

# INVESTIGACIÓN

## Influence of the extraction procedure on the antioxidative activity of lentil seed extracts in a $\beta$ -carotene-linoleate model system

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### RESUMEN

#### Influencia del procedimiento de extracción en la actividad antioxidante de extractos de semilla de lenteja en un sistema modelo $\beta$ -caroteno linoleato

Los compuestos fenólicos fueron extraídos de semillas de lenteja usando tres sistemas de disolventes: acetona del 80% (v/v), metanol del 80% (v/v), y etanol del 80% (v/v). Cada extracto fue separado posteriormente en dos fracciones por cromatografía en columna con Toyo Pearl HW-40 usando agua (fracción I) y metanol (fracción II) para la elución. La actividad antioxidante de los extractos y de sus fracciones respectivas fueron examinadas en un sistema modelo  $\beta$ -caroteno-linoleato. Los tres extractos exhibieron actividad antioxidante similar. Atendiendo al nivel de compuestos fenólicos en los extractos parece que los compuestos del extracto acetónico eran menos activos que los metanólicos y etanólicos. Debido a que el contenido de fenoles era aproximadamente 16 veces más bajo en la fracción I de los extractos metanólico y etanólico comparado con la fracción II, los fenoles en la fracción I de los extractos del metanol y del etanol de las semillas de lenteja son mucho más activos que éstos en la fracción II.

Una actividad antioxidante más fuerte de la fracción I del extracto acetónico comparado con el extracto acetónico crudo fue observada durante el posterior periodo de incubación. La razón fue el relativamente alto nivel de compuestos fenólicos en esta fracción. El espectro UV confirmó que los compuestos fenólicos del extracto de acetona fueron diferentes comparados con los extractos metanólicos y etanólicos.

**PALABRAS-CLAVE:** Actividad antioxidante –  $\beta$ -caroteno linoleato – Extracción – Semilla de lenteja (extracto).

### SUMMARY

#### Influence of the extraction procedure on the antioxidative activity of lentil seed extracts in a $\beta$ -carotene-linoleate model system.

Phenolic compounds were extracted from lentil seeds using three solvent systems: 80% (v/v) acetone, 80% (v/v) methanol, and 80% (v/v) ethanol. Each extract was subsequently separated into two fractions by chromatography on a column with Toyo Pearl HW-40 using water (fraction I) and methanol (fraction II) for elution. Antioxidative activity of extracts and their respective fractions were examined in a  $\beta$ -carotene-linoleate model system. All three extracts exhibited similar antioxidant activity. Considering the level of phenolic compounds in extracts it seems that phenolic compounds from the acetone extract were less active than those

from either the methanolic and ethanolic ones. Because the content of phenolics was about 16-fold lower in fraction I of the methanolic and ethanolic extracts compared to fraction II, the phenolics in fraction I of the methanol and ethanol extracts from lentil seeds are much more active than these in fraction II. A stronger antioxidant activity of fraction I from the acetone extract compared to the crude acetone extract was observed during the latter incubation stage. The reason was a relatively high level of phenolic compounds in this fraction. UV spectra confirmed that the phenolic compounds from the acetone extract were different compared to methanolic and ethanolic extracts.

**KEY-WORDS:** Antioxidative activity –  $\beta$ -carotene linoleate – Extraction – Lentil seed (extract).

### 1. INTRODUCTION

Plant phenolics encompass a wide variety of compounds characterized by the presence of an aromatic ring with one or more hydroxyl groups and a variety of substituents. Many phenolic compounds are primary antioxidants as they act as free radical receptors and chain breakers (Shahidi and Wanasundara, 1992). Few publications deal with the antioxidant properties of phenolic compounds of legumes. However, Tsuda *et al.* (1993) reported antioxidant activity of phenolic compounds in a bean (*Phaseolus vulgaris*) extract. The results of a  $\beta$ -carotene-linoleate test indicated that the extracts from pea, faba bean, lentil, everlasting pea, and broad bean seeds had a similar antioxidative activity whereas extract from white bean seeds was clearly less active (Amarowicz *et al.*, 1996b). The navy bean (*Phaseolus vulgaris*) hull extract was found to possess better antioxidant efficacy than a mixture of BHA-BHT when used at the same concentration (Onyeneho and Hettiarachchy, 1991). Antioxidant properties were reported for the phenolic fractions separated using Sephadex LH-20 column chromatography from the extracts of everlasting pea (*Lathyrus latifolius*), faba bean (*Vicia faba minor*) and broad bean (*Vicia faba maior*) (Amarowicz *et al.*, 1996c). 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; Hempel and Bohm, 1996). Hydrophilic oxygen radical scavengers in leguminous seeds were investigated by an EPR spin trapping method (Yoshiki *et al.*, 1996).

The aim of present study was to examine the antioxidant activity of a lentil seed extracts from different solvent systems and their respective fractions in a  $\beta$ -carotene-linoleate model system, as a first step for their evaluations in their potential use in lipid containing foods as a substitute for synthetic antioxidants.

## 2. MATERIALS AND METHODS

Materials investigated were seeds of lentil (*Lens culinaris*) obtained from the Institute of Plant Genetics and Breeding of the Agricultural University in Lublin, Poland.

Defatted milled seeds were extracted twice with 80% (v/v) acetone, 80% (v/v) methanol and 80% (v/v) ethanol for 15 min at 80°C (Amarowicz *et al.*, 1995a).

Following evaporation of the organic solvents in a rotary evaporator at 45°C the remaining aqueous solution was lyophilized.

A portion of the extract (1 g) was dissolved in 10 ml of methanol and then applied onto a Toyo Pearl HW-40 column (40 x 2.5 cm). Water followed by methanol were used to wash the column. The water eluate (fraction I) was lyophilized and the methanol eluate (fraction II) was evaporated to dryness.

The content of phenolic compounds in the extract and fractions was determined using the *Folin - Ciocalteu* reagent (Naczek and Shahidi 1989) and (+)-catechin was used as a standard. UV spectra of the crude extract and fractions were recorded using a Beckman DU 7500 diode array spectrophotometer.

Antioxidative activity of the crude extract and its two fractions, separated by column chromatography, was determined by measuring the coupled oxidation of  $\beta$ -carotene and linoleic acid (Miller 1971). This method is simply and was used by many authors for evaluation of the antioxidant activity of phenolic compounds extracted from natural plant sources. Using Miller's test also synthetic antioxidants BHA, BHT and TBHQ were tested (Karamac and Amarowicz 1995). About 2 mg of  $\beta$ -carotene was dissolved in 10 ml of chloroform and 2 ml of this solution was pipetted into a round-bottom flask. After removing the chloroform with a rotary evaporator, 20 mg of linoleic acid, 200 mg of Tween 40 and 50 ml of oxygenated distilled were added to the flask with vigorous stirring. Aliquots (5 ml) of the prepared emulsion were transferred to a series of

tubes containing either 2 mg of extract or fraction I and 0.5 mg of fraction II or the synthetic antioxidant (BHA). As soon as the emulsion was added to each tube, absorbance readings at 470 nm were recorded at 15 min intervals while keeping the samples in a water bath at 50°C for 120 min.

## 3. RESULTS AND DISCUSSION

The content of total phenolic compounds in lentil seed extracts depended upon the solvent employed (Fig. 1). The acetone extract contained more phenolics (22.6 g/100 g of extract) than either methanol (7.2 g/100 g of extract) or ethanol (8.2 g/100 g of extract) extracts. Fractions I of the methanol and ethanol extracts eluted from the Toyo Pearl HW-40 column contained small amounts of phenolics (i.e., respectively 1.29 and 1.05 g/100 g) whereas Fraction I of the acetone extract contained substantially more phenolics (i.e. 6.06 g/100 g of fraction I). Fraction II obtained from the acetone, methanol and ethanol extracts contained 19.7, 18.7, and 18.8 g phenolics/100 g of fraction II, respectively.

Different phenolic compounds were present in the extracts so obtained as confirmed by UV spectroscopy. UV spectra of the crude extract from acetone showed a maximum at 275 nm (Fig. 2). Absorption maxima for UV spectra of the methanolic and ethanolic extracts were noted at a shorter wavelength of 271 nm. All extracts exhibited a shoulder at 280 nm which are characteristic of catechins or procyanidins (Amarowicz and Shahidi, 1996). The presence of catechins and procyanidins in lentil extracts was detected using thin layer chromatography by Amarowicz *et al.* (1995a) and by high performance liquid chromatography by Bartolome *et al.* (1994). High absorbance for extracts in the 310-320 nm region was most probably due to the presence of phenolic acids (Naczek *et al.*, 1992). Phenolic acids such as protocatechuic, *p*-hydroxybenzoic, vanillic,

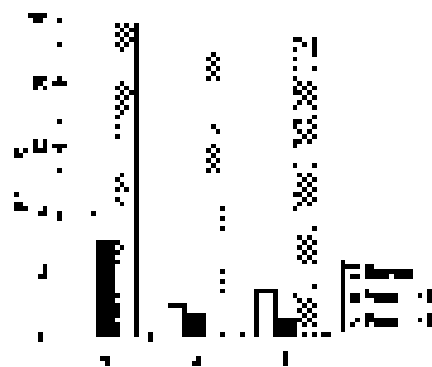


Figure 1

Content of phenolic compounds in crude extracts from lentil seeds and their fractions; extracts were obtained using 80% (v/v) solution of acetone (1), methanol (2) and ethanol (3)

*p*-hydroxyphenylpropionic, gentisic, ferulic and *p*-hydroxycinnamic acid were found for lentil seeds (Bartolome *et al.*, 1994). Some differences in the UV spectra of fractions I are presented in Fig.3. Fraction I with contained hydrophilic phenolic compounds separated from the methanolic and ethanolic extracts showed a maximum at 266 nm whereas the maximum in the spectrum of fraction I from the acetone extract was shifted slightly to 270 nm. UV spectrum of fraction II from the acetone extract was characterized by a maximum at 282 nm whereas for fractions II from the both methanolic and ethanolic extracts, maxima were observed at 270 nm (Fig. 4) ; an additional shoulder at 282 nm was recorded for this fraction. Different UV spectra recorded for the acetone extract resulted from acetone's greater ability to extract tannins from the lentil seeds. Previously this observation had been confirmed by Amarowicz *et al.* (1995a) by TLC on silica gel.

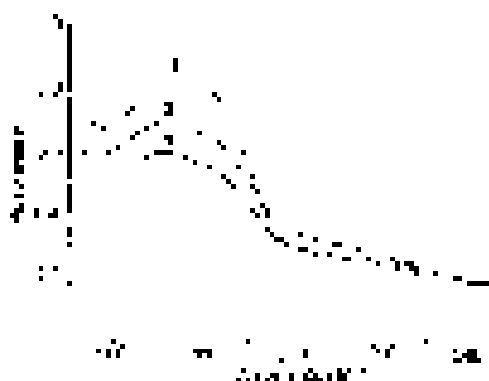


Figure 2  
UV spectra of crude extracts from lentil seeds and their fractions; extracts were obtained using 80% (v/v) solution of acetone (1), methanol (2) and ethanol (3)

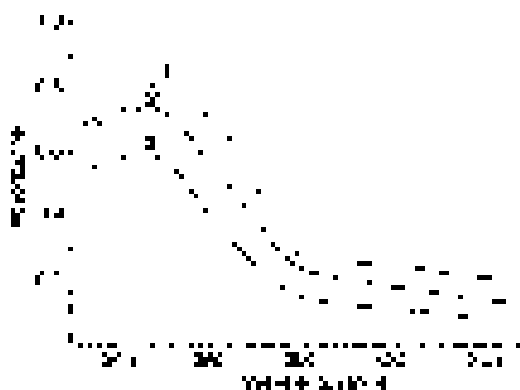


Figure 3  
UV spectra of fractions I separated from crude extracts; extracts were obtained using 80% (v/v) solution of acetone (1), methanol (2) and ethanol (3)

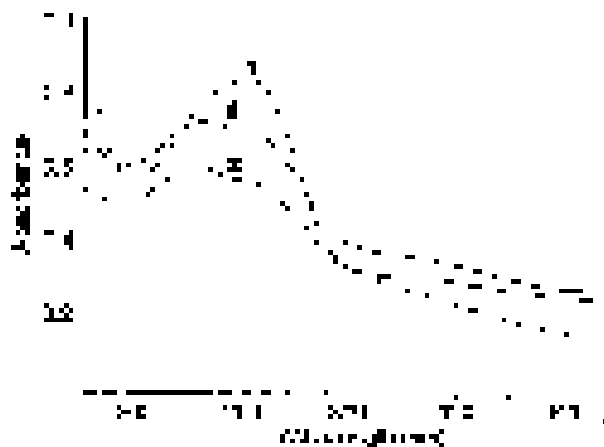


Figure 4  
UV spectra of fractions II separated from crude extracts; extracts were obtained using 80% (v/v) solution of acetone (1), methanol (2) and ethanol (3)

Results of the test with  $\beta$ -carotene and linoleic acid are presented as diagrams in Figs 5-7. All three extracts exhibited similar antioxidant activity. After 120 min of incubation absorbance for extract-treated systems was ca.0.5 while that for the control sample (without extract) almost the zero. Considering the level of phenolic compounds in extracts it seems that phenolic compounds from the acetone extract were less active than those from either the methanolic and ethanolic ones. Because the content of phenolics was about 16-fold lower in fraction I of the methanolic and ethanolic extracts compared to fraction II, the phenolics in fraction I of the methanol and ethanol extracts from lentil seeds are much more active than these in fraction II. A stronger antioxidant activity of fraction I from the acetone extract compared to the crude acetone extract was observed during the latter incubation stage (Fig.5). The reason was a relatively high level of phenolic compounds in this fraction.

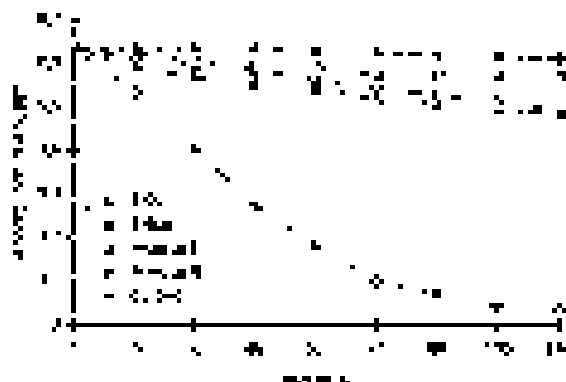


Figure 5  
Antioxidative activity of acetone extract from lentil seeds and its two fractions

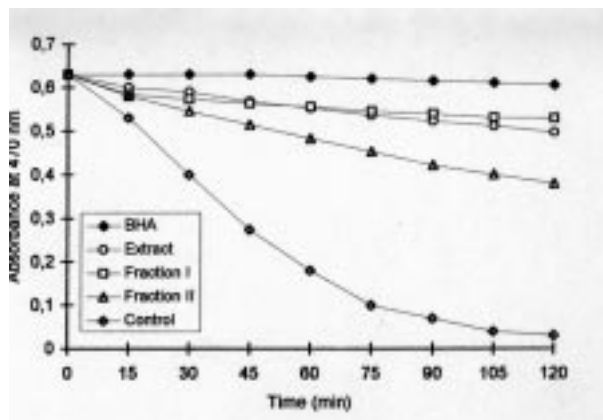


Figure 6

Antioxidative activity of methanol extract from lentil seeds and its two fractions

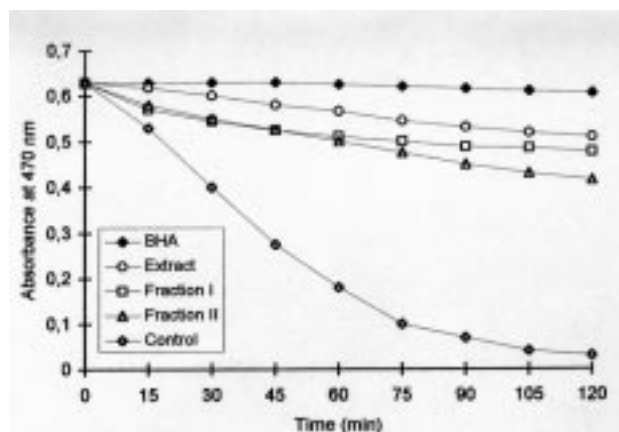


Figure 7

Antioxidative activity of ethanol extract from lentil seeds and its two fractions

Antioxidative properties of extracts from lentil seeds, especially for fractions eluted with water from the Toyo Pearl HW-40 column, can be regarded as very high.

They are comparable to the activity of extracts of green tea (Amarowicz and Shahidi 1995) and rapeseed (Amarowicz *et al.*, 1995b), and with phenolics fractions from canola (Wanasundara *et al.*, 1994), white mustard (Amarowicz *et al.*, 1996a) and flax (Amarowicz *et al.*, 1993; Amarowicz *et al.*, 1997). Antioxidative activity of lentil seed extract evaluated by chemiluminescence methods was reported by Amarowicz and Raab (1997). Antioxidant activity evaluated by the enhanced chemiluminescence technique was 1772 nmol Trolox/mg total phenolics in extract. For the photochemiluminescence method, activities measured were 2904 nmol ascorbic acid/mg total phenolics in extract and 3965 nmol Trolox/mg total phenolics in extract. Polyphenolic compounds in the lentil seed coat possessed antioxidative activity measured using a

liposome method (Troszynska *et al.* 1997). A stronger antioxidative activity observed for the hydrophilic fractions (the fractions I) is in accordance with results of the present investigation and to those of Tsuda *et al.* (1993). Tsuda *et al.* (1993) noted a strong antioxidant activity for the hydrophilic phenolic extract of bean (*Phaseolus vulgaris*) while the hydrophobic fraction showed only a weak one.

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