Antioxidant Activity of Phenolic Fraction of Pea (*Pisum sativum*)

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Abstract

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An extract of seeds of pea was prepared using 80% (v/v) acetone. Six fractions (I–VI) were separated from the crude extract on a Sephadex LH-20 column using methanol as the mobile phase. The antioxidant activity of fractions was investigated in a β-carotene-linoleate model system. For individual fractions, UV spectra were recorded and the content of total phenolics was determined. Results of the β-carotene-linoleate model system indicated that antioxidant activities of separated fractions correlated with their content of total phenolic compounds and they decreased in the order of IV ≈ VI > V > III > I > II. The antioxidant activity of fractions IV and VI was very strong as compared with that of butylated hydroxyanisole (BHA). Absorption maxima from UV spectra showed that flavonoids, and not phenolic acids, were the main phenolic compounds in separated fractions.

Keywords: pea; phenolic compounds; antioxidant activity; column chromatography

Evaluation of the antioxidant activity of natural substances has been of interest in recent years but only few publications deal with the antioxidant properties of phenolic compounds of legumes. A strong antioxidant activity was found in the hydrophilic phenolic extract of pea bean (TSDUDA *et al.* 1993). Navy bean hull extracts proved to offer a superior antioxidant activity than that of a mixture of BHA and BHT when used at similar concentration in storage studies of oils (ONYENHO & HETTIARACHCHY 1991). The extracts from pea, faba bean, lentil, everlasting pea, and broad bean seeds had a similar antioxidative activity in a β-carotene-linoleate model system, whereas the extract from white bean seeds was less active (AMAROWICZ *et al.* 1996a).

Antioxidant properties were determined for the phenolic fractions separated from the extracts of everlasting pea, faba bean and broad bean using Sephadex LH-20 column chromatography (AMAROWICZ *et al.* 1996c). The antioxidative activity of quercetin and kaempferol from green bean was studied by RAAB *et al.* (1996). An EPR spin trapping method was used for monitoring hydrophilic oxygen radical scavengers in leguminous seeds (YOSHIKI *et al.* 1996). The antioxidative efficacy of leguminous seed extracts evaluated by chemiluminescence methods did not depend upon their content of phenolic compounds (AMAROWICZ & RAAB 1997).

Content of polyphenols in pea seeds is affected by many factors such as their deterioration (controlled ageing treatment), by fungi (e.g. *Epicoccum purpurascens* Ehrenb. ex Schlecht.) elicitors or by UV and gamma-irradiation (LACHMAN *et al.* 1997, 1999; ORSÁK *et al.* 2000). The constituents of the pea extracts are mainly polyphenols, mainly isoflavonoids. LAPČIK *et al.* (1999) using radioimmunoassays (RIAs) combined with liquid chromatography presented evidence of daidzein, formononetin, isoformononetin and prunetin in *Pisum sativum.* ORSÁK *et al.* (2000) reported 2,3-dihydroxybenzoic, sinapic, *m*-hydroxybenzoic, veratric, and vanillic acids as the phenolic acids with highest representation in pea seeds.

The aim of the present study was to separate phenolic fractions from pea seeds using column chromatography and to examine the antioxidant properties of fractions obtained in this way.

MATERIAL AND METHODS

Samples: Investigated materials were seeds from a Polish cultivar of pea (*Pisum sativum*). Seeds were obtained

from the Institute of Plant Genetics and Breeding of Agricultural University in Lublin, Poland.

Extraction: To a 1000ml dark glass bottle, 35 g of ground seeds were weighed and suspended in 300 ml of 80% (v/v) acetone. We observed in our former investigations (AMAROWICZ & SHAHIDI 1995) that an acetone-water system extracted markedly higher amounts of phenolic compounds from lentil seeds compared with methanol-water or ethanol-water systems. The tightly capped bottle was placed in a shaking water bath at 80°C. After 15 min the bottle was removed from the bath, the extract was cooled and filtered under partial vacuum. The residue left on the filter was re-extracted with 300 ml of the fresh solvent. The extraction was repeated 3 times. Following the evaporation of acetone in a rotary evaporator at 45°C, the remaining water solutions were lyophilised.

Column chromatography: A 0.5 g portion of the extract prepared in this way was dissolved in 8 ml of methanol and applied to a chromatographic column (2 × 80 cm) packed with Sephadex LH-20 (Pharmacia) and eluted with methanol. Fractions (6 ml) were collected using a fraction collector and their absorbance was measured at 280 nm. Eluates were then pooled into major fractions, solvent evaporated and residues weighed.

Total phenolics determination: The content of total phenolic compounds in each fraction was estimated using the Folin-Denis reagent (NACZK & SHAHIDI 1989). (+)-Catechin was used as a standard in this work.

UV spectra: UV spectra of individual fractions were recorded using a Beckman DU 7500 diode array spectrophotometer.

Antioxidant activity: The antioxidant activities of separated fractions were evaluated using a β-carotene-linoleate model system (MILLER 1971). Methanolic solutions (0.2 ml) containing 2 mg of each of the dried fraction or 0.3 mg of butylated hydroxyanisole (BHA) were added to a series of tubes with 5 ml of prepared emulsion of linoleate and β-carotene.

RESULTS AND DISCUSSION

Six fractions (I–VI) containing phenolic constituents were obtained by the chromatography of acetone extract of pea seeds (Fig. 1). The chromatogram was characterised by three main peaks (I, II and VI) and three smaller ones (III, IV and VI). The relative proportions of fractions II and III were higher than those of fractions I, IV, V and VI (Table 1). The content of total phenolics in fractions I–III was lower than in fractions IV–VI. The highest content was found in fraction IV (23.6 mg/g). In former investigations, five fractions were separated from an extract of everlasting pea, faba bean and broad bean (AMAROWICZ *et al.* 1996c) while four fractions were obtained from an extract of lentil (AMAROWICZ & KARAMAĆ 2000) and white bean (AMAROWICZ *et al.* 2000). In these studies a low

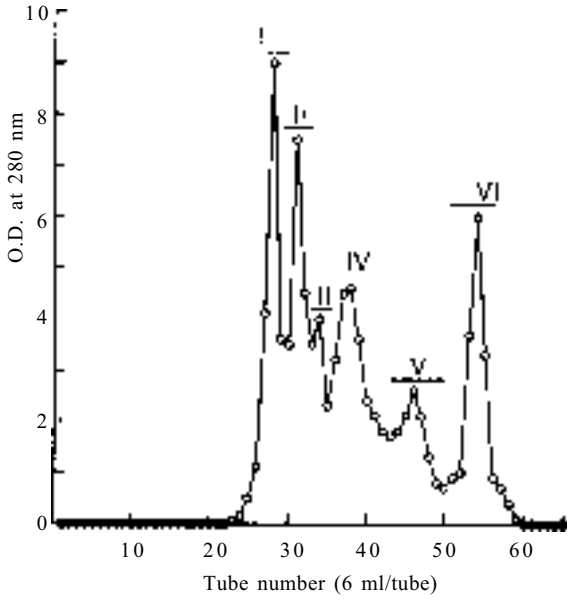

Fig. 1. Separation of pea extract on a Sephadex LH-20 column

Table 1. Relative content of separated fractions in the extract (%) and total phenolics content* (mg/g) in this material

Fraction	Relative content	Total phenolics
I	17.3	9.2
II	21.1	2.7
III	29.9	3.3
IV	14.4	23.6
V	9.1	19.0
VI	21.1	24.7

*As (+)-catechin equivalents

content of phenolic compounds was always observed in the first fraction. In general, the content of phenolic compounds in separated fractions from the pea extract was lower than those of extracts from everlasting pea, faba bean, broad bean (AMAROWICZ *et al.* 1996c) and lentil (AMAROWICZ & KARAMAĆ 2000). A low content of phenolic compounds in the crude extract of pea was reported by AMAROWICZ and RAAB (1997).

UV spectra of separated fractions (Fig. 2, Table 2) indicated that absorption bands were in the range 250–264 nm. Absorption maxima of UV spectra from fractions II and III were determined at a slightly longer wavelength. The UV spectrum of fraction I had no maximum. Sugars were probably the main compounds in this fraction. Furthermore, the first fraction separated on the Sephadex LH-20 column from everlasting pea did show any maximum (AMAROWICZ *et al.* 1996a, b, c). Previously, UV spectra of phenolic compounds from fractions separated from extracts of ev-

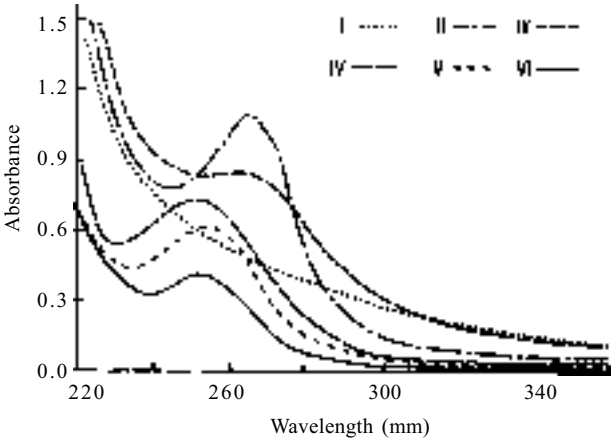

Fig. 2. UV spectra of individual fractions of pea extract

erlasting pea, faba bean and broad bean using Sephadex LH-20 column chromatography indicated absorption bands at a shorter wavelength, generally in the range 272–280 nm. The absence of absorption bands at approximately 326 nm indicates that no phenolic acid derivatives were present in fractions I–VI or that their levels were too low for detection (NACZK *et al.* 1992). This suggests that flavonoids are the main phenolic compounds in the acetone extract of pea. Phenolic acids were detected in the extract of lentil by UV spectroscopy (AMAROWICZ & KARAMAĆ 2000) and HPLC method (BARTOLOME *et al.* 1994).

Fig. 3 shows the antioxidant activity of each fraction as compared with that of BHA. The antioxidant properties of

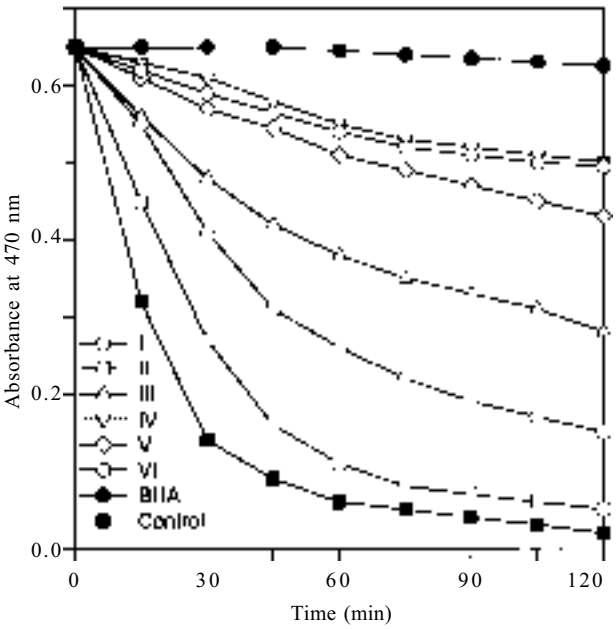

Fig. 3. Antioxidant activity of individual fractions of pea extract

Table 2. UV spectral data of separated fractions

Fraction	λ_{max} (nm)	Fraction	λ_{max} (nm)
I	–	IV	250
II	264	V	254
III	264	VI	252

fractions IV and VI were similar and were the strongest of all fractions collected. Their activity may be compared with the extract of rapeseed (NOWAK *et al.* 1992), crude catechins from green tea (AMAROWICZ & SHAHIDI 1995) or active fractions from extracts of flaxseed (AMAROWICZ *et al.* 1993, 1997) and mustard (AMAROWICZ *et al.* 1996b). Slightly lesser antioxidant efficacy was observed for fraction V. The remaining fractions were characterised by a weaker antioxidant activity in the order III > I > II. This activity was similar to that of fractions separated from an extract of white bean (AMAROWICZ *et al.* 2000). No fractions separated from extracts of everlasting pea, faba bean, broad bean (AMAROWICZ *et al.* 1996a, b, c) and lentil (AMAROWICZ & KARAMAĆ 2000) possessed such a strong activity as that of fractions IV and VI obtained from the pea extract in these investigations. The antioxidant activity of separated fractions correlated well with the content of total phenolics (Fig. 3, Table 1).

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Souhrn

AMAROWICZ R., KARAMAĆ M., WEIDNER S. (2001): **Antioxidační aktivita fenolocké frakce hrachu (***Pisum sativum***)**. Czech J. Food Sci., **19**: 139–142.

S použitím 80% (obj.) acetonu jsme připravili extrakt ze semen hrachu. Separací na koloně Sephadex LH-20 při užití mobilní fáze methanol jsme ze surového extraktu získali šest frakcí (I–VI). Antioxidační aktivitu frakcí jsme zjišťovali v modelovém systému β-karoten-linolanu. U jednotlivých frakcí jsme zjišťovali jejich UV spektra a stanovili jsme obsah celkových fenolických sloučenin. Výsledky modelového systému β-karotenyl-linoleátu ukázaly, že antioxidační aktivita frakcí vzniklých separací vykazuje korelaci s obsahem celkových fenolických sloučenin, které klesaly v pořadí IV ≈ VI > V > III> I > II. Antioxidační aktivita frakcí IV a VI byla ve srovnání s aktivitou butylovaného hydroanisolu (BHA) velmi silná. Absorpční maxima UV spekter ukázala, že hlavními fenolickými sloučeninami ve frakcích vzniklých separací jsou flavonoidy, nikoliv fenolické kyseliny.

Klíčová slova: hrách; fenolické sloučeniny; antioxidační aktivita; sloupcová chromatografie

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