); absorbance of 10 mL fractions was measured at 280 nm.

The solution of 12S globulin was prepared at a concentration of 2 mg/mL in 40% acetonitrile (v/v) containing 0.1% of trifluoroacetic acid (TFA); this solutions was then filtered through a 0.45 μm membrane. A sample (20 μL) was injected into an HPLC (Shimadzu Corp. Kyoto, Japan) system equipped with a Shimadzu LC-10AD pump, SCL-10A system controller, SPD-M 10A photodiode array UV-VIS detector, CTO-10AD column oven and CLASS-VP. C_{18} Hypersil Bakerbond BDC, 4.6 × 250 mm, 5 μm column (Mallincrodt Baker Inc., Phillipsburg, NJ, USA) was used for the separation. A flow rate of 1 mL/min, and gradient elution of 0.1% TFA in water (solvent A), 0.1% TFA in 40% (v/v) acetonitrile (solvent B), 0 to 30 min solvent B from 0 to 100% were employed. Temperature of separation was 25°C. Detection of the separated compounds was monitored at 280 and 330 nm.

RESULTS AND DISCUSSION

The HPLC chromatogram of rapeseed 12S globulin recorded at 280 nm was characterised by several sharp peaks of phenolic compounds and one broad peak originated from protein (Figure 1A). The main phenolic peaks were noted at 7.28 min (1), 8.12 min (2) and 20.09 min (3). Retention time of protein peak (4) was 24.65 min. When the detector was set at 330 nm, the majority of peaks disappeared (Figure 1B) and the chromatogram was characterised by the main peak with retention time of 20.09 min (3). One more phenolic compound was observed on chromatogram at longer retention time of 23.85 min (5). Figure 1C depicts a chromatogram of pure sinapic acid. Retention time of this phenolic acid was 16.95 min.

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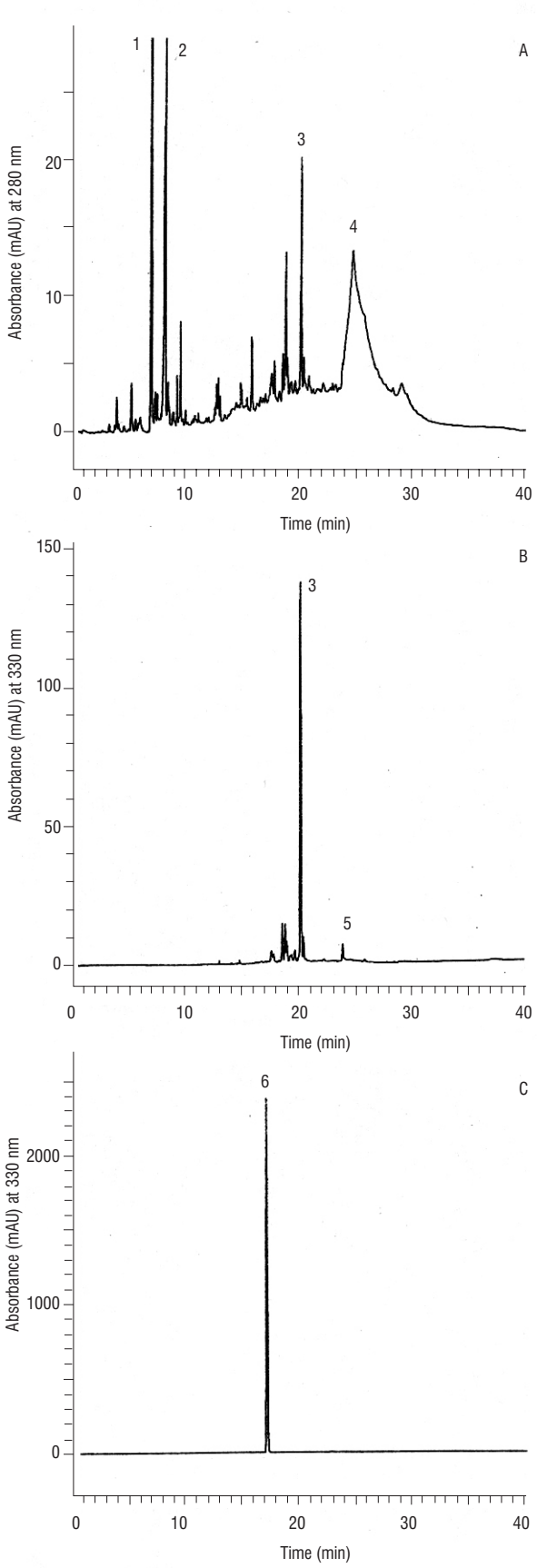

FIGURE 1. HPLC chromatogram of rapeseed 12S globulin recorded at 280 (A) and 330 nm (B) and chromatogram of pure sinapic acid (C).

UV-DAD spectra of compounds giving peaks 1–6 are present in Figure 2. Spectrum of sinapic acid had the maximum at 321 nm (Figure 2.6). Absorption bands of compounds 3 and 4 were recorded at 328 and 336 nm, respectively. Probably they were the derivatives of sinapic acids. Their retention times longer than that of sinapic acid indicate their less polar properties. From the theoretical point of view, they could be esters or depsides of sinapic acid [Zadernowski, 1987]. UV-DAD of compounds (1) and (2) was characterised by the maxima at 270 nm and at 254 nm, respectively. A shoulder at 273 nm was noted at spectrum of compound (2). UV data of phenolic acids of rapeseed published by Zadernowski [1987] pointed out that compounds (2) and (3) do not belong to the phenolic acids. The presence of the same compounds was observed also on HPLC chromatogram of low molecular rapeseed proteins [Karamać *et al.*, 2004]. In the cited study rapeseed albumins were eluted with retention time longer than this of globulins in this report.

CONCLUSIONS

The presence of peaks originated from phenolic acids and other phenolics confirmed the observation that phenolic compounds can occur as a complex with rapeseed 12S globulin. Under conditions of HPLC separation (pH 2, the presence of TFA and acetonitrile in mobile phase), they liberate from the complex and appear as free constituents. It is worth noting that they could not be separated from globulin either with gel filtration or dialysis.

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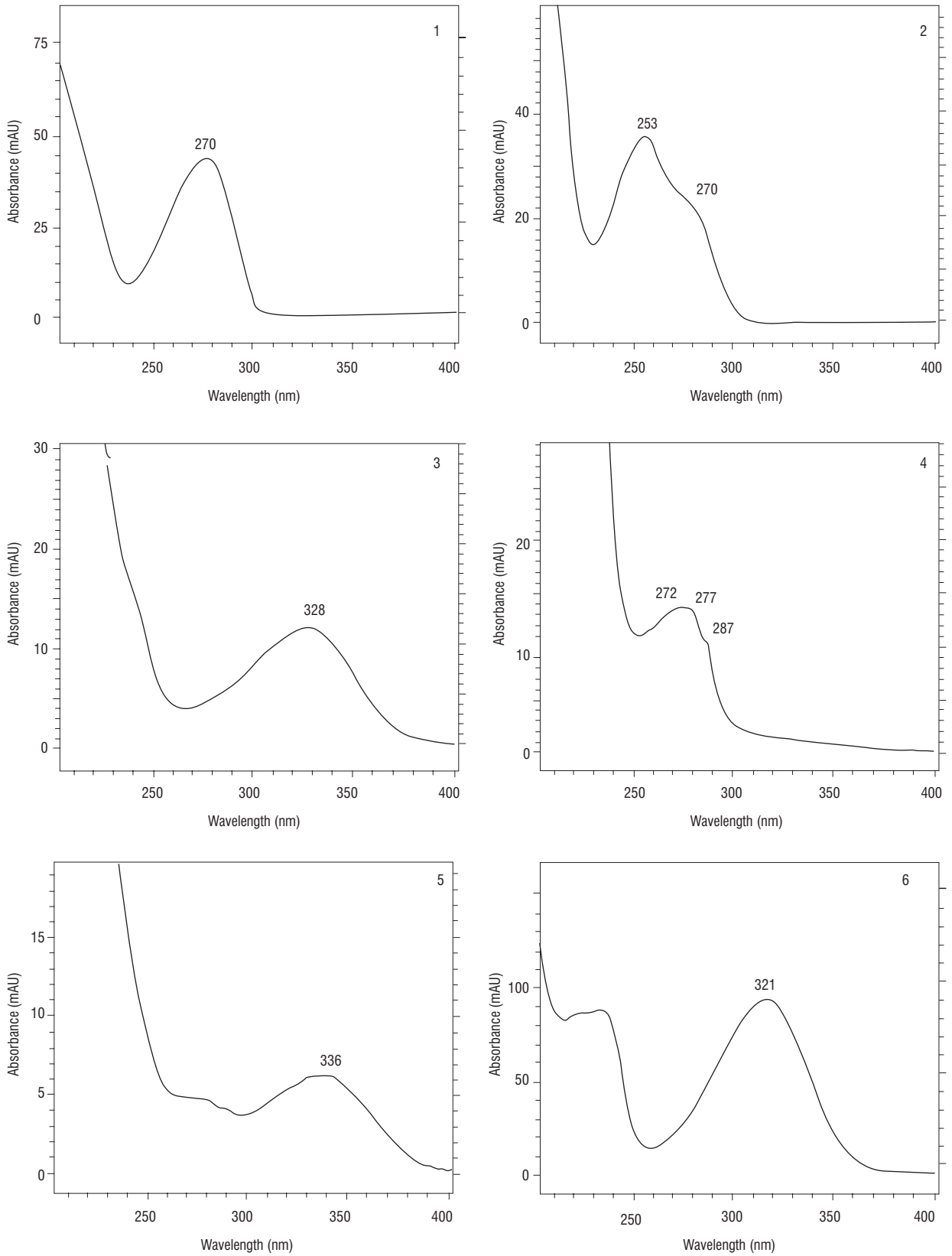

FIGURE 2. UV-DAD spectra of compounds separated using HPLC method; numbers like in Figure 1A–C.

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ZASTOSOWANIE HPLC-DAD DO DETEKCJI ZWIĄZKÓW FENOLOWYCH ZWIĄZANYCH Z 12S GLOBULINĄ RZEPAKU – KRÓTKI KOMUNIKAT

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Krucyferynę (12S globulinę) pochodzącą z nasion rzepaku analizowano metodą HPLC z detekcją fotodiodową. Do rozdziału białka i składników fenolowych zastosowano kolumnę w odwróconej fazie i gradient acetonitrylu w wodzie. Otrzymany chromatogram (rys. 1) charakteryzował się szerokim pikiem globuliny o czasie retencji 24.65 min. Obecność dodatkowych pików wskazuje, że związki fenolowe mogą występować w nasionach rzepaku w kompleksie z 12S globuliną. Dwóm związkom fenolowym przypisano strukturę pochodnych kwasu sinapowego. Maksima ich UV-DAD widm charakteryzowały się maksimami przy 328 i 336 nm (rys. 2).