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ANTIOXIDANT CAPACITY OF ROASTED HEALTH-PROMOTING PRODUCTS

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Key words: antioxidant capacity, antiradical activity, reducing power, phenolics, roasted products

Roasted sesame seeds, pumpkin seeds, sunflower seeds, soy beans, and wheat germs were obtained from *Ekoprodukt* company (Częstochowa, Poland). Phenolic compounds were extracted with 80% aqueous methanol. The extract of roasted sunflower seeds was characterised by a higher content of total phenolics (158 mg/g). The Total Antioxidant Capacity was the highest in the extract of roasted sunflower seeds (0.478 mmol Trolox/g) followed by the extract of roasted wheat germs (0.066 mmol Trolox/g). The extracts of roasted sunflower seeds and wheat germs were strong scavengers of DPPH radical. Their reducing power was strong as well.

INTRODUCTION

Antioxidants are compounds that inhibit or delay the oxidation of molecules by inhibiting the initiation or propagation of oxidizing chain reaction [Velioglu *et al.,* 1998; Shahidi & Ho, 2005]. Natural antioxidants are often present in plant material in combinations involving a number of different compounds. The mode of action of natural antioxidants involves multiple mechanisms of action, depending on the type and source of the material used [Shahidi, 2000]. Many of the natural antioxidant compounds exhibit a wide range of such biological effects as antibacterial, antiviral, anti-inflammatory and antiallergic [Cook & Samman, 1996]. Phenolics of plant origin provide protection against harmful free-radicals and have been known to reduce the risk of certain types of cancer, coronary heart disease (CHD), cardiovascular disease (CVD), stroke, atherosclerosis, osteoporosis, inflammation, and other neurodegenerative diseases associated with oxidative stress [Hertog *et al*., 1993; Ness & Powles, 1997; Joseph *et al*., 1999; Mazza *et al*., 1999; Surh, 2003]

Roasted plant seeds possess very attractive sensory properties and can be a valuable source of natural antioxidants in a human diet. Therefore, the objective of this study was to investigate antioxidant capacity of several roasted health products from the Polish market.

MATERIAL AND METHODS

Chemicals. All solvents used were of analytical grade. Methanol, potassium persulfate, potassium ferricyanide, ferric chloride and trichloroacetic acid were acquired from the P.O.Ch. Company (Gliwice, Poland). Folin & Ciocalteau's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'azino-bis-(3-ethylbenzthizoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8,-tetramethyl-chroman-2--carboxylic acid (Trolox), and catechin were obtained from Sigma Ltd. (Poznań, Poland).

Material. Roasted sesame seeds, pumpkin seeds, sunflower seeds, soy beans, and wheat germs were obtained from *Ekoprodukt* company (Częstochowa, Poland).

Fat extraction. Ground samples were heated with hexane (1:10, w/v) under reflux for 30 min, and then filtrated. Hexane with extracted oil was collected. The process was repeated twice more with a new portion of hexane. Extracted oil after hexane evaporation was weighed. Finally, the defatted extract was dried for few h at room temperature.

Preparation of extracts. Defatted material was transferred to dark-coloured flasks, extracted using 100 mL of 80:20, v/v methanol/water at a material-to-solvent ratio of 1:8 (w/v), and subsequently placed in a shaking bath at 70°C for 15 min [Amarowicz *et al*., 1995a]. The resulting slurry was filtrated. The residue was re-extracted twice under the same conditions, and filtrates were combined. Then, the combined solvent was removed under vacuum at 40°C and the remaining water solution was lyophilised.

Determination of total phenolics. The content of total phenolics in extracts was determined using the Folin & Ciocalteau's phenol reagent [Naczk & Shahidi, 1989]. (+)-Catechin was used as a standard for sesame seeds, pumpkin seeds and soy beans and ferulic acid for sunflower seeds and wheat germs.

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UV spectra. UV spectra of phenolic compounds present in the extracts were recorded in methanol using a Beckman DU 7500 diode array spectrophotometer.

Determination of Total Antioxidant Capacity (TAC). The TAC in extracts and in raw material was determined according to the Trolox equivalent antioxidant activity (TEAC) assay described by Re *et al.* [1999]. The TAC was expressed as mmol Trolox equivalents (TE) per gram of extract, g of material, and g of defatted material.

Determination of DPPH radical scavenging activity. The method described by Amarowicz *et al*. [2002] was used to assess the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts. Briefly, an aliquot (0.1 mL) of a methanolic solution containing 0.04 to 4.0 mg of extracts was mixed with 2 mL of methanol and then a methanolic solution of DPPH radical (1 mol/L, 0.250 mL) was added. The mixture was vortexed for 15 sec and then left to stand at room temperature for 20 min. Finally, the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Results were expressed as a plot of content of the extract content (mg/assay) *vs* absorbance at 517 nm.

Determination of reducing power. The reducing power of the extracts was determined as described by Oyaizu [1986]. Briefly, a suspension of each extract (0.2–5.0 mg) in 1 mL of distilled water was mixed with 2.5 mL of a 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. After incubation in a water bath at 50°C for 20 min, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 1750 *g* for 10 min. Following this, a 2.5-mL of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride. Finally, absorbance of the reaction mixture was measured spectrophotometrically at 700 nm; increased absorbance of the reaction mixture indicates greater reducing power. Results were expressed as a plot of the extract content (mg/assay) *vs* absorbance at 700 nm.

Statistical analysis. Each extract was considered as a "treatment". All measurements were replicated three times for each treatment and their means are reported.

RESULTS AND DICUSSION

The contents of total phenolics in the extracts and in products are presented in Table 1. The extract of roasted sunflower seeds was characterised by a higher content of total phenolics (158 mg/g). This product exhibited the highest content of total phenolics expressed in relation to crude and defatted product. The content of total phenolics in other extracts was much lower and ranged from 10.4 (pumpkin seeds) to 19.6 mg/g of extracts (soy beans). The high content of phenolic compounds is typical of oil seeds and was reported by Amarowicz *et al.* [1995b] as well as Schmidt & Pokorný [2005]. The content of total phenolics in wheat germs was higher than that reported by Amarowicz *et al.* [2002] and Karamać *et al.* [2002; 2004] for the extracts of phenolic compounds obtained from the embryos of wheat, triticale, and rye. The content of total phenolics in the extract of white sesame seeds accounted for 10.6 mg/g [Shahidi & Liyana-Pathirana, 2005], whereas in sunflower seeds and wheat germs for 16.0 and 3.49 mg/g of product, respectively [Velioglu *et al.*, 1998].

TABLE 1. Content of total phenolics in extracts and products.

Product	Total phenolics in		
	mg/g of extract	mg/g of product	mg/g of defatted product
Roasted sesame seeds	15.9	0.58	1.17
Roasted pumpkins seeds	10.4	0.39	0.91
Roasted soy beans	19.6	0.51	2.51
Roasted sunflower seeds	158.0	14.8	27.4
Roasted wheat germs	17.3	0.43	4.79

Figure 1 depicts UV spectra of phenolic compounds extracted from the investigated material. The different maxima recorded for the extracts (Table 2) show that phenolic compounds present in the extracts belong to different classes. The UV spectrum of extract of sunflower seeds is very similar to that of ferulic acid [Amarowicz & Weidner, 2001]. The presence of phenolic acids in the extract of wheat germs confirms a strong absorption bound at a wavelength of 320 nm. Similar UV spectra of wheat embryo extract were recorded by Amarowicz *et al.* [2002]. UV spectra of the extracts of sesame and pumpkin seeds and soy bean were characterised by maxima at a shorter wavelength (Table 2), which indicates the absence of phenolic acids and flavonols in these extracts.

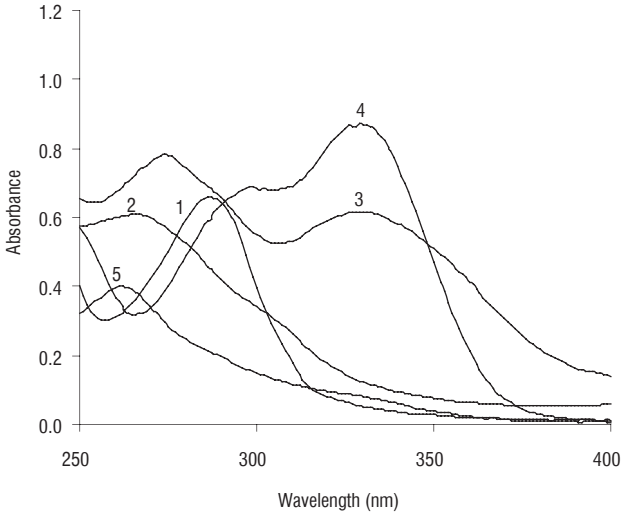

FIGURE 1. UV spectra of the extracts; 1 – roasted sesame seeds, 2 – roasted pumpkin seeds, 3 – roasted wheat germs, 4 – roasted sunflower seeds, and 5 – roasted soy beans.

TABLE 2. UV spectral data of phenolic compounds present in extracts.

Product	λ_{max} (nm)
Roasted sesame seeds	286
Roasted pumpkins seeds	267
Roasted soy beans	262
Roasted sunflower seeds	299, 329
Roasted wheat germs	274, 328

TABLE 3. Total antioxidant capacity of the extracts and products.

Product	TAC in		
	mmol Trolox/g of extract	mmol Trolox/g of product	mmol Trolox/g of defatted product
Roasted sesame seeds	0.048	0.002	0.004
Roasted pumpkins seeds	0.045	0.002	0.004
Roasted soy beans	0.059	0.002	0.008
Roasted sunflower seeds	0.478	0.043	0.083
Roasted wheat germs	0.066	0.002	0.018

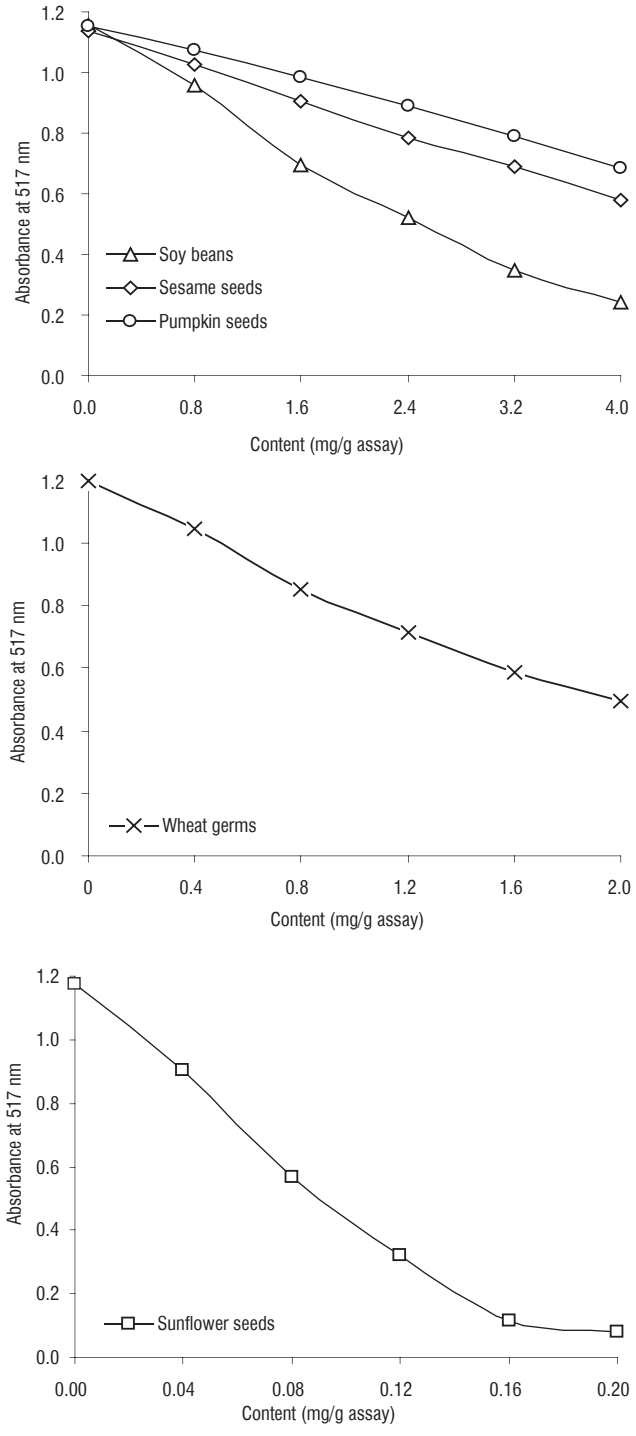

FIGURE 2. Scavenging effect of the extracts on DPPH radical.

Total Antioxidant Capacity of the extracts and products is shown in Table 3. The TAC was the highest in the extract of roasted sunflower seeds (0.478 mmol Trolox/g) followed by the extract of roasted wheat germs (0.066 mmol Trolox/g). Roasted sunflower seeds exhibited the highest antioxidant capacity expressed in relation to the mass of the product (0.043 mmol Trolox/g of product). Zieliński & Kozłowska [2000] reported the values of TAC to range from 0.054 to 0.222 mmol Trolox/g for methanolic extracts of wheat, barley, rye and oat. The extracts of leguminous seeds were characterised by TAC ranging from 0.30 (pea) to 1.76 mmol Trolox/g (adzuki bean) [Amarowicz *et al.* 2004]. TAC of white sesame seed extract accounted for 0.005 mmol Trolox/g [Shahidi & Liyana-Pathirana, 2005].

The radical-scavenging activity (RSA) of the extracts prepared was examined using the free radical, DPPH·. A freshly prepared DPPH· solution exhibits a deep purple colour with an absorption maximum at 517 nm. This colour disappears when an antioxidant is added to assayed solution. Figure 2 depicts the concentration-dependent response curves for the RSA of the extracts; results are expressed as the decrease in absorbance of the DPPH· solution at 517 nm. The RSA of the extract from roasted sunflower seeds, which contained the greatest amount of total phenolics, was the highest and

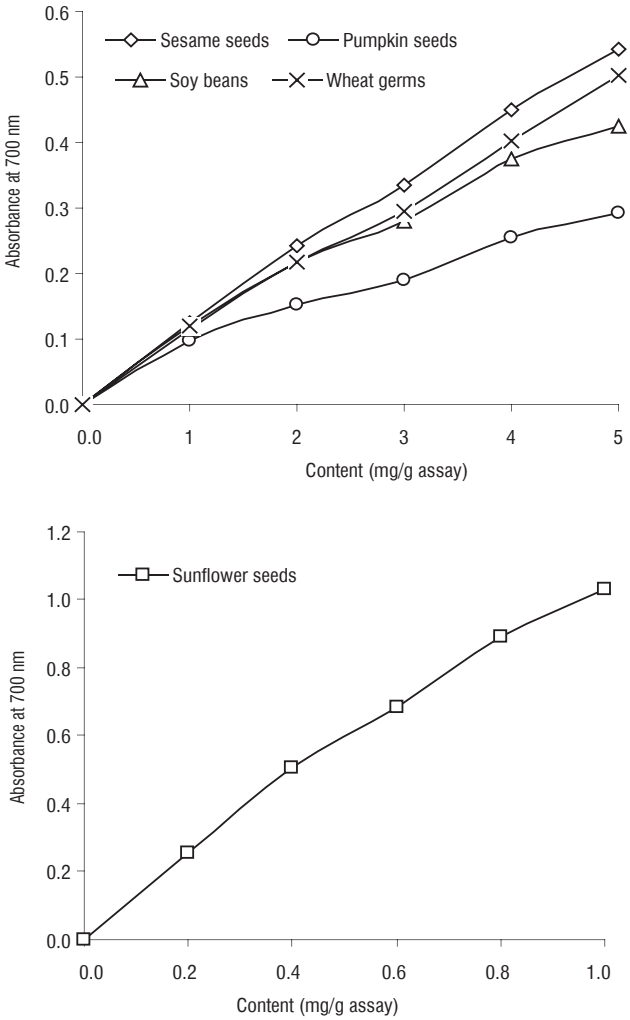

FIGURE 3. Reducing power of the extracts.

thus far superior to any of the other extracts investigated. The RSA of the other examined extracts was in the following order: wheat germs > pumpkin seeds > sesame seeds > soy beans. The antiradical activity of sunflower seeds extract was similar or higher than that reported for of the extracts of beach pea, canola hulls, evening primrose, faba bean, and almonds [Amarowicz *et al.*, 2000; 2005; Siriwardhana & Shahidi, 2002]. The RSA of the wheat germ extract was a bit stronger than that of wheat, triticale, and rye embryo extracts [Amarowicz *et al.,* 2002; Karamać *et al.,* 2002, 2004].

Figure 3 depicts the reducing powers of the extracts examined. In this assay, the presence of reductants (*i.e.* antioxidants) in the extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, it can be monitored by measuring the formation of Perl's Prussian blue coloration having maximum absorbance at 700 nm. The highest reducing power was noted for sunflower seeds extract. Other extracts exhibited 10–15 times lower reducing properties. The weakest reducing power was noted for the extract of pumpkin seeds. The reducing power of the extract of wheat germs was stronger than that of the wheat, triticale, and rye embryo extracts [Amarowicz *et al.,* 2002; Karamać *et al.,* 2002; 2004]. The reducing power of sunflower seeds was similar to the results measured for plant extracts rich in polyphenolics [Amarowicz *et al.*, 2000; 2005].

CONLUSIONS

The roasted products examined were characterised by a different content of total phenolics. Therefore their antioxidant activity was differentiated. The highest content of total phenolics was observed for extracts of roasted sunflower seeds and roasted wheat germs. The extracts of both these products exhibited the highest values of the Toatal Antioxidant Capacity. Their antiradical activity against DPPH radical and reducing power were also the highest.

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POJEMNOŚĆ PRZECIWUTLENIAJĄCA PRAŻONYCH PRODUKTÓW PROZDROWOTNYCH

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Ekstrakty związków fenolowych uzyskano z prażonych nasion sezamu, dyni, słonecznika, soi oraz zarodków pszenicy – analizowany materiał pochodził z firmy Ekoprodukt. Związki fenolowe ekstrahowano 80% metanolem. Ekstrakt z prażonych nasion słonecznika charakteryzował się najwyższą zawartością fenoli ogółem (158 mg/g). Całkowita Pojemność Przeciwutleniająca ekstraktu z nasion słonecznika i zarodków pszenicy były najwyższe – odpowiednio 0.478 i 0.066 mmol Trolox/g. Obydwa wyżej wymienione ekstrakty były silnymi zmiataczami wolnego rodnika DPPH. Ich zdolność redukcyjna była również wysoka.