Antioxidant and Radical Scavenging Activities of a Barley Crude Extract and its Fractions

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Abstract

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Phenolic compounds were extracted from the Candle variety of hull-less waxy barley with 80% (v/v) methanol to yield a crude preparation. Seven fractions (I–VII) were separated from the barley extract so obtained on a Sephadex LH-20 column using methanol as the mobile phase. Nearly 80% of the phenolics extracted from barley were comprised in the first three fractions. The measurements of the antioxidant activity using a β-carotene-linoleate model system, radical scavenging capacity against DPPH•, and reducing power based on the reduction of a $Fe^{3+}/K_3Fe(CN)_6$ complex to the ferrous state were assessed in the barley crude extract and its fractions. The results indicated that barley possess marked antioxidant and antiradical capacities as compared to other grains such as wheat, rye, and triticale. Furthermore, the methanolic extract of the waxy barley sample and its fractions resembled in the aforementioned activities those from leguminous seeds, rapeseed and pulses. Phenolic constituents contained in barley may have a future role as ingredients in the development of functional foods.

Keywords: barley; phenolics; antioxidant activity; radical scavenging activity; column chromatography

The antioxidant properties of phenolic compounds in grains have been associated with the health benefits attributed to these crops and the value-added products derived from them. Antioxidants may play an important role in the chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital biomolecules such as DNA, lipids, and proteins (Hollman 2001).

The antioxidant and antiradical capacities of cereal extracts were thoroughly investigated using a variety of model systems including bulk oils (Onyeneho & Hettiarachchy 1992), scavenging of DPPH radicals (DPPH•) (Amarowicz *et al.* 2002a, b; Karamać *et al.* 2002; 2004), coupled oxidation of β-carotene and linoleic acid (Amarowicz *et al.* 2002a, b; Karamać *et al.* 2002, 2004), liposomes (Baublis *et al.* 2000), the total antioxidant activity (TAA) assay (Zieliński & Kozłowska 2000), and reducing power (Amarowicz *et al.* 2002a, b; Karamać *et al.* 2002, 2004).

One of the richest sources of phenolics among the grains is barley. In beer, for example, 70 to 80% of the phenolic constituents originate from

malted barley while the remaining 20 to 30% come from the hops (Gerhäuser 2005). The scavenging activity of barley phenolics against DPPH• and $ABTS^{\bullet+}$ were comparable to a synthetic antioxidant, butylated hydroxytoluene (BHT) (Ragaee *et al.* 2006). Bonoli *et al.* (2004) also observed the scavenging effect of barley extracts against DPPH•. The relationship between the observed antioxidant activity of barley with the contents of flavan-3-ols, hydroxycinnamic acid derivatives, and flavonols was described by Maillard *et al.* (1996). Holasova *et al.* (2002) also reported a prominent antioxidant activity in a barley extract using the Schaal oven test at 70°C with gravimetric indication and lard as the substrate. The antiradical activity of 2''-*O*-glycosylisovitexin (2''-*O*-GIV) isolated from green barley leaves was investigated using EPR spectroscopy by Arimoto *et al.* (2000). These authors reported that 2''-*O*-GIV inhibited superoxide formation by 97% at the level of 25mM, and hydroxyl radical formation by 91% at the level of 500mM; the inhibitory activity of 2''-*O*-GIV was dose-dependent. The flavone *C*-glycosides saponarin and luteorin were found to be the major flavonoid antioxidants in young green barley leaves (Markham & Mitchell 2003). The results of the investigation of the antioxidative and hypolipidemic effects of barley leaf essence in a rabbit model of atherosclerosis suggested that this material can be utilised in the prevention of cardiovascular disease in which atherosclerosis is important (Yu *et al.* 2002). The antioxidative properties of barley tea (a well-known drink used during the summer season in Japan) were evaluated by measuring its peroxynitrite-scavenging activities (Etoh *et al.* 2004). Among the five phenolics identified, 3,4-dihydroxybenzaldehyde, *p*-coumaric acid, quercetin, and isoamericanol A showed stronger activities than is that of BHT at 400μM.

The object of the present study was to investigate the antioxidant and antiradical activities of a hull-less waxy barley (Candle var.) crude extract and its fractions obtained chromatographically by means of a Sephadex LH-20 column.

MATERIAL AND METHODS

Material. All solvents used were of analytical grade. Methanol, potassium ferricyanide, ferric chloride and trichloroacetic acid were acquired from the P.O.Ch. Company (Gliwice, Poland). Butylated hydroxyanisole (BHA), β-carotene, linoleic acid, (+)-catechin, vanillin, Folin & Ciocalteu's phenol reagent, polyoxyethylenesorbitan monopalmitate (Tween 40), Sephadex LH-20, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) were obtained from Sigma Ltd. (Poznań, Poland).

The Candle variety of hull-less waxy barley was generously provided from authenticated voucher specimens of the Crop Development Centre, University of Saskatchewan (Saskatoon, Canada).

Extraction. Barley grains were ground in a Moulinex coffee mill. Phenolic compounds were extracted from the ground material using 80% (v/v) methanol at the solids-to-solvent ratio of 1:10 (w/v) at 50°C for 30 min (Amarowicz *et al.* 1995). The extraction was carried out in dark-coloured flasks in a shaking water bath. The extraction was repeated twice more, the supernatants were combined and methanol was evaporated under vacuum at 40°C using a rotary evaporator. Residual water was removed from the extract by lyophilisation. The crude extract was stored at –20°C until used.

Column chromatography. An 800-mg dried sample of the barely crude extract was dissolved in 8 ml of methanol, applied to the top of the chromatographic column (20 × 800 mm [I.D. × length], Kontes, Vineland, NJ, USA) packed with Sephadex LH-20 (bead size: 25–100 μ), and then eluted from the column with methanol as the mobile phase. Fractions (8 ml) were collected using a LKB Bromma 2112 RediFrac fraction collector (Pharmacia, Uppsala, Sweden) and their absorbances were measured at 280 and 320 nm. Eluates were then pooled into major fractions, the solvent was removed *in vacuo* in a rotary evaporator, and the products were weighed.

Total phenolics. The contents of total phenolic compounds in the barley crude extract and each of its fractions were estimated using the Folin & Ciocalteu's reagent (Naczk & Shahidi 1989). (+)-Catechin was used as the standard in this work.

Antioxidant activity. The antioxidant activities of the barley crude extract and its fractions were determined using a β-carotene-linoleate model system (Miller 1971). Methanolic solutions (0.2 ml) containing 2 mg of the crude extract or its fractions were transferred to a series of tubes. To these, 5-ml aliquots of the previously prepared emulsion of linoleic acid and β-carotene stabilised with Tween 40 were added. Immediately after adding the emulsion to each tube, the zero-time absorbance at 470 nm was recorded. Samples were held in a 50°C water bath and their absorbance

values were recorded over a 120-min period at 15-min intervals.

Scavenging of DPPH radical. The scavenging effects of phenolics in the crude extract and its fractions were monitored as described by Yen and Chen (1995). A 2.0-ml methanolic solution containing 0.5 mg of the barley extract or one of its fractions was added to a methanolic solution of DPPH• (1mM, 0.25 ml). The mixture was vortexed for 30 s and then left to stand at room temperature for 30 min. The absorbance of the resulting solution was read spectrophotometrically at 517 nm. The capacity to scavenge free radicals (AA %) was calculated as follows:

$$A\ (\%) = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{sample}}{\text{Absorbance}_{control}} \times 100$$

For the control, pure methanol without antioxidant was employed.

Reducing power. The reducing power of the phenolics in the crude extract and its fractions was determined according to the method of Oyaizu (1986) and Yen & Chen (1995). A 1-ml aqueous suspension of 0.5-mg barley extract or one of its fractions was mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) $K_3Fe(CN)_6$ solution. The mixture was placed in a water bath at 50°C for 20 min. Following the incubation, 2.5 ml of 10% (w/v) trichloroacetic acid solution were added and the mixture was then centrifuged at 1750 × g for 10 min. A 2.5-ml aliquot of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) $FeCl_3$ solution. The absorbance of the reaction mixture was read spectrophotometrically at 700 nm.

Statistical analysis. Correlation analysis between the total phenolic content and unoxidised β-carotene, scavenging effect, and reducing power was performed using Microsoft Excel software.

RESULTS AND DISCUSSION

Seven phenolic-containing fractions (I–VII) were obtained from the 80% (v/v) methanolic extract of barley using Sephadex LH-20 column chromatography with methanol as the mobile phase (Figure 1). Most fractions absorbed UV radiation at both 280 and 320 nm wavelengths, thus indicating the presence of flavonoids and phenolic acids in each fraction. However, fractions V–VII were exceptions which did not absorb at the 320 nm band. The majority of the phenolics in the barley crude extract (~ 80%) were eluted from the column in the first three fractions (i.e., fractions I–III); the masses were 200, 191, and 244 mg, respectively. The relatively high content of the first fraction (i.e., 200 mg) is typical for Sephadex LH-20 chromatography: it is caused by the quick elution of sugars and lesser polar phenolics. This phenomenon was described with the extracts of leguminous seeds investigated by Amarowicz *et al.* (1996, 2001b).

The content of total phenolics in the barley crude extract was 92 mg/g (Figure 2), whereas the values in the fractions ranged from a low content of 36 mg/g

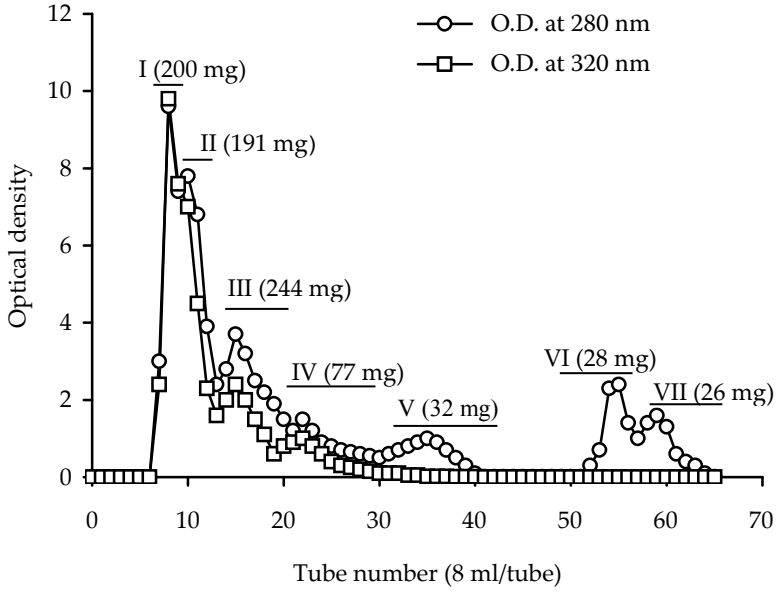

Figure 1. Separation of phenolic compounds from a barley crude extract on Sephadex LH-20 column with methanol as the mobile phase; the mass of individual fractions separated from 800 mg of the extract is listed in brackets

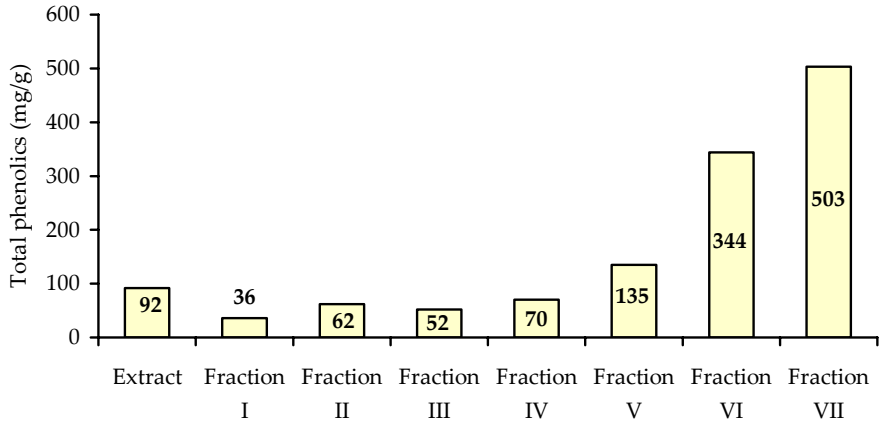

Figure 2. Contents of total phenolics (mg/g) in a barley crude extract and its fractions

(fraction I) to a high one of 503 mg/g (fraction VII). The crude extract of barley possessed *ca.* a 20-times higher content of total phenolics than the extracts prepared under identical conditions from caryopses and embryos of rye, wheat, and triticale (Karamać *et al.* 2002, 2004; Amarowicz *et al.* 2002a, b). The contents of total phenolics in the dominant fractions (i.e., I–III) were similar to or higher than those in the fractions separated from the extracts of leguminous seeds (Amarowicz *et al.* 1996), canola (Wanasundara *et al.* 1994), rapeseed (Amarowicz *et al.* 2001a, 2003), soybean (Karamać & Amarowicz 2002), and flax (Amarowicz *et al.* 1993).

The antioxidant efficacy of the barley crude extract against the coupled oxidation of linoleic acid and β-carotene is depicted in Figure 3. A 2-mg addition of the crude extract to the emulsion effectively inhibited oxidation. Even after 2 h of incubation, 59.3% of β-carotene remained unoxidised whereas in the control sample devoid of antioxidant practically all of the β-carotene was bleached/oxidised. Fractions II, VI and VII were characterised as possessing the best antioxidant activity in the emulsion system, unlike fraction IV which demonstrated the weakest protection against β-carotene bleaching (Figure 4). Even at the 4-mg level of addition, methanolic extracts prepared from rye, wheat, or triticale caryopses and embryos (Amarowicz *et al.* 2002a, b; Karamać *et al.* 2002, 2004) were less effective in retarding oxidation in the model emulsion than those containing 2 mg of the barley fractions. A similar finding was made with methanolic extracts of lentil, faba bean, broad bean, pea, and white bean (Karamać *et al.* 2002, 2004), as they were also less active than the barley crude extract examined in this work. Only in methanolic extracts of phenolic compounds prepared from canola hulls (Amarowicz *et al.* 2000a, b) and acetonic extracts of condensed tan-

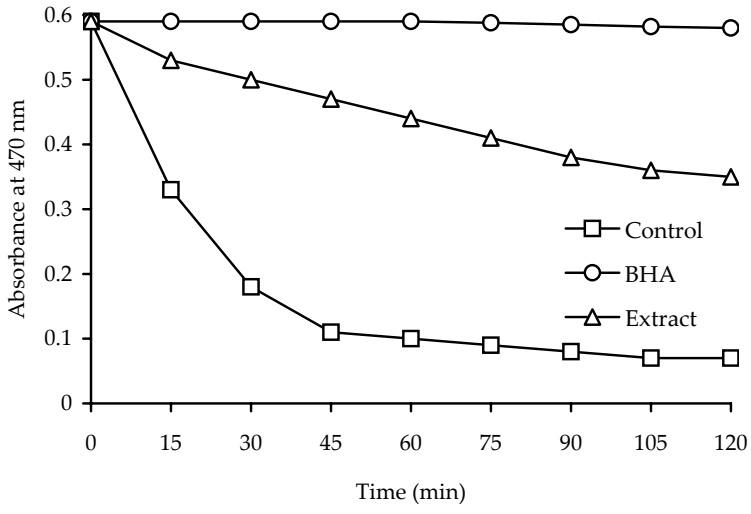

Figure 3. Antioxidant activity of a barley crude extract assessed using a β-carotene-linoleate model system, as reflected by changes in absorbance values at 470 nm

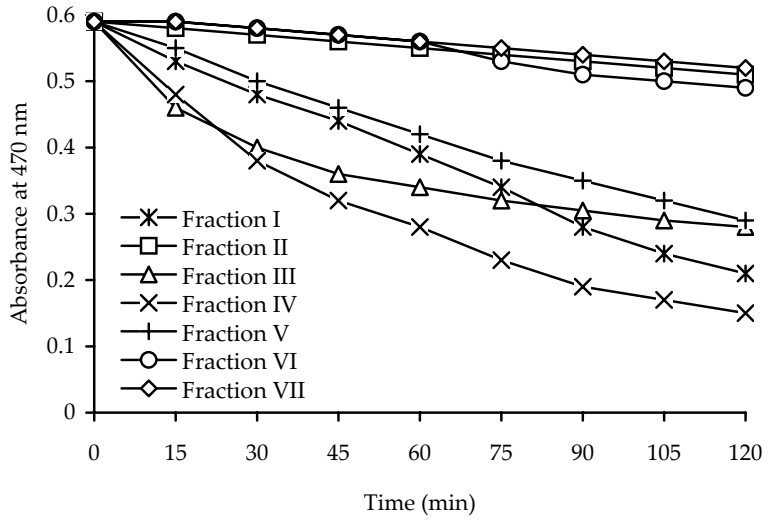

Figure 4. Antioxidant activities of individual fractions from a barley crude extract assessed using a β-carotene-linoleate model system, as reflected by changes in absorbance values at 470 nm

nins from beach pea, canola hulls, and faba beans (Amarowicz *et al.* 2000c) was found a similar antioxidant efficacy.

The radical scavenging activities of the barley crude extract and its fractions toward DPPH• are tabulated in Figure 5. The barley crude extract as well as fractions VI and VII were found to afford the strongest free-radical scavenging activities at 43.6, 41.8 and 47.4% efficacy, respectively, based on the conditions of the assay. The antiradical capacities noted in this study were much stronger than those of the extracts prepared from rye or wheat as well as triticale caryopses and embryos (Amarowicz *et al.* 2002a, b; Karamać *et al.* 2002, 2004). The radical scavenging activities of the barley crude extract and its fraction are comparable to the phenolic fractions separated *via* Sephadex LH-20 column chromatography from leguminous seeds (Amarowicz *et al.* 1996), rapeseed (Amarowicz *et al.* 2003), and almond (Amarowicz *et al.* 2005).

Figure 6 depicts the reducing powers of phenolic compounds present in the barley crude extract and its fractions. Data so obtained at a 0.5-mg dose indicate the magnitude of the reducing power in descending order: fraction VII > fraction VI >> fraction V > fraction II ≈ fraction III ≈ crude extract ≈ fraction IV > fraction I. In previous studies by Amarowicz and co-workers (Amarowicz *et al.* 2002a, b; Karamać *et al.* 2002, 2004), the reducing powers of extracts prepared from wheat, rye as well as triticale caryopses and embryos were markedly less potent than those of the extract and its fractions as described in this work.

The results of correlation analyses between the total phenolics content, antioxidant and antiradical

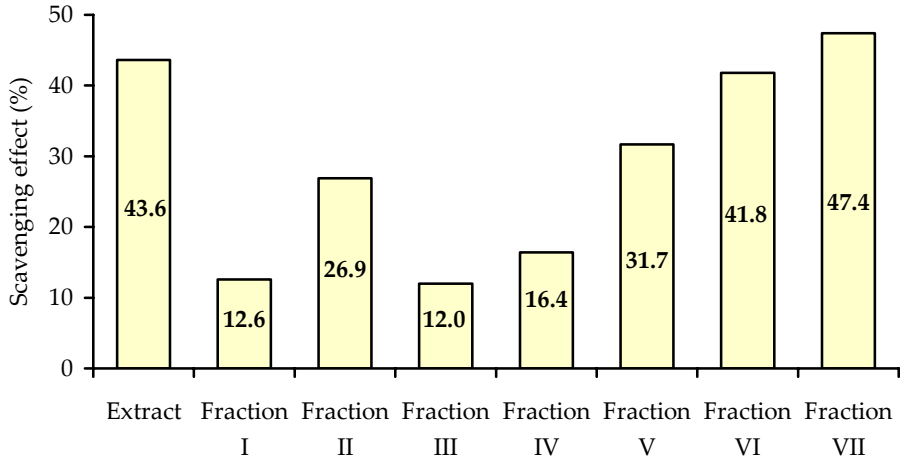

Figure 5. Scavenging effects (%) of a barley crude extract and its fractions on DPPH• radical, as measured by changes in absorbance values at 517 nm; 0.5 mg of the crude extract or its fractions was used per assay

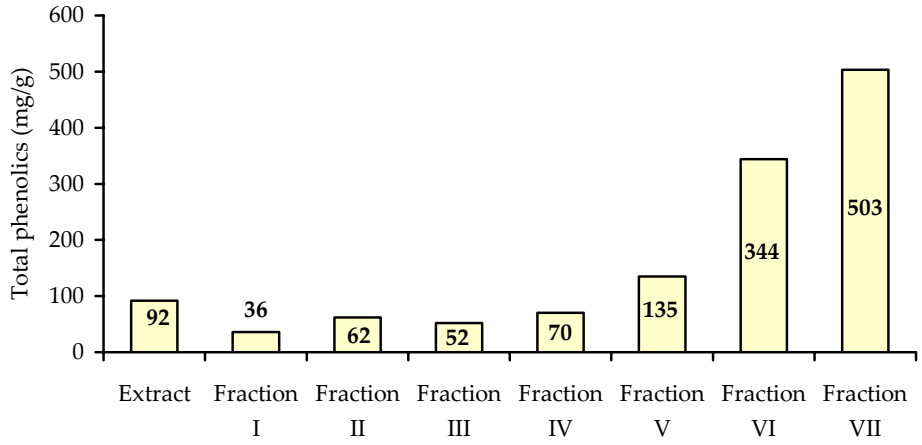

Figure 6. Reducing powers of a barley crude extract and its fractions as reported by optical density readings at 700 nm; 0.5 mg of the crude extract or its fractions was used per assay

activities and reducing powers are depicted in Figure 7. Statistically significant ($P \leq 0.01$) correlation was found with total phenolics versus antiradical activity and reducing power. Using a 7-point correlation between total phenolics and antioxidant activity, the data were significant at $P \leq 0.05$. With the rejection of the data obtained with fraction II from the correlation analysis, the calculated R^2 value increased from 0.4568 to 0.9883. In the case of leguminous seeds extracts, a statistically significant ($P \leq 0.01$) correlation was determined for total phenolics versus total antioxidant activ-

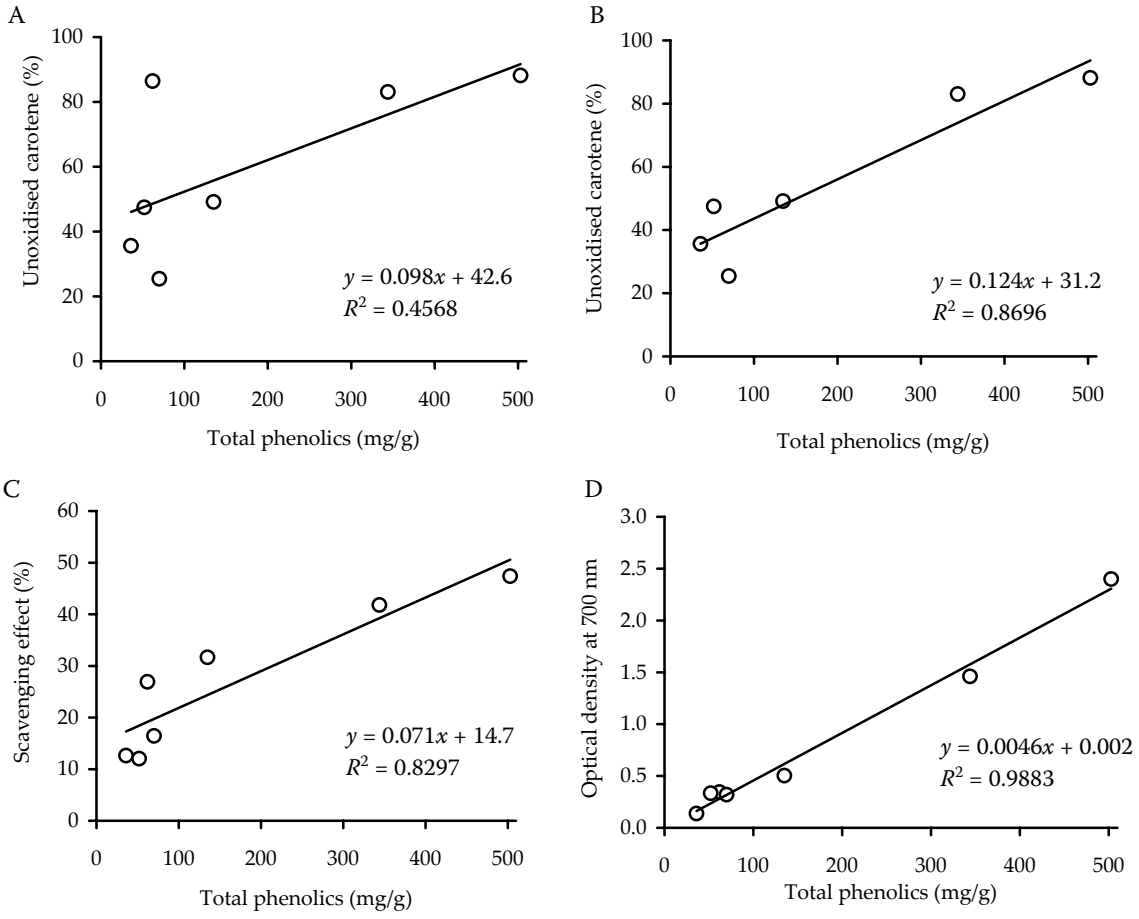

Figure 7. Correlations between the total phenolics content and observed antioxidant activity from the β-carotene-linoleate model system (A and B; in A correlation was calculated for 7 points, in B one point was omitted), antiradical activity against DPPH radical (C), and reducing power (D)

ity (TAA) (Amarowicz *et al.* 2004a). The strong correlation between the content of total phenolics and the reducing power was found in the extracts of selected plant species from the Canadian prairies as reported by Amarowicz *et al.* (2004b). Velioglu *et al.* (1998) examined 28 plant products and found a significant relationship between the total antioxidant activity and total phenolics in flaxseed and cereal products.

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