Antioxidative Activity of Leguminous Seed Extracts Evaluated by Chemiluminescence Methods

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Z. Naturforsch. **52c,** 709–712 (1997); received March 5/July 21, 1997

Leguminous Seed Extract, Antioxidative Activity, Free Radical Scavengers, Chemiluminescence

The antioxidant properties of extracts from seeds of white bean, pea, lentil, everlasting pea, faba bean, and broad bean were investigated by enhanced chemiluminescence (ECL) and photochemiluminescence (PCL) methods. All extracts exhibited antioxidative activity. The antioxidative efficacy of the extracts investigated varied markedly and did not dependent upon their content of phenolic compounds. Results of TLC analysis of the extracts indicated that the antioxidant activity originated from several phenolic compounds, partly similar in their polarity.

Introduction

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 (*Phaseolus vulgaris*), while the hydrophobic fraction showed only a weak activity (Tsuda *et al.*, 1993). In storage studies of soy and sunflower oils, navy bean (*Phaseolus vulgaris*) hull extracts proved to be a better antioxidant than a mixture of butylated hydroxyanisole-butylated hydroxytoluene (BHA-BHT) when used at a similar concentration (Onyeneho and Hettiarachchy, 1991). The results of a β-carotene-linoleate test indicated that the extracts from pea, faba bean, lentil, everlasting pea, and broad bean seeds have similar antioxidative activity, whereas the extract from white ban seeds is clearly less active (Amarowicz *et al.*, 1996a).

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Antitioxidant properties were noted for the phenolic fractions separated on a Sephadex LH-20 column from extracts of everlasting pea, faba bean and broad bean (Amarowicz *et al.*, 1996b). Antioxidative activities of quercetin and kaempferol as well as their glucuronides and rutinosides, characterized as the main flavonoids of yellow and green beans (*Phaseolus vulgaris*), were evaluated by an enhanced chemiluminescence (ECL) technique (Raab *et al.*, 1996). Hydrophilc oxygen radical scavengers in leguminous seeds were investigated by an ESR spin traping method (Yoshiki *et al.*, 1996).

The aim of present study was to examine and to compare the effectivenees of leguminous extracts as natural antioxidants by chemiluminescence methods, as a first step for their evaluation in their potential use in lipid containg foods as a substitute for synthetic antioxidants.

Materials and Methods

Materials investigated were seeds of Polish cultivars of white bean (*Phaseolus vulgaris*), pea (*Pisum sativus*), lentil (*Lens culinarus*), everlasting pea (*Lathyrus latifolius*), faba bean (*Vicia faba minor*) and broad bean (*Vicia faba maior*). Seeds were obtained from the Institute of Plant Genetics and Breeding of Agricultural University in Lublin.

Extraction of phenolic compounds was conducted in the following way (Amarowicz *et al.*, 1995): to a 1000 ml dark glass bottle, 35 g of ground seeds were weighed and suspended in 300 ml of 80% (v/v) acetone. The tightly capped bottle was placed in water bath at 80 °C. After 15 min, during which the content wase shaken twice, the extract was cooled and filtered under partial vacuum. The material left on the filter was re-extracted with 300 ml of the fresh solvent. This activity was triplicated. Following evaporation of the acetone in a rotary evaporator at 45 °C, the remaing water solutions were lyophilized and dry extracts were weighed.

Antioxidant activities of the lyophilized extracts obtained were examined by enhanced chemiluminescence (ECL) and photochemiluminescence (PCL) methods.

0939–5075/97/0900–0709 $ 06.00

The ECL method of Whitehead *et al.* (1992) was employed using a LS-50B Luminescence spectrometer (Perkin Elmer) in the bioluminescence mode. Briefly, the cuvette containinga mixture of 1.6 ml of deionised water, 0.2 ml of signal reagent (buffer, luminol, p-indophenol, sodium perborate – Amersham Buchler, Braunschweig, Germany) and 0.2 ml of horseradish peroxidase (HRP) conjugate (mouse IgR HRP-linked whole antibody from sheep – Amersham Buchler) dilution was placed into the spectrometer. The solution was stired and time drive started immediately. After stabilization of the light emission (about 1 min), 0.04 ml of the antioxidant standard solution (Trolox – 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid; Aldrich) or the aqueous solution of lyophilizates were added. Time elapsed until 10% recovery of the initial light output before addition of the antioxidant was measured and used for calculation of the antioxidative activities as trolox equivalents.

The PCL method based on photo-induced chemiluminescence of luminol was carried out using a photoluminometer "Photochem" (FAT GmbH, Berlin, Germany) as well as the substance KITS ACW and ACL (FAT GmbH) for water- or lipid-soluble antioxidants, respectively, according to aqueous (Popow and Lewin, 1994) and methanolic (Popow and Lewin, 1996) systems. The PCL assay mixture for measuring of water-soluble antioxidants consisted of 1.5 ml of deionised water, 1 ml of buffer, 0.025 ml photosensitizer, and 0.01 ml of ascorbic acid standard solution or and aqueous solution of extracts, those for lipid-soluble substances of 2.4 ml methanol, 0.1 mlof buffer, 0.05 ml photosensitizer, and 0.01 ml of calibration standard (Trolox) or methanolic solution of extracts. The presence of water-soluble antioxidants led to a temporary inhibition of PCL. The resulting lag-phase was used to calculate the antioxidative activity in comparison with the known amounts of ascorbic acid. The presence of lipid-soluble antioxidants in the methanolic system resulted in a decrease of the integral of PCL intensity. The percentage of inhibition was used as a parameter for quantification, expressed as Trolox equivalents.

In all experiments, samples were analysed in triplicated and mean value ± standard deviation were recorded.

UV spectra of extracts were recorded on a Beclkman DU 7500 diode array spectrophotometer. Extracts were also examined by TLC using silica gel plates (Merck) with chloroform-methanol-water (65:35:10, v/v/v, lower phase) as the developing system (Amarowicz *et al.*, 1995b). To visualize phenolic compounds, plate was sprayed with a solution of ferric chloride (Reio, 1958). Compounds posessing antioxidant activity were visualised by spraying the plate with a solution of β-carotene and linoleic acid (Philip, 1974).

The content of total phenolics in extracts was determined using the FOLIN-DENIS reagent (Naczk and Shahidi, 1989); catechin was used as a standard in this work.

Results and Discussion

Using 80% (v/v) acetone, similar amounts of extracts were recovered from leguminous seeds (Table I). The lagest amounts of extract obtained was noted for lentil (8.76 g / 100 g dry weight) and the lowest for white bean (6.95 g / 100 g dry weight). The content of total phenolics compounds in the extracts from investigatede seeds was diversified. The hoghest content was found in the extract from lentil seeds, while the lowest from everlasting pea and white bean.

Table I. The content of extracts in leguminous seeds (g / 100 g dry weight) and the content of total phenolics in the extracts (g / 100 g).

Leguminous seeds	Extract	Total phenolics
White bean	6.95	1.08
Pea	7.25	3.48
Lentil	8.76	6.01
Everlasting pea	8.02	0.97
Faba bean	8.12	8.09
Broad bean	7.15	6.01

All extracts of leguminous seeds investigated by the luminescence methods described above exhibited antioxidative properties. Results of antioxidant activity of leguminous seed extracts evaluated by the ECL method and expressed in proportion to the mass of the extract varied widely (Table II). Highest activities were observed for the extracts of lentil and faba bean, while the lowest activity was found for white bean. Results recalculated according to the content of total phenolics

Table II. Antioxidant activity of leguminous seeds extracts evaluated by the ECL method.

Leguminous seeds	nmol Trolox*/ mg extract	nmol Trolox/ mg total phenolics
White bean	13 ± 1	1213 ± 93
Pea	74 ± 7	2112 ± 200
Lentil	202 ± 19	1772 ± 167
Everlasting pea	18 ± 1	1825 ± 101
Faba bean	170 ± 16	2101 ± 198
Broad bean	34 ± 3	561 ± 49

* Trolox – 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

compounds present in the lyophilized extracts investigated are characterized by a narrower range. Similar results were observed for extracts of pea, lentil, everlasting pea and faba bean. Activity for water-soluble antioxidants, data higher than 200 nmol / mg extract were observed for broad bean, lentil and faba bean (Table III). Weaker activities were noted for pea and everlasting pea. The extract from white bean had only a weak efficacy. The activity of water-soluble antioxidants calculated in proportion to the total phenolic compounds present in the extracts investigated showed the following trend: faba bean > broad bean > everlasting pea ≈ lentil > pea > white bean. The strongest antioxidant properties, due to lipid-soluble antioxidants determined by the PCL method, were from extracts of lentil and pea, while less activity was noted for extracts of everlasting pea and faba bean. Moreover extracts from broad bean and white bean showed only an activity equivalent to 31 nm Trolox / mg extract. Activities of lipid-soluble antioxidants presented in proportion to the total phenolic compounds were highest for pea and everlasting pea, followed by lentil, white bean and faba bean. The lowest activity was noted for broad bean.

Phenolics extracted from leguminous seeds were mixtures of unknown composition but possessed their UV spectra exibited maxima ranging from 262 (everlasting pea) to 276 nm (faba bean and broad bean). Several phenolic compounds were visualized on the TLC plate and chromatograms obtained were similar to one another. For all extracts, the positions of spots from antioxidant active compounds on TLC plate were similar for developing system used.

Based on the results obtained, the antioxidant activity of leguminous extracts appears to be generated by a great number of phenolic compounds and does not depend directly of their total content in the seeds. The main phenolic compounds of legumes are glycosides of quercetin, kaempferol, and myricetin (Tomas-Lorente *et al.*, 1990; Hempel and Bohm, 1996), isoflavonoids (Adesanya *et al.*, 1985), isoflavonoid phytoalexins (O'Neill, 1983; Robeson, 1983), catechins (Amarowicz *et al.*, 1995; Bartolome *et al.*, 1994), and anthocyanins (Tsuda *et al.*, 1994). Most of these compounds possess antioxidative efficiacy (Negre-Salvare and Salvare, 1992; Aruoma, 1993; Aruoma *et al.*, 1994; Sanz *et al.*, 1994; Tsuda *et al.*, 19994; Amarowicz and Shahidi, 1995; Rice-Evants *et al.*, 1995; Raab *et al.*, 1996).

Table III. Antioxidant activity of leguminous seeds extracts evaluated by the PCL method.

Leguminous seeds	nmol ascorbic acid / mg extract	nmol ascorbic acid / mg total phenolics	nmol Trolox / mg extact	nmol Trolox / mg total phenolics
White bean	9 ± 1	833 ± 92	31 ± 2	2833 ± 183
Pea	66 ± 4	1897 ± 115	261 ± 13	7500 ± 373
Lentil	331 ± 15	2904 ± 132	452 ± 22	3965 ± 193
Everlasting pea	31 ± 2	3196 ± 206	62 ± 3	6392 ± 309
Faba bean	480 ± 29	5933 ± 358	96 ± 5	1187 ± 62
Broad bean	238 ± 12	3960 ± 200	31 ± 1	516 ± 17

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