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SEPARATION OF LOW MOLECULAR RAPESEED PROTEINS BY CAPILLARY ELECTROPHORESIS

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Low molecular proteins were extracted and isolated from rapeseed. The main protein was characterised with molecular mass of 14 000 determined using a capillary electrophoresis-SDS technique. UV spectrum showed that this protein appears as a complex with phenolic acids.

INTRODUCTION

Low molecular 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 separation of low molecular rapeseed proteins, HPLC method with ion-exchange columns Shodex IEC-CM 825 [Raab *et al.*, 1992] and Mono Q [Amarowicz *et al.*, 1993] were used.

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]. The application of an UV diode array detectors offers very useful, from the analytical point of view, recording of UV spectra of individual proteins separated in the capillary. The CE-SDS technique separates SDS-protein complexes using a dynamic sieving mechanism. Incorporation of a hydrophilic polymer in the CE-SDS protein run buffer causes a sieving effect so that SDS-protein complexes migrate according to their molecular size, with small proteins migrating faster and larger proteins migrating more slowly [Schwartz & Prichett, 1994].

The aim of this work was to separate the low molecular proteins of rapeseed using CE-SDS technique.

MATERIALS AND METHODS

Rapeseeds of the double improved Bolko variety defatted with hexane were used in this study. Material was obtained from the Department of Food Science, University of Marmia and Mazury in Olsztyn. For extraction and salting out of low molecular proteins with ammonium sulphate the method described by Raab & Schwenke [1984] was applied. For a final separation of low molecular proteins and rapeseed globulins, a preparative Sephadex G-100 gel filtration with distilled water as a mobile phase was used. Briefly, 2 g of low protein lyophilisate were dissolved in 20 mL of distilled water and applied onto a 95 x 3.5 column; absorbance of 10 mL fractions was measured at 280 nm.

Capillary electrophoresis-SDS (CE-SDS) separation of so obtained low molecular rapeseed proteins 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 same procedure was applied for the protein size standard (a mixture of α-lactoalbumin, carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase B, β-galactosidase).

Analysis conditions: capillary – 39.9/46.5 cm x 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

Electropherogram of the protein size standard (Figure 1) was characterised by peaks with migration 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 (phosphorylase B), and 25.95 (β-galactosidase). The calibration plot of the molecular mass *versus* retention time was described by the linear regression equation: $y = 10905 x – 169550$ ($r = 0.993$) (Figure 2).

Three peaks with migration times of 13.82, 15.44 and 16.25 min were recorded on the electropherogram of the sample of rapeseed low molecular proteins (Figure 3). The dominant was one with migration time of 16.25 min. Unfortunately, the application of the regression equation

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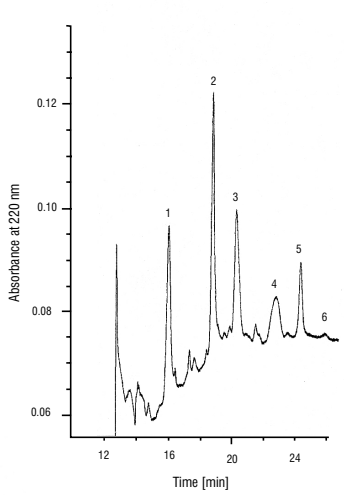

FIGURE 1. EC-SDS electropherogram of the protein size standard (1– α-lactoalbumin, 2 – carbonic anhydrase, 3 – ovalbumin, 4 – bovine serum albumin, 5 – phosphorylase B, 6 – β-galactosidase).

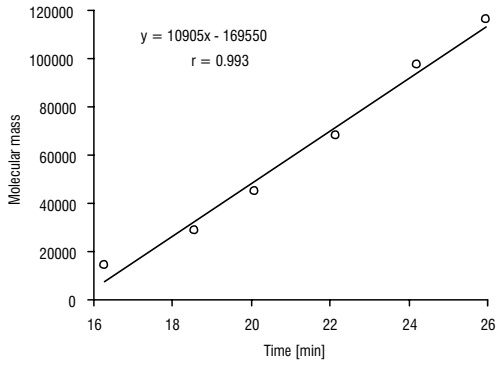

FIGURE 2. Molecular mass calibration report.

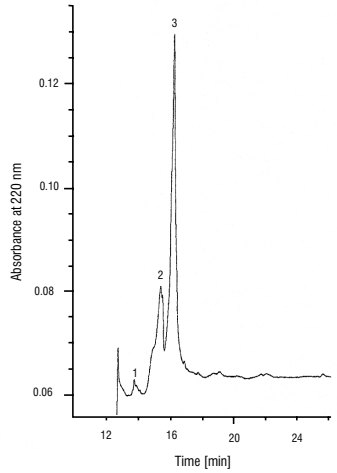

FIGURE 3. EC-SDS electropherogram of rapeseed low molecular proteins.

for calculation of the molecular mass of protein with the above-mentioned migration time was impossible because it discontinued its linearity for proteins with low molecular mass. The result obtained by means of this equation was <10 000, which is not in accordance with literature data. Migration time of the main protein was almost the same as that of α-lactoalbumin. Therefore its molecular

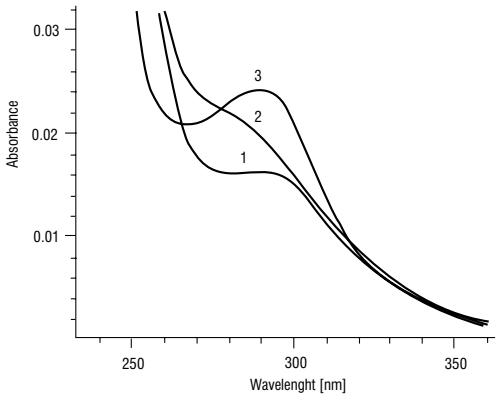

FIGURE 4. UV-DAD spectra of rapeseed low molecular proteins separated using an EC-SDS technique (1, 2, 3 – numbers of peaks from Figure 3).

mass must approximate 14 000. Similar molecular mass of the main low protein isolated from rapeseed was determined by means of SDS-PAGE [Amarowicz *et al.*, 1993]. Molecular mass of proteins or polypeptides which were recorded on electropherogram as peaks 1 and 2 seems to be less than 14 200.

UV-DAD spectrum of the main rapeseed low molecular protein was characterised by the intensive band approximately at 290 nm. The spectrum originating from peak 1 possessed also the maximum at the same wavelength but it was less noticeable (Figure 4). Low content of aromatic amino acids in protein/polypetide 2 was caused by a low content of aromatic amino acids in its composition. The shift of maximum of spectra 3 and 1 towards the longer wavelength was probably effected by the run buffer. On the other hand, this shift could be caused by phenolic compound: sinapic acid and/or sinapine formed complexes with protein. The presence of such structures in the case of low molecular rapeseed proteins was confirmed by Amarowicz *et al.* [1993] using HPLC method with detection at 280 and 330 nm. A similar conclusion was presented by Smyk *et al.* [1991] who investigated (by UV spectroscopy) low molecular proteins of rapeseed separated on a Sephadex G-25 column. UV spectrum of phenolics found in the extract of low molecular weight nitrogen compounds in rapeseed was recorded at 330 nm [Leman *et al.*, 1991].

CONCLUSIONS

Molecular mass of the main low molecular protein separated from rapeseed and determined using a CE-SDS method is approximately 14 000. UV-DAD spectra confirmed the presence of phenolic acids bound with this protein.

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