Original article

Comparison of composition and antioxidant capacity of some cereals and pseudocereals

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Summary Polyphenols, phenolic acids, fibres and antioxidant capacity were determined in water, acetone and methanol extracts of buckwheat, rice, soybean, quinoa and 3 amaranth cultivars. Their antioxidant activities were comparatively assessed by total radical-trapping antioxidative potential (TRAP), ferric ion-reducing antioxidant power (FRAP), cupric-reducing antioxidant capacity (CUPRAC) and nitric oxide ($NO^{\bullet}$) assays, which comprised of contributions from polyphenols and phenolic acids (especially from the most abundant ferulic acid). The correlation coefficients between polyphenols and antioxidant activities of cereal and pseudocereal methanol extracts with FRAP, $NO^{\bullet}$, CUPRAC and TRAP were 0.99, 0.97, 0.96 and 0.77, respectively. The weakest correlation was with dietary fibres, an average one exhibited with tannins and marked correlation was shown with the phenolics. All the applied methods have shown that pseudocereals have higher antioxidant activity than some cereals (*rice and buckwheat*) and can be successfully replaced by cereals in case of allergy.

Keywords Antioxidant potentials, cereals, polyphenols, pseudocereals.

Introduction

Coronary heart disease is the leading cause of death in most developed nations and is rapidly increasing in prevalence in developing countries. Proper diets, which include fruits, vegetables, legumes, whole grain cereals and pseudocereals may be an independent contributor to cardiovascular protective effect (Anderson *et al*., 2000; Truswell, 2002; Czerwinski *et al*., 2004). It was shown that foods rich in dietary fibre tend to be a rich source of vitamins, minerals, phytochemicals, natural antioxidants and other micronutrients (Anderson *et al*., 2000). Among these foods, cereals and pseudocereals play an important role (Baublis *et al*., 2000; Steadman *et al*., 2001; Decker *et al*., 2002; Yu *et al*., 2002). So, in Norwegian, Mediterranean and Korean diets, cereals contribute 11.7% of the total intake of plant antioxidants (Lee *et al*., 2001; Simopoulos, 2001; Halvorsen *et al*., 2002). However, more than 2% of the adult population of the developed countries suffers from IgE-mediated hypersensitivity reactions after ingestion of foods, including wheat products (Simonato *et al*., 2001; Tsuji *et al*., 2001). Cereals are a typical example of foods whose consumption could cause gastrointestinal symptoms (Simonato *et al*., 2001). Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and others, have restricted use in food as they are suspected to be carcinogenic (Diaz & Cabanillas, 2004). Therefore, investigations of allergy-

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free pseudocereals were intensified (Czerwinski *et al.,* 2004).

Some cereals (rice, maize and others) and pseudocereals (amaranth, buckwheat and quinoa), rich in proteins, do not contain gluten, which is the main cause of celiac disease. Recent findings suggest that Western diets based on highly palatable foods are likely to be much less satiating than more ethnic foods or those typical of less-developed countries. In particular, some alternative crops (e.g. buckwheat, oat, barley, spelt, rye, quinoa, amaranth) seem to be of great nutritional interest and to represent important recipes for healthier and typical regional foods (Berti *et al*., 2005). Therefore, in the last decade, the use of pseudocereals was increased not only in special diets for people allergic to cereals, but also in healthy diets.

It has been shown that pseudocereals contain high concentration (13.2–18.2%) of protein and a good balanced amino acid composition (Gorinstein *et al*., 2004b). Some research has been conducted in the functionalities and properties of buckwheat proteins, flavonoids, flavones, phytosterols, thiamin-binding proteins, and other rare compounds in buckwheat seeds. Buckwheat proteins have unique amino acid composition with special biological activities of cholesterol-lowering effects, antihypertension effects, and improving the constipation and obesity conditions by acting similar to dietary fibre and interrupting the *in vivo* metabolisms (Quettier-Deleu *et al*., 2000; Steadman *et al*., 2001; Holasova *et al*., 2002).

However, no detailed data were published about the antioxidant potential of the cereals and pseudocereals. In order to determine if pseudocereals could be a good substitute for cereals in allergic persons, it was decided to compare allergy-free pseudocereals: quinoa, buckwheat and three cultivars of amaranth (*cruentus*, *hybridus* and *hypochondriacus*) with cereal (rice) and legume (soybean) on the basis of their antioxidant status. Therefore, in this investigation, the antioxidant activities of the studied samples were determined by total radical-trapping antioxidative potential (TRAP), ferric ion-reducing antioxidant power (FRAP), cupric-reducing antioxidant capacity (CUPRAC) and nitric oxide ($NO^{\bullet}$) assays. As far as we know, no results of such investigations are published to date.

Materials and methods

Chemicals

6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox); BHT; BHA; caffeic, ferulic and *p*-coumaric acids; Griess reagent; sodium nitroprusside, Folin-Ciocalteu reagent, α-amylase A-3306, protease P-3910 and amyloglucosidase A-9913; 2,4,6-tripyridyl-*s*-triazine (TPTZ); $FeCl_3x6H_2O$; ammonium acetate, $CuCl_2x2H_2O$ and neocuproine (2,9-dimethyl-1, 10-phenanthroline), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,2-azo-bis-2-amidinopropane hydrochloride (ABAP) was delivered by Polyscience, Warington, PA, USA. All reagents were of analytical grade.

Samples

Common buckwheat [(*Fagopyrum esculentum Mnch*. Polygonaceae, Peru), rice (*Oryzea sativa*, Brazil), soybean (*Glycine max L. Merr*. Fabaceae, low oil content, Brazil), quinoa (*Chenopodium quinoa Wild* Chenopodiaceae, Peru), and three cultivars of amaranth (*Amaranthus cruentus,* Amaranthaceae, USA; *Amaranthus hybridum,* Amaranthaceae, Pakistan; *Amaranthus hypochondriacus*, Amaranthaceae, Mexico)] were used. The samples were ground on a mill through a 60-mesh screen, defatted in a Soxhlet extractor with *n*-hexane for 10 h, and were then stored at 5 °C after the removal of hexane.

Unconjugated plus conjugated ('total') polyphenols (TP) and tannins were extracted from defatted samples (50 mg) with 5 mL of 1.2-M HCl in 40% methanol/water and heated at 90 °C for 3 h (Vinson *et al*., 2001). Polyphenols were also extracted in the same proportion of the solvent to the sample with water (PolyphW) and with acetone/water [(70:30 v/v) (PolyphAC)] (Perez-Jimenez & Saura-Calixto, 2005).

After centrifugation, each of the supernatant was analysed separately for total polyphenols and tannins.

Determination of the total polyphenol content

Total polyphenols were determined by Folin-Ciocalteu method and measured at 765 nm. The results are given in μg of gallic acid equivalents (GAE) per g dry weight (DW) (Singleton *et al.,* 1999). Tannins were estimated at 500 nm after reaction with vanillin-HCl. The values were expressed as per cent catechin equivalents.

Individual antioxidants

Phenolic (Gorinstein *et al*., 2004a) acids were determined by high-performance liquid chromatography (HPLC) with injection volume of 20 μL, using a Pye Unicam PU 4002-Video Liquid Chromatograph with a Spherisorb 5 ODS column (250 × 4.6 mm), using two solvents: A – 5-mM citric acid + 5-mM sodium dihydrogen orthophosphate + 0.3-mM caprylic acid (adjusted to pH 2.0 by phosphoric acid) and B – 80% (v/v) methanol. Elution conditions were as follows: flow rate 0.5 mL min^{-1}, linear gradient from 10% to 35% B for 70 min, then from 35% to 50% B for 15 min, from 50% to 100% B for 5 min and finally for 5 min 100% B and 5 min from 100% to 10% B. The column eluate was monitored at 260 and 300 nm using a Multichannel detector PU 4021.

Dietary fibre determination

The samples were treated (Mañas *et al*., 1994; Gorinstein *et al*., 2004a) with heat-stable α-amylase, protease and amyloglucosidase, followed by centrifugation (15 min, 3000 × ***g***) to separate the soluble and insoluble fractions and dialysis against water, which substituted ethanol precipitation of soluble dietary fibre (SDF).

Determination of the total antioxidant potentials

Four different scavenging tests were used:

1. Total radical-trapping antioxidative potential

The reaction was initiated by mixing 475-μL phosphate-buffered saline, 50-μL 10-mM luminol in 100-mM borate buffer (pH 10), and 50-μL ABAP. Then 20 μL of water and acetone plant extracts were added directly into the cuvette, and the samples were measured for another period of time (τ) until a 50% recovery of the original steady state chemiluminescence (CL) signal. The TRAP value measured on the luminometer Bio-Orbit 1251 (BioOrbit, Finland) for 15 min. During this period of time, a steady state of the CL signal was reached and was obtained from the equation: TRAP = 2.0 [trolox]. $\tau_{sample}/f \,.\tau_{\ trolox}$, where f is the dilution of sample measured.

The results obtained are expressed as nM of peroxyl radicals trapped by 1 mL of sample (Slavíková *et al*., 1998).

2. Scavenging activity against nitric oxide ($NO^{\bullet}$ test)

0.5-mL portion of a mixture (0.4 mL of extract and 0.1 mL of sodium nitroprusside solution) was diluted with 0.3 mL of Griess reagent. The absorbance of the chromophore formed during the diazotation of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent (Marcocci *et al*., 1994).

3. Ferric-reducing/antioxidant power

This assay measures the ability of the antioxidants contained in the plant samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against μM Trolox equivalent (TE) per g (Benzie & Strain, 1996).

4. Cupric reducing antioxidant capacity (CUPRAC)

This assay is based on utilising the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidising agent. To the mixture of 1 mL of Cu (II), Nc and NH_4Ac buffer solution, antioxidant sample (or standard) solution (x mL) and H_2O [(1.1 - x) mL] were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank (Apak *et al*., 2004).

The antioxidant activities for all samples and standards (Trolox, BHA, BTH, caffeic, ferulic and *p*-coumaric acids) were determined by the aforementioned methods.

Statistical analysis

Values are given as the means ± SD of five measurements. Where appropriate, data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA, USA, followed by Duncan's new multiple range test to assess the differences among groups means. Spearman correlation coefficient (R) and p-value were used to show correlations and their significance. Differences of $P < 0.05$ were considered significant.

Results

Dietary fibres, polyphenols, tannins and phenolic acids

The results of the determination of soluble, insoluble and total dietary fibre and tannins are summarised in the Table 1. Our data are in correspondence with others (Mañas *et al*., 1994; Steadman *et al*., 2001; Truswell, 2002), showing the highest percentage of total dietary fibre in buckwheat (2.45%) and the lowest in *A. cruentus* (1.30%). The amount of the phenols and tannins varied between the samples, with the highest one in buckwheat (Tables 1 and 2).

Table 1 Dietary fibres and tannins [% dry weight (DW)] in some of the cereals and pseudocereals studied

Plants	IDF	SDF	TDF	Tannin
Buckwheat	2.04 ± 0.23^{a}	0.40 ± 0.09^{b}	2.45 ± 0.21^{a}	0.322 ± 0.08^{a}
Soybean	1.61 ± 0.12^{b}	0.54 ± 0.23^{a}	2.15 ± 0.16^{b}	0.034 ± 0.05^{e}
A. hybridus	1.21 ± 0.16^{c}	0.37 ± 0.06^{b}	1.58 ± 0.14^{d}	0.087 ± 0.14^{c}
A. hypochondriacus	1.08 ± 0.17^{d}	0.34 ± 0.08^{b}	1.42 ± 0.11^{e}	0.060 ± 0.11^{d}
A. cruentus	1.02 ± 0.21^{d}	0.28 ± 0.09^{c}	1.30 ± 0.31^{e}	0.120 ± 0.04^{b}
Quinoa	1.39 ± 0.14 ^{c}	0.39 ± 0.02^{b}	1.78 ± 0.18^{c}	0.051 ± 0.01^{d}
Rice	1.28 ± 0.18 ^{c}	0.31 ± 0.06^{c}	1.59 ± 0.13^{d}	0.035 ± 0.01^{e}

Data are means (M) ± standard deviations (SD) of five measurements.
Values in columns with different superscript letters are significantly different ($P < 0.05$).
A., amaranthus; DW, dry weight; IDF, SDF, TDF, insoluble, soluble and total dietary fibres.

Table 2 Polyphenols and antioxidant activities of different extracts in the studied cereals and pseudocereals

Plants	PolyphAC (% DW)	PolyphW (% DW)	TRAPW (nM mL^{-1})	TRAPAC (nM mL^{-1})	$NO^{\bullet}$ (%)	FRAP (μM TE g^{-1})	CUPRAC, μMTEg^{-1}
Buckwheat	0.210 ± 0.04[a]	0.110±0.01[a]	2561 ± 102[a]	1213 ± 65.1[a]	85.0 ± 7.1[a]	3.35 ± 0.7[a]	9.14±2.8[a]
Soybean	0.022 ± 0.001[c]	0.012 ± 0.001[c]	561 ± 35.8[c]	224 ± 13.1[c]	38.5 ± 3.2[b]	2.44 ± 0.4[b]	4.49±1.1[b]
A. hyb	0.03 ± 0.001[b]	0.017 ± 0.001[b]	388 ± 23.1[d]	309 ± 21.7[b]	23.0 ± 1.9[c]	1.96 ± 0.3[c]	3.11±0.7[d]
A. hyp	0.027 ± 0.001[b]	0.011 ± 0.001[c]	373 ± 23.1[d]	288 ± 17.1[b]	23.0 ± 1.9[c]	1.96 ± 0.3[c]	3.25±0.8[d]
A. cru	0.034 ± 0.002[b]	0.018 ± 0.001[b]	384 ± 23.5[d]	345 ± 22.9[b]	25.0 ± 1.9[c]	1.99 ± 0.3[c]	3.94±0.8[c]
Quinoa	0.022 ± 0.001[c]	0.019 ± 0.001[b]	1686 ± 81.3[b]	251 ± 13.9[c]	32.0 ± 3.0[b]	2.30 ± 0.4[b]	4.96±1.1[b]
Rice	0.016 ± 0.001[d]	0.009 ± 0.001[d]	301 ± 31.3[e]	183 ± 13.9[d]	20.0 ± 1.7[d]	1.78 ± 0.3[d]	3.21±0.7[d]

Data are means (M) ± standard deviations (SD) of five measurements.
Values in columns with different superscript letters are significantly different ($P < 0.05$).
A., Amaranthus; *A. hyb., A. hybridus*; *A. hyp., A. hypochondriacus*; *A. cru, A. cruentus*; PolyphW, polyphenols extracted with water (hydrophilic); PolyphAC, polyphenols extracted with acetone (lipophilic); DW, dry weight; TRAPW, total radical-trapping antioxidative potential in water extract; TRAPAC, total radical-trapping antioxidative potential in acetone extract; $NO^{\bullet}$, scavenging activity against nitric oxide; FRAP, ferric ion-reducing antioxidant power; CUPRAC, cupric-reducing antioxidant capacity; polyphenols extracted with 40% methanol/water/ 0.2-M HCl (40% methane/HCl); μg GAE (gallic acid equivalent)g^{-1} DW (dry weight); TE, trolox equivalents.

Table 3 The contents of some phenolic acids in methanol extracts of cereal and pseudocereal samples (μg g^{-1} dry matter)

Samples	Ferulic acid	*p*-Coumaric acid	Caffeic acid
Buckwheat	1213.2 ± 99.1[a]	0.8 ± 0.1[d]	6.95 ± 0.5[b]
Rice	485.1 ± 43.2[b]	1.6 ± 0.3[a]	8.52 ± 0.7[a]
Soybean	224.8 ± 21.1[e]	0.5 ± 0.1[e]	5.21 ± 0.9[d]
Quinoa	251.5 ± 22.2[e]	1.1 ± 0.1[c]	6.31 ± 0.5[c]
Amaranthus cruentus	345.0 ± 27.2[c]	1.4 ± 0.1[b]	6.61 ± 0.7[c]
Amaranthus hybridus	309.8 ± 26.1[d]	1.2 ± 0.1[c]	6.41 ± 0.8[c]
Amaranthus hypochondriarus	288.5 ± 23.2[d]	1.2 ± 0.1[c]	6.49 ± 0.9[c]

Data are means (M) ± standard deviations (SD) of five measurements.
Values in columns with different superscript letters are significantly different ($P < 0.05$).

The phenolic acid content also was the highest in the buckwheat (Table 3); however, the phenolic acid content for all the studied samples of cereals and pseudocereals was estimated to be of the same order of their contents: ferulic acid > *p*-coumaric acid > caffeic acid. The same relation was found in 17 rye varieties by Andreasen *et al*. (2000).

Antioxidant potentials

TRAP values (nM mL^{-1}) were estimated in water and acetone extracts. There were no significant differences ($P > 0.05$) in the antioxidant values of TRAP in acetone extracts (Fig. 1a, Table 2) among samples 1, 2, 3, 4 and 5 (*A. cruentus* = 345, *A. hypochondriacus* = 288, *A. hybridus* = 309, quinoa = 251 and soybean = 224), although their antioxidant capacity increased from samples 1 to 5. Sample 6 (buckwheat = 1213) has markedly higher antioxidant capacity in comparison with samples 1–5. As can be seen, the antioxidant potential of the buckwheat sample is the highest among all the studied crops. Rice showed similar values as for the amaranth varieties. We also determined separately in buckwheat samples the contents of polyphenols in pericarps and in grains. It was found that the content of polyphenols in pericarps was ($P < 0.05$) significantly higher than in grains (0.208% and 0.143%) and TRAP values (nM mL^{-1}) were estimated as in water (6533 and 1487) and acetonic (2996 and 830) extract, respectively. These results were in accordance with others (Velioglu *et al*., 1998), showing that the polyphenols (mg $100g^{-1}$ DW) of buckwheat hulls and buckwheat seeds were 3900 and 726, respectively. The difference between the two parts of these plants was about four to five times higher. Also, other investigators found that buckwheat possesses a very high antioxidant capacity (Quettier-Deleu *et al*., 2000; Holasova *et al*., 2002). The water extracts [TPAP water (TRAPW)] showed different values than the acetone extracts (AC) (TRAPAC) and were approximately 2–2.5 times higher (Table 2, Fig. 2).

$NO^{\bullet}$ scavenging effects of methanol/HCl buckwheat (85%) and soybean (38.5%) extracts in concentration of 0.2 mg mL^{-1} were higher than those of the other extracts at the same concentration (Fig. 1b, Table 2).

Buckwheat was significantly higher ($P < 0.05$) than that of Trolox (66%) at the same concentration ($P < 0.05$). The $NO^{\bullet}$ scavenging effects of soybean and quinoa (Table 2, Fig. 1b) were nearly equal to that of BHA (40%). Rice and three varieties of amaranth (Table 2, Fig. 1b) exhibited modest scavenging effects and were equal to BHT (20%).

The best correlation (Fig. 2a) was between polyphenols in AC and TRAPAC ($R^2 = 0.9957$), following by tannins (Fig. 2b) and TRAPAC ($R^2 = 0.9695$), followed by polyphenols (Fig. 2c) in water extract

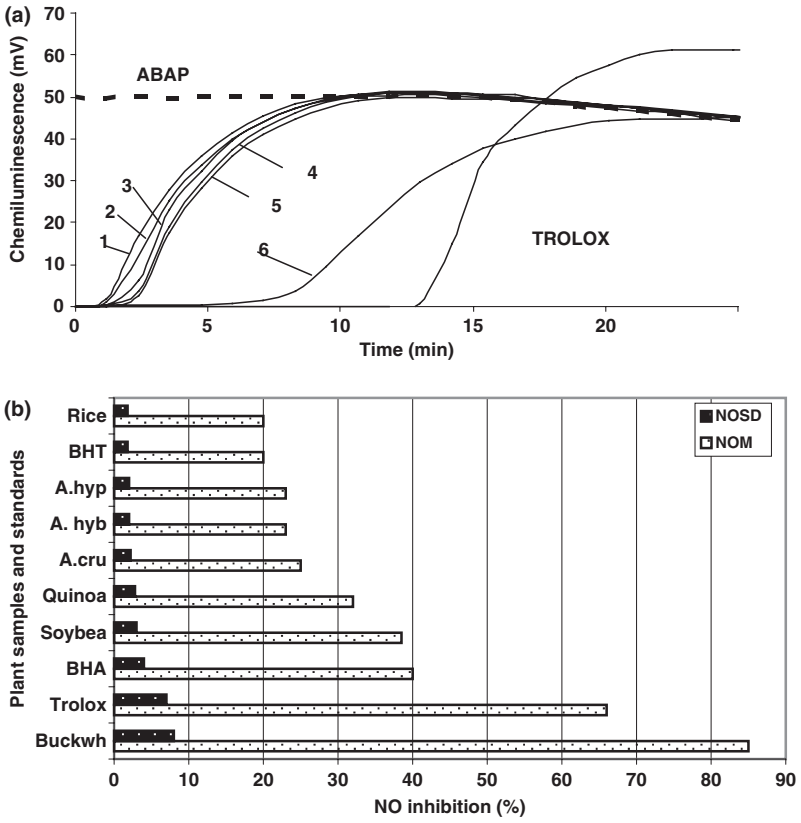

Figure 1 (a) Kinetics of ABAP-induced, luminol-enhanced CL in the presence of: 1, *Amaranthus cruentus*; 2, *Amaranthus hypochondriacus*; 3, *Amaranthus hybridus*; 4, quinoa; 5, soybean; and 6, buckwheat extracts, in addition to the reference antioxidant trolox. Dashed line represents control ABAP-induced CL. (b) $NO^{\bullet}$ scavenging effect (% inhibition) of plant extracts and standards. M ± SD (two horizontal bars for every studied variable). For comparison, trolox, BHA and BHT were used. CL, chemiluminescence; ABAP, 2-azo-bis-2-amidinopropane hydrochloride; trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.

and TRAPW (R^2 = 0.7433) and total dietary fibre (Fig. 2d) and TRAPW (R^2 = 0.568).

The highest correlation (Fig. 3a) was between polyphenols in methanol extracts and FRAP (R^2 = 0.988), followed by polyphenols and $NO^{\bullet}$ (R^2 = 0.9724), followed by polyphenols (Fig. 3b) and CUPRAC (R^2 = 0.9579) and then by polyphenols and TRAPAC (R^2 = 0.7682). The best correlation with all the four methods was achieved with FRAP. These data are in accordance with others, who claim that high total polyphenol content increases the total antioxidant activity (Kahkonen *et al*., 1999; Baublis *et al*., 2000; Halvorsen *et al*., 2002; Conforti *et al*., 2005).

Discussion

The present report is based on the evidence that polyphenols are the most abundant antioxidants in the diet and are widespread constituents of fruits, vegetables, cereals, dry legumes, chocolate and beverages, such as tea, coffee or wine (Scalbert *et al*., 2005). Experimental studies on animals or cultured human cell lines support a role of polyphenols from plants in the prevention of cardiovascular diseases (Anderson *et al*., 2000; Truswell, 2002). More human studies are needed to provide clear evidence of their health protective effects and to evaluate the risks possibly resulting from too high a polyphenol consumption (Scalbert *et al*., 2005). As was written in the Introduction, there is a need of pseudocereals, which could be a valuable substitute of cereals in allergic patients. This study was undertaken to investigate the free radical-scavenging and antioxidant activities of various structure-related phenols extracted from cereals and pseudocereals with different solvents in order to utilise these significant sources of natural antioxidants. Further characterisation of the phenolic composition is needed. Therefore, in this investigation comparisons were made among allergy-free pseudocereals – quinoa, buckwheat and three cultivars of amaranth with cereals (rice) and legume (soybean). Amaranth and soybean were closely similar in the distribution of protein fractions and their microscopic structure. As an addition to chemical analyses, microscopy (Gorinstein *et al*., 2004b) helped to understand and visualise the structural changes and textural differences in protein fractions. It was concluded that pseudocereals can be used as a nutritive substitute of some cereals in functional foods (Gorinstein *et al*., 2004b), but the present research is based on the comparison of the same plants on their antioxidant potentials.

It has been shown time and again that the bioactivity of individual compounds does not reflect the value of

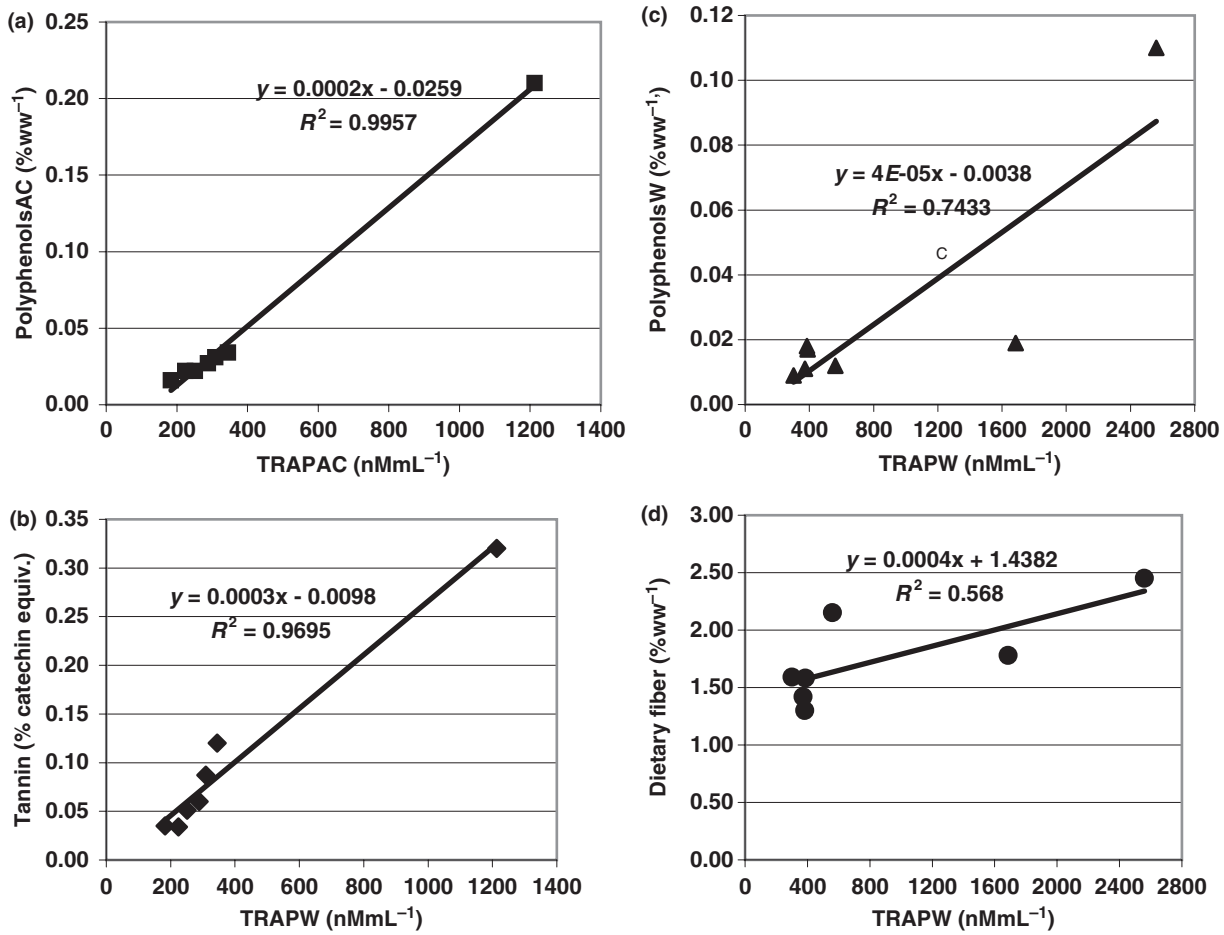

Figure 2 Correlation between: (a) ■ polyphenols and TRAPAC in acetone extract; (b) ▲ tannins and TRAPAC in acetone extract; (c) ◆ polyphenols and TRAPW in water extract; (d) • total dietary fibre and TRAPW in water extract. TRAPAC, total radical-trapping antioxidative potential in acetone extract; TRAPW, total radical-trapping antioxidative potential in water extract.

the total antioxidant potential of the studied samples (Ou *et al.,* 2002). In this investigation, four different antioxidant tests were used in order to compare and substitute each other, in case if not all of them are available for the investigation. The applied assays are based on different mechanisms: TRAP involves hydrogen atom transfer reactions, and FRAP, CUPRAC and Folin-Ciocalteu reagent represent electron-transfer reaction. The use of TRAP, which is generally employed to evaluate the antioxidant status of biological fluids, for cereal antioxidants, showed (Fig. 1a) that this method was based on the measurements of induction times in the oxidation of a lipid dispersion exposed to a free radical source (ABAP). The light profiles obtained after the addition of antioxidants (Fig. 1a) corresponded to other reports (Lissi *et al.*, 1995). The results of all applied methods were compared using the correlation coefficients between polyphenols and antioxidant activities (Fig. 3a, b), because these methods can be classified not only as hydrogen atom transfer and electron transfer reactions, but also TRAP user organic radical producer and FRAP and CUPRAC work with metal ions for oxidation. Another difference between these assays is the reaction procedure. TRAP (Fig. 1a) is based on the delay in oxidation and determines the lag phase as a parameter for the antioxidant activity (Fig. 1, lines 1–6). In contract, FRAP and CUPRAC analyse the ability to reduce the radical cation. Despite these differences, the results of these *in vitro* assays indicate that the obtained data might not be the same, but the relation between the polyphenols and antioxidants remains similar. Therefore, it is obvious to use more than two different methods for antioxidant determination. Our conclusions are in the same line with others (Schlesier *et al.*, 2002). The calculated data (Fig. 3a, b) showed that the best correlation was found with the FRAP method. Therefore, TRAP (Figs 1a and 2a) and FRAP (Fig. 3a) can be used, and could replace the other methods, such as $NO^{\bullet}$

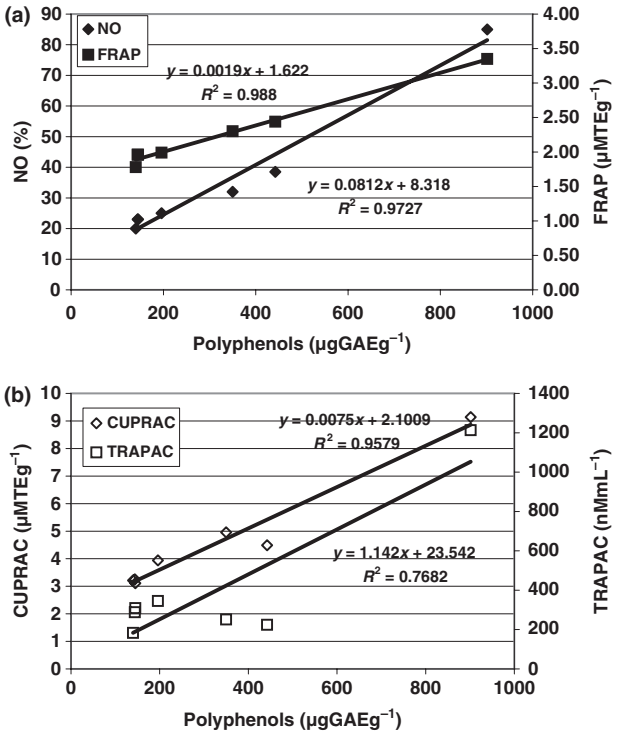

Figure 3 Relationship, calculated by linear regression analysis for plant extracts between: (a) ◆ Polyp in 40% Met/HCl (μgg^{-1} DW, *X*) to NO$^{\square}$ scavenging effects (%, Y_1) and ■ Polyp in 40% Met/HCl (μgg^{-1} DW, *X*) to FRAP (μMTE g^{-1}, Y_2). (b) ◇ Polyp in 40% Met/HCl (μgg^{-1} DW, *X*) to CUPRAC (μMTE g^{-1}, Y_1) and □ polyp in 40% Met/HCl (μgg^{-1} DW, *X*) to CUPRAC (μMTE g^{-1}, Y_1). and □ polyp in 40%Met/HCI (μgg^{-1} DW, *X*) to TRAPAC (μMml^{-1}, Y_2). Abbreviations: polyphenols expressed as μg gallic acid equivalent (GAE); TE, Trolox equivalent; TRAP, total radical-trapping antioxidative potential; NO$^{\bullet}$, scavenging activity against nitric oxide; FRAPAC, ferric-reducing/antioxidant power in acetone extract; CUPRAC, cupric reducing antioxidant capacity; Met, methanol.

and CUPRAC (Kahkonen *et al*., 1999; Baublis *et al*., 2000; Halvorsen *et al*., 2002; Schlesier *et al*., 2002; Conforti *et al*., 2005).

Some authors have studied the antioxidant compounds and the antioxidant activity of cereals, particularly in buckwheat, oat, rice and amaranth (Yu *et al.,* 2002; Conforti *et al.,* 2005). However, the aforementioned authors did not apply the antioxidant tests used by us. These antioxidant tests have shown that buckwheat and allergy-free quinoa possess the highest antioxidant potential among the studied cereals and pseudocereals, respectively. These data support the finding of others (Kahkonen *et al.,* 1999; Quettier-Deleu *et al*., 2000).

Our results are also in correspondence with others about the use of cereals, legumes (McConnell & van Dyke, 2000) as a source *in vitro* of dietary fibre and antioxidants (Anderson *et al*., 2000). The results of this report support the conclusions of Perez-Jimenez and Saura-Calixto (2005) that the most efficient antioxidant extraction was achieved by using successively acidic methanol/water (50:50 v/v, pH 2), followed by acetone extraction (70:30 v/v), and the antioxidant capacity of the plant extracts ranged from 1.1 to 4.4 μM TE g^{-1} DW. The hydrolysable phenolics have higher correlation with the antioxidant activity than those extracted with acetone and water. The extraction with methanol/HCl is based on the data of others (Hertog *et al*., 1992), showing that under acid hydrolysis with 2.0-M HC1 in boiling 50% aqueous methanol, flavonol 3-*O*-glucosides are hydrolysed completely within a few minutes, whereas complete hydrolysis of flavonol 3,7- and 4′-*O*-glucuronides takes 60–250 min. Optimisation of extraction and hydrolysis has shown that the highest yield was found using 1.2-M HC1 and a reaction period of 2 h. It appeared that extraction was most efficient with 50% aqueous methanol. The flavonoid glycosides were hydrolysed to their corresponding aglycons by refluxing in 1.2-M HCl containing 40% MeOH so as to exert maximal reducing power towards scavenging radicals used in the antioxidant assays. Increasing acid concentration and reaction time led to a significant degradation of quercetin; therefore, such extraction was suggested in this study for the extraction of total phenols (Hertog *et al*., 1992). In the present report, the percentage of methanol was decreased from 50% to 40%. The yield of extracted polyphenols was slightly decreased. These results are exactly in line with Hertog *et al.* (1992), reporting that the extraction efficiency could thus depend on the water/methanol ratio.

The calculated correlations in acetone and water extracts showed that the dietary fibre's contribution to the antioxidant potential of cereals and pseudocereals was minimal; of tannins, moderate; and of total phenols, decisive. The best correlation was between polyphenols in methanol in all the used scavenging assays. We did not find published data by others for such comparison, but in the study of Conforti *et al.* (2005), reports on the biological properties, antioxidant and antidiabetic, of the two varieties of *Amaranthus caudatus* seeds (Oscar blanco and Victor red), showing high bioability of this pseudocereal.

Hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic) were abundant in investigated plants. The antioxidant capacity of ferulic, *p*-coumaric and caffeic acids were as follows: in NO (% inhibition) as 70, 60 and 80, respectively; FRAP (μM TE g^{-1}) as 1.25, 1.09 and 1.19, respectively. Our data were supported by others, showing that much higher amounts of hydroxycinnamic acids are bound to the cell walls of buckwheat and Pa91 calli; ferulic and *p*-coumaric acids in maize, and ferulic and sinapic acids in buckwheat (Kahkonen *et al*., 1999; Andreasen *et al*., 2000; Quettier-Deleu *et al*., 2000; Holasova *et al*., 2002).

Phenolic acids from cereals and pseudocereals possess strong antioxidant activity *in vitro* and depend on their concentrations. They share some common structural features – phenolic nature and high molecular weight – with phenolic polymers found in black tea and red wine (Baublis *et al*., 2000). All the phenolic compounds, including proanthocyanidins and tannin-like compounds are strong antioxidants (Scalbert *et al*., 2005).

In the reviewed reports, it was shown that soybean seeds are an important source of dietary tocopherols, but like the seeds of other dicotyledonous plants, they contain relatively little alpha-tocopherol, the form with the greatest vitamin E activity (Britz & Kremer, 2002; Yoshida *et al*., 2006). In all probability, the obtained relatively high value of antioxidant activity of soybean in this report mostly relates to its polyphenol content (Takahashi *et al*., 2005; Ekor *et al*., 2006).

In conclusion: (i) water, acetone and methanol buckwheat extracts showed the highest contents of total polyphenols and the highest antioxidant potential, and water extract of quinoa revealed the highest antioxidant potential among the studied samples; (ii) therefore, quinoa and buckwheat could be a potential substitute of cereals in diets for allergic subjects.

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References

Anderson, J.W., Hanna, T.J., Peng, X.J. & Kryscio, R.J. (2000). Whole grain foods and heart disease risk. *Journal of the American College of Nutrition*, **19**, 291S–299S.

Andreasen, M.F., Christensen, L.P., Meyer, A.S. & Hansen, A. (2000). Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (*Secale cereale* L.) varieties. *Journal of Agricultural and Food Chemistry*, **48**, 2837–2842.

Apak, R., Guclu, K., Ozyurek, M. & Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, **52**, 7970–7981.

Baublis, A.J., Lu, C.R., Clydesdale, F.M. & Decker, E.A. (2000). Potential of wheat-based breakfast cereals as a source of dietary antioxidants. *Journal of the American College of Nutrition*, **19**, 308S–311S.

Benzie, I.F.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Analytical Biochemistry*, **239**, 70–76.

Berti, C., Riso, P., Brusamolino, A. & Porrini, M. (2005). Effect on appetite control of minor cereal and pseudocereal products. *British Journal of Nutrition*, **94**, 850–858.

Britz, S.J. & Kremer, D.F. (2002). Warm temperatures or drought during seed maturation increase free alpha-tocopherol in seeds of soybean (*Glycine max [L.] Merr*.). *Journal of Agricultural and Food Chemistry*, **50**, 6058–6063.

Conforti, F., Statti, G., Loizzo, M.R., Sacchetti, G., Poli, F. & Menichini, F. (2005). *In vitro* antioxidant effect and inhibition of α-amylase of two varieties of *Amaranthus caudatus* seeds. *Biological and Pharmaceutical Bulletin*, **28**, 1098–1102.

Czerwinski, J., Gorinstein, S., Bartnikowska, E., et al. (2004). Oat (*Avena sativa L.*) and amaranth (*Amaranthus hypochondriacus*) meals positively affect plasma lipid profile in rats fed cholesterol-containing diets. *Journal of Nutritional Biochemistry*, **15**, 622–629.

Decker, E., Beecher, G., Slavin, J., Miller, H.E. & Marquart, L. (2002). Whole grains as a source of antioxidants. *Cereal Foods World*, **47**, 370–373.

Diaz, T.G. & Cabanillas, A.G. (2004). Analysis of synthetic food antioxidants. In: *Handbook of Food Analysis* (edited by L. M. L. Nollet). Pp 1577–1642. vol. **2**. New York, NY: Marcel Dekker.

Ekor, M., Farombi, E.O. & Emerole, G.O. (2006). Modulation of gentamicin-induced renal dysfunction and injury by the phenolic extract of soybean (*Glycine max*). *Fundamental and Clinical Pharmacology*, **20**, 263–271.

Gorinstein, S., Cvikrova, M., Machackova, I., et al. (2004a). Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chemistry*, **84**, 503–510.

Gorinstein, S., Pawelzik, E., Delgado-Licon, E., et al. (2004b). Use of scanning electron microscopy to indicate the similarities and differences in pseudocereal and cereal proteins. *International Journal of Food Science and Technology*, **39**, 183–189.

Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition*, **132**, 461–471.

Hertog, M.G.L., Hollman, P.C.H. & Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, **40**, 1591–1598.

Holasova, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, J. & Vavreinova, S. (2002). Buckwheat – the source of antioxidant activity in functional foods. *Food Research International*, **35**, 207–211.

Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, **47**, 3954–3962.

Lee, L., Kang, S.A., Lee, H.O., et al. (2001). Relationships between dietary intake and cognitive function level in Korean elderly people. *Public Health*, **115**, 133–138.

Lissi, E., Salim-Hanna, M., Pascual, C. & Del Castillo, M.D. (1995). Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements. *Free Radical Biology and Medicine*, **18**, 153–158.

Mañas, E., Bravo, L. & Saura-Calixto, F. (1994). Sources of error in dietary fibre analysis. *Food Chemistry*, **50**, 331–342.

Marcocci, L., Packer, L., Droylefaix, M.T., Sekaki, A. & Gardesalbert, M. (1994). Antioxidant action of Ginkgo-Biloba extract EGB-761. *Oxygen Radicals in Biological Systems, PT D Methods in Enzymology*, **234**, 462–475.

McConnell, P. & van Dyke, K. (2000). Soybean and green tea antioxidants counteract oxidation via peroxynitrite (from SIN-1) as measured by chemiluminescence of luminol. *FASEB Journal*, **14**, 1088–1092.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. & Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, **50**, 3122–3128.

Perez-Jimenez, J. & Saura-Calixto, F. (2005). Literature data may underestimate the actual antioxidant capacity of cereals. *Journal of Agricultural and Food Chemistry*, **53**, 5036–5040.

Quettier-Deleu, C., Gressier, B., Vasseur, J., et al. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum*

esculentum Moench) hulls and flour. *Journal of Ethnopharmacology*, **72**, 35–42.

Scalbert, A., Manach, C., Morand, C., Remesy, C. & Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, **45**, 287–306.

Schlesier, K., Harwat, M., Bohm, V. & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research*, **36**, 177–187.

Simonato, B., Pasini, G., Giannattasio, M., Peruffo, A.D.B., de Lazzari, F. & Curioni, A. (2001). Food allergy to wheat products: the effect of bread baking and in vitro digestion of wheat allergic proteins. A study with bread dough, crumb, and crust. *Journal of Agricultural and Food Chemistry*, **49**, 5668–5673.

Simopoulos, A.P. (2001). The Mediterranean diets: what is so special about the diet of Greece? The scientific evidence. *Journal of Nutrition*, **131**, 3065S–3073S.

Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology*, **299**, 152–158.

Slavíková, H., Lojek, A., Hamar, J., et al. (1998). Total antioxidant capacity of serum increased in early but not late period after intestinal ischemia in rats. *Free Radical Biology and Medicine*, **25**, 9–18.

Steadman, K.J., Burgoon, M.S., Lewis, B.A., Edwardson, S.E. & Obendorf, R.L. (2001). Buckwheat seed milling fractions: description, macronutrient composition and dietary fibre. *Journal of the Cereal Science*, **33**, 271–278.

Takahashi, R., Ohmori, R., Kiyose, C., Momiyama, Y., Ohsuzu, F. & Kondo, K. (2005). Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. *Journal of Agricultural and Food Chemistry*, **53**, 4578–4582.

Truswell, A.S. (2002). Cereal grains and coronary heart disease. *European Journal of Clinical Nutrition*, **56**, 1–14.

Tsuji, H., Kimoto, M. & Natori, Y. (2001). Allergens in major crops. *Nutritional Research*, **21**, 925–934.

Velioglu, Y.S., Mazza, G., Gao, L. & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, **468**, 4113–4117.

Vinson, J.A., Su, X., Zubic, L. & Bose, P. (2001). Phenol antioxidant quantity and quality of foods: fruits. *Journal of Agricultural and Food Chemistry*, **49**, 5315–5321.

Yoshida, H., Kanrei, S., Tomiyama, Y. & Mizushina, Y. (2006). Regional characterization of tocopherols and distribution of fatty acids within soybean seeds (glycine max L.). *Journal of Food Lipids*, **13**, 12–26.

Yu, L., Perret, J., Davy, B., Wilson, J. & Melby, C.L. (2002). Antioxidant properties of cereal products. *Journal of Food Science*, **67**, 2600–2603.