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SEPARATION OF RAPESEED GLOBULIN BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC) – A SHORT REPORT

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Cruciferin was separated from the rapeseed crude proteins using salting out with ammonium sulphate and Sephadex G-200 gel filtration. Then, so obtained protein fraction was separated using a micellar electrokinetic chromatography (MEKC) with SDS as a the surfactant. Nine peaks with migration times between 14.33 and 20.48 min were recorded on the chromatogram. The main cruciferin subfractions were characterised with molecular mass of 22 000 and 33 000 determined using MEKC technique. UV spectra showed that cruciferin protein appears as a complex with phenolic acids.

INTRODUCTION

Rapeseeds contain two predominant classes of seed storage proteins: 12S globulin (cruciferin) and 2S albumin (napin) [Schwenke *et al.,* 1981; Schmidt *et al.*, 2004]. Cruciferin makes up 25–65% of nitrogen compounds in rapeseed [Raab *et al.,* 1992]. The mean molecular mass of cruciferins was estimated to be around 300 000 and their pI to be about 7.2 [Schwenke *et al.*, 1981].

Capillary electrophoresis (CE) of proteins is directly comparable to traditional slab or tube electrophoresis. The CE format can offer a number of advantages over traditional slab gel electrophoresis, including the use of 10–100 times higher electric fields without the deleterious effects of Joul heating, on-capillary detection, and instrumental automation [Heiger, 1992]. An effective way to achieve better selectivity is the addition of micelle-forming reagents to the buffer. These can be either anionic (*e.g.* sodium dodecyl sulphate – SDS) or cationic (*e.g.* cetyltrimethylammonium chloride – CTAB). The resulting separation, known as micellar electrokinetic chromatography (MEKC), resembles reversed-phase high performance liquid chromatography (RP-HPLC) in that the analytes partition between a mobile phase and a pseudo-stationary phase ("micellar phase") [Schwartz & Prichett, 1994].

The aim of this work was to separate cruciferin (the rapeseed globulin) using MEKC technique.

MATERIALS AND METHODS

Rapeseeds of the double improved *Bolko* variety defatted with hexane were used in this study. Material was obtained from Department of Food Science, University of Warmia and Mazury in Olsztyn. For extraction and salting out of globulins with ammonium sulphate use was made of the method described by Raab & Schwenke [1984]. For a final separation of globulin a preparative Sephadex G-200 gel filtration with 0.9% (m/v) water solution of NaCl as a mobile phase was used. Briefly, a sample (2 g) of globulins lyophilisate was dissolved in 20 mL of mobile phase and applied onto a 95 × 3.5 column. Fractions (10 mL/tube) were collected using a fraction collector and absorbance of eluate was measured at 280 nm.

A micellar electrokinetic chromatography (MEKC) separation of so obtained rapeseed globulins was performed using a Beckman P/ACE 5510 instrument with UV diode array detection. The sample (1 mg) was dissolved in 0.1 mL of CE-SDS protein sample buffer and 5 μL of 2-mercaptoethanol and placed in a boiling water bath for 5 min. The protein size standard (a mixture of α-lactoalbumin, carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase B, β-galactosidase, Sigma) was prepared the same way.

Analytical conditions: capillary – 39.9/46.5 cm × 100 μm SDS-Beckman (installed in user-assembled cartridge); polarity – negative to positive; buffer – CE-SDS protein run buffer (Beckman SDS kit 14–200); run voltage – 18.5 kV; injection – pressure; cartridge temp. – 20°C; run time – 26 min; detection – 220 nm.

RESULTS AND DISCUSSION

MEKC, a hybrid of electrophoresis and chromatography, is the only one electrophoretic technique that can be used for the separation of neutral analytes as well as charged

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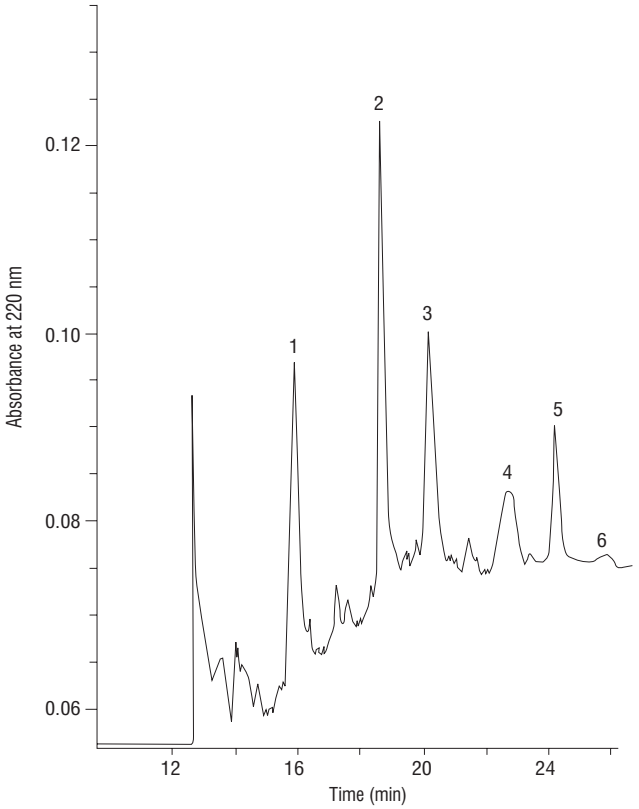

FIGURE 1. MEKC chromatogram of the protein molecular weight standards (1 – α-lactoalbumin, 2 – carbonic anhydrase, 3 – ovalbumin, 4 – bovine serum albumin, 5 – phosphorylase B, 6 – β-galactosidase).

ones. In this work, SDS was the surfactant employed as the micelle-forming species. Because SDS is an anionic surfactant and carries a large negative charge, the micelles migrate "upstream" towards the anode, which is in the opposite direction to the electroosmotic flow (EOF).

Chromatogram of the protein size standard (Figure 1) was characterised by peaks with retention times of 16.25 min (α-lactoalbumin), 18.55 min (carbonic anhydrase), 20.08 min (ovalbumin), 22.15 min (bovine serum albumin), 24.10 min

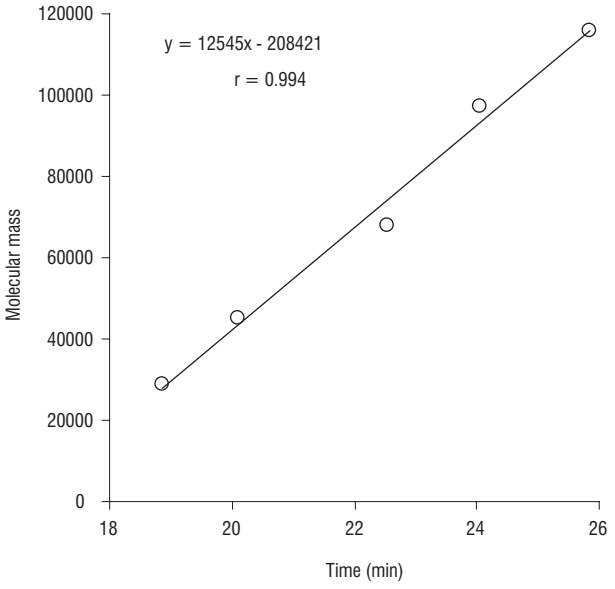

FIGURE 2. Molecular mass calibration curve.

(phosphorylase B), and 25.95 (β-galactosidase). The calibration plot of the molecular mass *versus* retention time was described by the linear regression equation: y = 12545 x – 208421 (r = 0.994), (Figure 2). This equation was calculated without retention time of α-lactoalbumin.

Nine peaks with migration times of 14.33, 14.72, 16.24, 17.84, 18.35, 19.24, 19.76, 20.16, and 20.48 min were recorded on the chromatogram of the sample of rapeseed globulins. The dominant were peaks with retention time of 18.35 and 19.24 min. The calculation of molecular mass for pep-

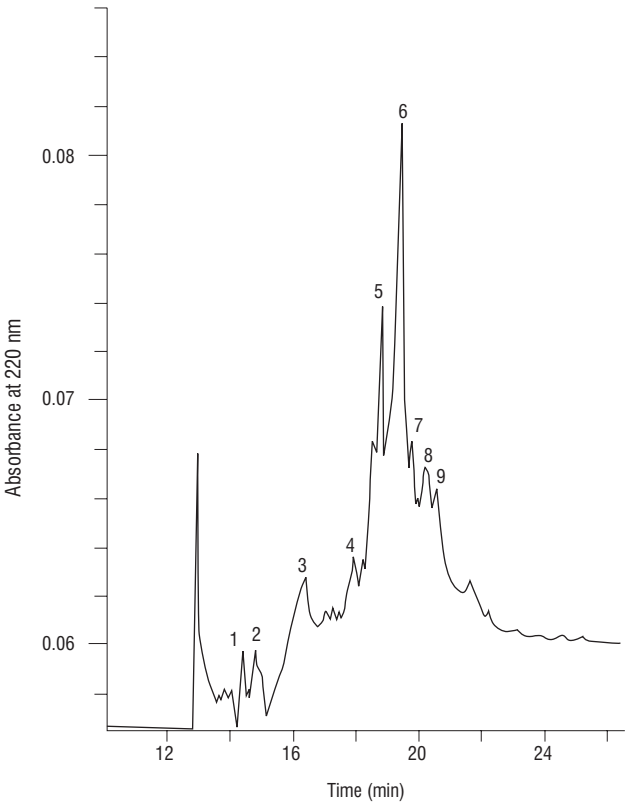

FIGURE 3. MEKC chromatogram of rapeseed globulin.

tides/proteins 1, 2, and 3 (Figure 3) was impossible because the regression equation discontinued its linearity for proteins with low molecular mass. Proteins 4–9 were characterised with molecular mass of 15 000, 22 000, 33 000, 39 000, 44 000, and 49 000. The results obtained using MEKC method are in accordance with literature data. At extreme pH and in urea solution, cruciferin dissociates into six subunits, each of them being composed of two polypeptide chains (α and β) of about 20 000 and 30 000. The SDS-PAGE electrophoregram of rapeseed globulins reported by Bérot *et al.* [2005] showed four main bands characterised with molecular mass of ~ 21 000 and 30 000. These results are similar to molecular mass of main proteins (peak 5 and 6 in Figure 3). The peaks 1–3 could originate from some minor proteins, such as thionins and lipid transfer protein (LTP) [Pearce *et al.*, 1998] or from the impurities of the napin. Napin exhibits molecular mass between 12 500 and 14 500 [Schmidt *at al.*, 2004] and comprises two polypeptide chains (with molecular mass of 4 500 and 10 000) held together by two disulfide bonds [Neumann *et al.,* 1996].

UV-DAD spectra of rapeseed globulins were character-

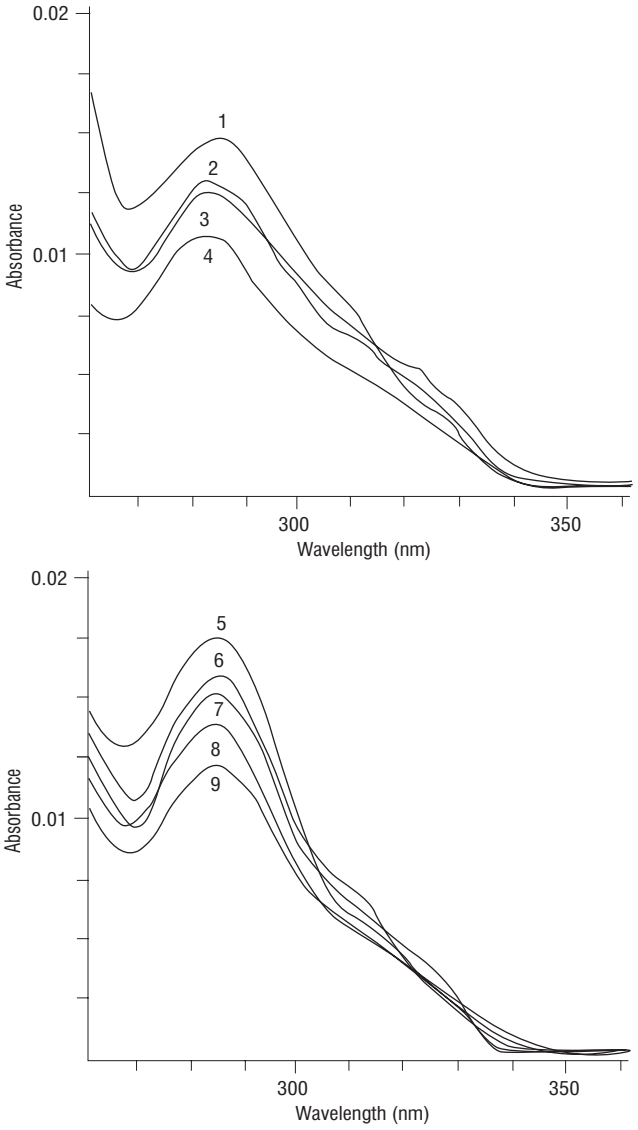

FIGURE 4. UV-DAD spectra of rapeseed globulin subfractions separated using a MEKC technique: (1–9) – numbers of peaks.

ised by the intensive band approximately at 282-286 nm (Figure 4). All spectra exhibited the relative high absorbance at the region between 310 and 330 nm. This absorption band could be caused by phenolic compound: sinapic acid and/or sinapine formed complexes with protein. Till now the presence of such complexes was known only for low molecular rapeseed proteins and was investigated using a Sephadex G-25 gel filtration and UV spectroscopy [Smyk *et al.*, 1991], HPLC method with detection at 280 and 330 nm [Amarowicz *et al.*, 1993], and capillary electrophoresis [Amarowicz *et al.* 2003], and HPLC-DAD method [Kosińska *at al.*, 2006].

CONCLUSIONS

Molecular mass of the main subunits of cruciferin separated from rapeseed and determined using a MEKC method is approximately 22 000 and 33 000. UV-DAD spectra confirmed the presence of phenolic acids bounded with this protein.

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ROZDZIAŁ GLOBULINY RZEPAKU METODĄ MICELARNEJ ELEKTROKINETYCZNEJ CHROMATOGRAFII (MEKC) – KRÓTKI KOMUNIKAT

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Krucyferyna została oczyszczona z ekstraktu białek rzepaku stosując wysalanie siarczanem amonu i filtrację żelową na żelu Sephadex G-200. Tak otrzymane białko rozdzielono metodą micelarnej elektrokinetycznej chromatografii (MEKC) z SDS jako związkiem powierzchniowo czynnym. Na chromatogramie zarejestrowano dziewięć pików o czasach retencji między 14,33 i 20,48 min. Główne subfrakcje krucyferyny charakteryzowały się masą cząsteczkową 22 000 i 33 000. Widmo UV wskazuje, że krucyferyna występuje w nasionach rzepaku jako kompleks z fenolokwasami.