

Separation of Low Molecular Weight Rapeseed Proteins by RP-HPLC-DAD – a Short Report

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Abstract

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Low molecular weight proteins were extracted and isolated from rapeseed and analysed using the HPLC-DAD method. The separation of proteins and phenolic compounds was done on the reversed phase C₁₈ column with a gradient of acetonitrile in water. The chromatogram was characterised by two peaks of low molecular weight proteins with the retention times of 19.92 and 23.24 min. Additional three main peaks of phenolic constituents were recorded on the chromatogram. One of them with maximum of UV spectrum at 328 nm was identified as sinapic acid derivatives.

Keywords: HPLC; rapeseed; low molecular weight proteins; phenolics; phenolic acids

Low molecular weight proteins make up 40–50% of nitrogen compounds in rapeseed (MIETH *et al.* 1983). According to various authors, their molecular mass ranges from 12 000 to 18 000 (AMAROWICZ *et al.* 1993). For the separation of low molecular weight rapeseed proteins, HPLC method with ionexchange columns Shodex IEC-CM 825 (RAAB *et al.* 1992) and Mono Q (AMAROWICZ *et al.* 1993) as well capillary electrophoresis (MEKB with SDS as the surfactant) (AMAROWICZ *et al.* 2003a) were used.

The aim of this work was to apply the HPLC method with a diode array UV detector for the separation of low molecular weight rapeseed proteins.

MATERIALS AND METHODS

Purification of proteins. Rapeseeds of the double improved Bolko variety defatted with hexane were

used in this study. The material was obtained from the Department of Food Science, University of Warmia and Mazury in Olsztyn. For the extraction and salting out of low molecular weight proteins with ammonium sulphate, the method described by RAAB and SCHWENKE (1984) was applied. For the final separation of the low molecular weight proteins and rapeseed globulins, a preparative Sephadex G-100 gel filtration with distilled water as the mobile phase was used. Briefly, 2 g of low molecular weight protein lyophilisate was dissolved in 20 ml of distilled water and applied onto a 95 × 3.5 column; the absorbance of 10 ml fractions was measured at 280 nm.

HPLC analysis. The solution of low molecular weight proteins was prepared at the 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 (Shi-

madzu 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. C₁₈ 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 the gradient elution with 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. The temperature of separation was 25°C. The detection of the separated compounds was monitored at 280 and 330 nm.

RESULTS AND DISCUSSION

The HPLC chromatogram of low molecular weight rapeseed proteins recorded at 280 nm was characterised by three sharp peaks of phenolic constituents (1–3 in Figure 1A) and two not baseline-separated broad peaks which originated from proteins (4 and 5 in Figure 1). The retention times of the phenolic peaks were 6.15 min (1), 7.64 min (2), and 18.09 min (3). Peaks 4 and 5 were characterised by the retention times of 19.92 and 23.24 min, respectively.

One main peak (3 in Figure 1B) was present on the chromatogram when the detector was set at 330 nm. Peaks 1 and 2 were not detected at 330 nm. The signal of proteins was very weak at

this wavelength. The retention time of the low molecular weight proteins was shorter than that of 12S rapeseed globulins reported by KARAMAĆ *et al.* (2004). In the paper cited the same conditions of HPLC analysis were applied.

UV-DAD spectra of compounds 1 and 2 were characterised by the maxima at 270 and 254 nm, respectively (Figure 2). Additionally, a shoulder at 273 nm was recorded in the spectrum of compound 2. Compound 3 possessed the maximum at 328 nm. The same spectral data (maxima at 272 and 277 nm, a shoulder at 287 nm) were noted for both proteins.

According to UV data of phenolic acids of rapeseed (AMAROWICZ & FORNAL 1995; AMAROWICZ *et al.* 2003b, compounds 1 and 2 do not belong to this class of phenolic compounds. Compound 3 was identified as a derivative of sinapic acid. Under the same conditions of HPLC separation pure sinapic acid was eluted from the column after 16.75 min. Its UV-DAD spectrum possessed the maximum at 321 nm (KARAMAĆ *et al.* 2004).

The results obtained confirmed the observation that phenolic acid derivatives and other phenolic constituents can occur in rapeseed in the form of a complex with low molecular weight proteins. Under the conditions of gel filtration, the complex was stable. The release of phenolic constituents from the complex occurred only at low pH and the presence of TFA and acetonitrile

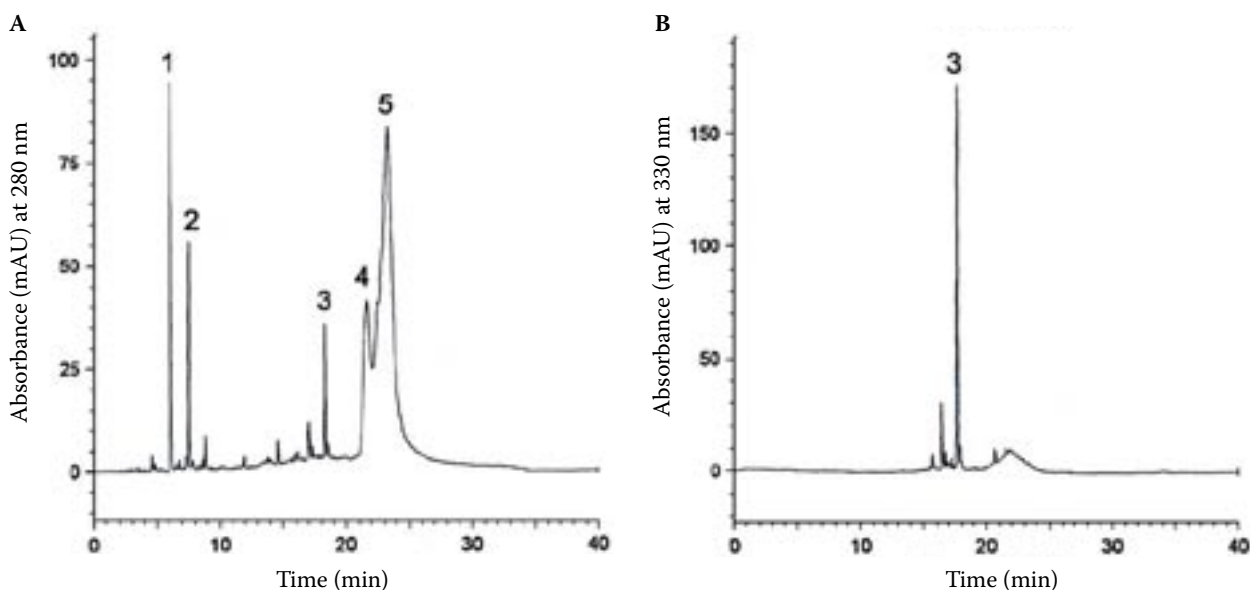


Figure 1. HPLC chromatogram of rapeseed low molecular weight proteins recorded at 280 and 330 nm

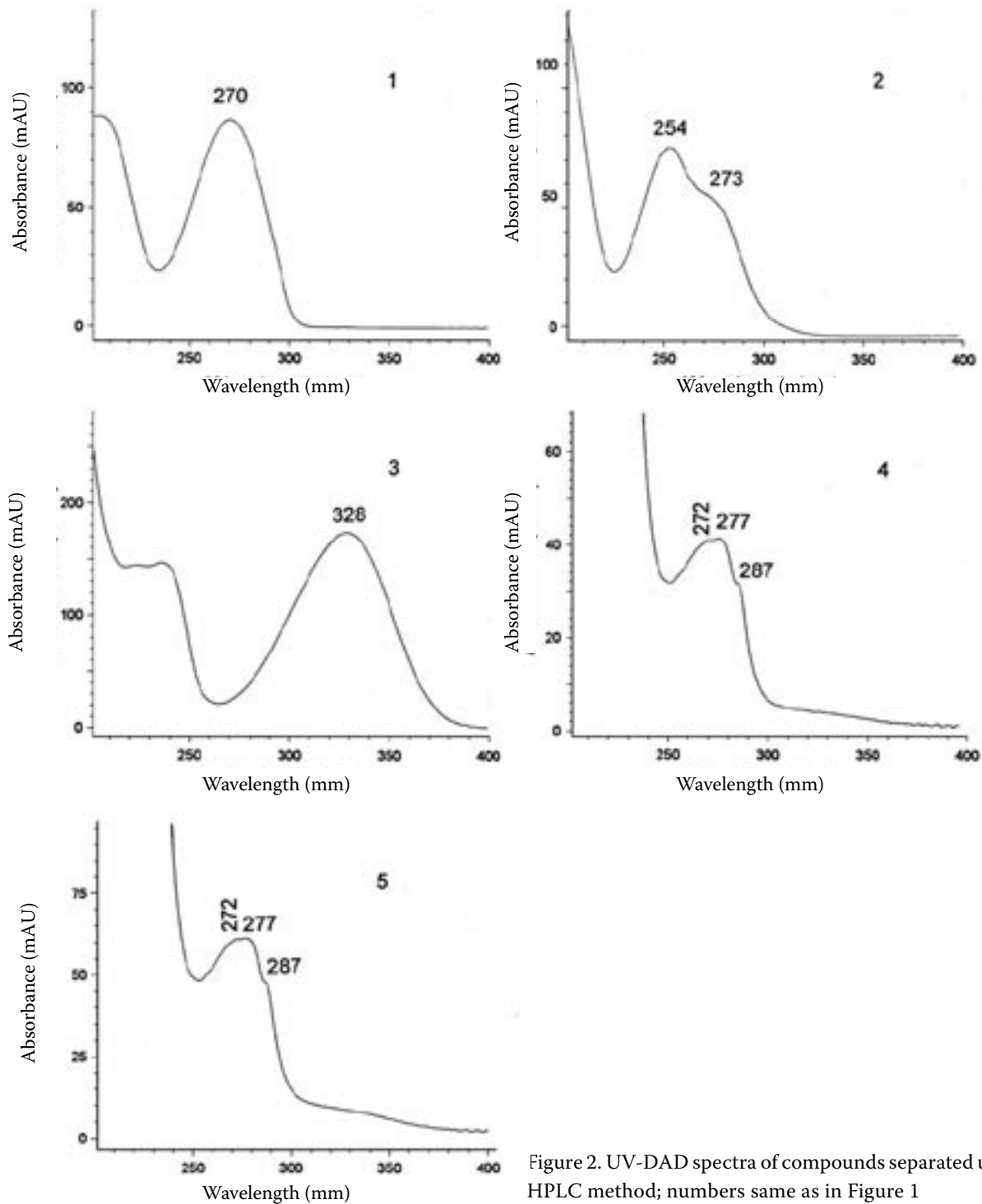


Figure 2. UV-DAD spectra of compounds separated using HPLC method; numbers same as in Figure 1

in the mobile phase. The presence of such complexes of rapeseed low molecular weight proteins with phenolic compounds was observed by AMAROWICZ *et al.* (1993) who used an HPLC method with the detection at 280 and 330 nm, and by SMYK *et al.* (1991) who applied gel filtration column chromatography on Sephadex G-25 column together with UV spectrometry and spectrofluorometry.

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