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ORIGINAL PAPER

The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals

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Abstract The objective of the present study was to investigate the effect of phenolic substances and proteins on the antioxidant potentials in some cereals and pseudocereals and to compare their bioability. The polyphenol dry matter extracts (PDME) from the investigated seeds of buckwheat, rice, soybean, amaranth and quinoa with 1.2 M HCl in 50% methanol/water (PDME50%Met/HCl) exhibited higher inhibition of lipid peroxidation than the ones extracted with 50% methanol/water (PDME50%Met) and were comparable to the antioxidant activity of butylated hydroxyanisole at concentration of 0.2 mg mL^{-1}. The antioxidant activities of these seed extracts determined by 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)-$ABTS^{\bullet+}$/$K_2S_2O_8$, β-carotene bleaching (β-carotene), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging methods showed high correlation coefficients (R^2) such as 0.9515, 0.9058 and 0.8723, respectively, with the presence of total polyphenols estimated by Folin-Ciocalteu assay. These results indicate that the major antioxidant components in these extracts mostly derived from the polyphenols, and proteins showed only minimal values of bioactivity. Based on high contents of polyphenols, anthocyanins, flavonoids and their antioxidant activities pseudocereals such as buckwheat, quinoa and amaranth can be a substitute for cereals for common and atherosclerotic diets and sometimes in the allergic cases.

Keywords Seeds · Selected cereals · Pseudocereals · Antioxidants

Introduction

Celiac disease is owing to an intolerance of certain amino acid sequences of wheat gluten and corresponding fractions of other cereals. Certain cereals (rice, maize, sorghum and millet) and pseudocereals (amaranth, buckwheat and quinoa), rich in proteins and carbohydrates do not contain gluten. The grains and the products of these plants proofed their qualification and in some cases readiness for marketing was achieved [1, 2]. Therefore, in the last decade, the use

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of pseudocereals was increased not only in allergic to cereals population, but also in common diets [2]. Some authors have assessed the nutritional and antioxidant properties of pseudocereals in order to recommend for use the most valuable among them [3–5]. One team of these authors compared oat meal, whole amaranth flour and amaranth seeds in vitro and in vivo and found that both flour and amaranth seeds have high antioxidant potential and positively affect plasma lipid profile and antioxidant activity in rats fed cholesterol-containing diets [3]. Also others had shown that pseudocereals possess high nutritional and functional values [6]. It was reported that quinoa had a suppressive effect on the increase in systolic blood pressure. These results suggest that quinoa would be useful as a functional food [6–8].

However, most of the recent published papers are focused mainly on the studies of different cereal products and their antioxidant potentials [9–12].

Most of these reports, concerning the determination of the cereal antioxidant potential, have two limitations: (a) the procedure used to extract antioxidants may be incomplete; (b) the solvents employed in these experiments are absolute ethanol or ethanol/water [12]. The use of more polar solvent such as methanol could improve the extraction of phenolic compounds, and therefore to lead to a reliable assessment of the antioxidant potential.

Also other investigators have shown that combination of a number of scavenging radical assays is preferable for determination of total antioxidant potentials [13–15].

However, as far as we know no such complementary methods were used to determine the antioxidant potential in pseudocereals. Therefore, we decided to compare the antioxidant potential of some selected cereals and pseudocereals by the following methods: Trolox equivalent antioxidant activity (TEAC) with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)-ABTS$^{\bullet+}$/$K_2S_2O_8$; β-carotene linoleate model system; 1,1-diphenyl-2-picrylhydrazyl radical (DPPH); and Folin-Ciocalteu. On the basis of the relatively high bioactivity of pseudocereals in comparison with cereal and legume the substitution of cereals was suggested.

Materials and methods

Chemicals

2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonate)-(ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); β-carotene; 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox); butylated hydroxytoluene (BHT); butylated hydroxyanisole (BHA); glutathione; rutin; gallic acid and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Samples and their preparation

Common buckwheat (*Fagopyrum esculentum Mnch*. Polygonaceae Peru), Jasmin rice (*Oryzea sativa*, Khao Dawk Mali, Thailand), rice bran (the product of Jasmin rice milling, Thailand), quinoa (*Chenopodium quinoa Wild*. Chenopodiaceae, Peru), and three cultivars of amaranth (*Amaranthus* (*A*.) *cruentus*, Amaranthaceae – USA; *A*. *hybridum,* Amaranthaceae – Pakistan; *A*. *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, were removed under liquid nitrogen to prevent oxidation and then were stored at 5 °C after removal of hexane.

Extraction and the polyphenol content

Defatted samples were extracted from a 50-mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for 3 h. Samples were cooled and then diluted to 10 mL with methanol and centrifuged for 5 min at 5000 rpm. The polyphenol dry matter extract was assigned as PDME50%Met. The second extract was obtained with 5 mL of 1.2 M HCl in 50% methanol/water (PDME50%Met/HCl) and treated as above with heating [16]. The clear supernatants obtained from two different extractions were used for determination of total polyphenols by the Folin–Ciocalteu method and the values were read at 675 nm [17].

Anthocyanins and flavonoids

Anthocyanins

Absorbance was measured in extracts (1 g of the defatted sample was extracted with 1 mL of acetonitrile containing 4% acetic acid) at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510}–A_{700})_{pH1.0}–(A_{510}–A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29 600. Results were expressed as milligrams of cyanidin-3-glucoside equivalent per 100 g DW [18].

Flavonoids

Flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH) were measured at 510 nm with known (+)-catechin concentration as a standard and expressed as milligrams of catechin equivalents per 100 g dry weight [17].

Total antioxidant potentials

1. Trolox equivalent antioxidant capacity (TEAC) was determined using 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)-ABTS$^{\bullet+}$/$K_2S_2O_8$. ABTS$^{\bullet+}$ radical cation was

generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μL of ABTS solution to 10 μL of investigated extracts (0.2 mg mL^{-1}) or Trolox standards (final concentration 0–20 μM) in ethanol or phosphate buffered saline (PBS), the absorbance was monitored exactly 1 and 6 min after the initial mixing. This solution was then diluted in a 5 mM phosphate buffered saline, pH 7.4 to an absorbance of 0.70. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox for the standard reference data. The results were expressed in μM of Trolox equivalent (TE) g^{-1} [19].

2. Antioxidant activity (AA) using β-Carotene Linoleate Model System was evaluated in terms of bleaching of the β-carotene, measuring the absorbance at 470 nm: $AA = 100\ [1 - (A_0 - A_t)/(A^\circ_0 - A^\circ_t)]$, where A_0 and A°_0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and A_t and A°_t are the absorbance measured in the test sample and control, respectively, after incubation for 180 min. The results were expressed in % of inhibition. BHA and BHT were used for comparison [20].
3. Radical scavenging activity (RSA) using 1,1-diphenyl-2-picrylhydrazyl (DPPH) test was performed as follow. The volume of cereal and pseudocereal extracts at 0.2 mg mL^{-1} was adjusted to 100 μL by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5 μL).

RSA was expressed as the inhibition percentage: % RSA = (control OD − sample OD/control OD) × 100, where OD is optical density. Changes in the absorbance of the samples were measured at 517 nm [20].

Extraction of proteins

Samples (1 g) were subjected to extraction with 0.05 M Na_2HPO_4 buffer (two portions of 20 mL each) at 4–6 °C for 48 h (2 × 24 h) under constant stirring. Centrifugation (K-24 D centrifuge, Janetzki, Germany) at 15,000 rpm for 45 min at 4–6°C was applied at the end of each extraction step, and the supernatants were combined and freeze-dried. Proteins were extracted with buffer and were used for further analyses.

Gel filtration chromatography

Ten milliliters portion of phosphate buffer extracts were applied on Sephadex G-25 column (bed volume 130 mL) at flow rate 40 mL h^{-1} at 20 °C. The effluent profile was monitored at 280 nm on LKB 2510 Uvicord SD. The column was preliminarily calibrated with 0.2 M NaCl and Blue Dextran.

Protein content

The chromatographic fractions were pooled, freeze-dried, and 5 mg of each sample were used for protein content determination [21].

Statistics

Values are given as 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, following by Duncan's new multiple-range test to assess differences between groups means. Spearman correlation coefficient (R^2) and p-value were used to show correlations and their significance. Differences of $p<0.05$ were considered significant.

Results and discussion

Total polyphenols

The contents of polyphenols (μg g^{-1} DW) extracted with 1.2 M HCl in 50% methanol/water varied significantly: from 920 ± 85.5 for rice bran till 330 ± 32.7 for Jasmin rice.

The determination of the content of total polyphenols (μg g^{-1} DW) extracted with 50% methanol/water showed significantly lower values: 293.0 ± 25.7 for rice bran and 95.0 ± 8.3 for Jasmin rice (Table 1).

Anthocyanins and flavonoids

The contents of anthocyanins (mg 100 g^{-1} DW) were 132.0.7 ± 13.7 for rice bran and 83.0 ± 7.4 for Jasmin rice. The contents of flavonoids (mg 100 g^{-1} DW) were 185.0 ± 17.9 and 38.0 ± 4.0 for rice bran and Jasmin rice, respectively (Table 1). As can be seen (Table 1), the methanol extracts revealed lower estimation in flavonoids: 86.1 ± 8.7 and 12.5 ± 1.7 for rice bran and Jasmin rice, respectively. The relationship between the contents of the anthocyanins and flavonoids was similar to the relationship shown for total polyphenols.

In all investigated cereals and pseudocereals the highest polyphenol content was achieved by extraction with 50% of methanol and HCl, following by 50% of methanol (Table 1). Buckwheat and rice bran had the highest polyphenol contents in all extracted fractions, following by soybean and pseudocereals.

Antioxidant potential

The obtained results of antioxidant inhibition in PDME50%Met/HCl were compared with the results in

Table 1 Polyphenol compounds in cereals and pseudocereals

Plants	T. polyphenols[a] (μg g^{-1} DW)[d]	T. polyphenols (μg g^{-1} DW)[e]	Anthocyanins[b] (mg 100 g^{-1} DW)	Flavonoids[c] (mg 100 g^{-1} DW)[d]	Flavonoids[e] (mg 100 g^{-1} DW)
Buckw	912 ± 81.9 d	290 ± 25.7 a	111.3 ± 9.8 a	146.0 ± 13.8 a	76.8 ± 8.2 a
Soybe	690 ± 65.8 e	120 ± 11.1 a	100.2 ± 9.3 a	105.0 ± 9.9 a	45.2 ± 4.7 a
A.hyb	405 ± 40.1 g	150 ± 13.7 a	83.5 ± 7.4 a	72.0 ± 6.4 a	21.9 ± 2.0 a
A. hyp	415 ± 42.1 g	154 ± 15.7 a	91.0 ± 9.0 a	73.5 ± 7.8 a	21.9 ± 2.2 a
A. cru	430 ± 44.4 g	160 ± 16.3 a	94.6 ± 8.1 a	75.0 ± 8.1 a	22.2 ± 2.4 a
Quinoa	600 ± 53.8 f	250 ± 23.8 a	96.4 ± 9.5 a	102.0 ± 11.1 a	38.6 ± 4.4 a
J. rice	330 ± 32.7 h	95 ± 8.3 a	83.0 ± 7.4 a	38.0 ± 4.0 a	12.5 ± 1.7 a
R. bran	920 ± 85.5 d	293 ± 26.7 a	132.0 ± 13.7 a	185.0 ± 17.9 a	86.1 ± 8.7 a

Abbreviations: A., *Amaranthus*; A. hyb, *A. hybridus*; A. hyp, *A. hypochondriacus*; A. cru, *A. cruentus*; Buckw, buckwheat; Soybe, soybean; J. rice, Jasmin rice; R. bran, rice bran; T, total

Means in columns with different letters are significantly different ($p<0.05$)

[a]Concentration of gallic acid equivalents μg g^{-1} DW

[b]Concentration based upon cyanidin 3 glucoside as standard expressed mg100 g^{-1} DW

[c]Concentration of (+)-catechin equivalents mg 100 g^{-1} DW

[d]In polyphenol dry matter extract with methanol/acid (PDME/MetAc) with 50% methanol/water/0.2 M HCl (50% Met/HCl)

[e]In PDME/Met with 50% methanol/water (50%Met)

Table 2 Antioxidant activities of cereals and pseudocereals in polyphenol and protein extracts

Plants	β-Carotene[a] (%)	DPPH[a] (%)	TEAC[a] (μM TE g^{-1})	TEAC[b] (μM TE g^{-1})	TEAC[c] (μM TE g^{-1})
Buckw	75.6 ± 6.7 a	80.0 ± 7.0 a	2.60 ± 0.24 a	2.37 ± 0.25 a	0.0123 ± 0.02 a
Soybe	35.8 ± 3.1 b	33.0 ± 3.1 b	1.97 ± 0.17 b	0.82 ± 0.08 c	0.0096 ± 0.01 b
A. hyb	24.6 ± 1.9 c	24.0 ± 1.9 b	1.50 ± 0.13 c	0.85 ± 0.07 c	0.0048 ± 0.001c
A. hyp	26.0 ± 2.0 c	24.3 ± 2.0 b	1.53 ± 0.14 c	0.88 ± 0.09 c	0.0046 ± 0.003 c
A. cru	26.2 ± 2.1 c	26.2 ± 2.1 b	1.59 ± 0.16 c	0.91 ± 0.07 c	0.0050 ± 0.001 c
Quinoa	34.0 ± 3.1 b	30.0 ± 2.8 b	1.71 ± 0.18 b	1.43 ± 0.15 b	0.0101 ± 0.001 b
J. rice	21.2 ± 3.0 c	20.0 ± 1.8 d	1.47 ± 0.13 c	0.54 ± 0.06 d	0.0036 ± 0.001 d
R. bran	78.0 ± 7.5 a	79.0 ± 6.9 a	2.67 ± 0.27 a	2.35 ± 0.25 a	0.0108 ± 0.03 a

Abbreviations: A., *Amaranthus*; A. hyb, *A. hybridus*; A. hyp, *A. hypochondriacus*; A. cru, *A. cruentus*; Buckw, buckwheat; Soybe, soybean; J. rice, Jasmin rice; R. bran, rice bran; β-Carotene, antioxidant assay with β-carotene linoleate model system; DPPH, radical scavenging activity with 1,1-diphenyl-2 -picrylhydrazyl; TEAC, Trolox equivalent antioxidant coefficient

Means in columns with different letters are significantly different ($p<0.05$)

[a]In polyphenol dry matter extract/methanol, acid (PDME/MetAc) with 50% methanol/water/0.2 M HCl (50%Met/HCl)

[b]In PDME/methanol (PDME/Met) with 50% methanol/water (50%Met)

[c]Protein extraction obtained with 0.05 M Na_2HPO_4 buffer (Buffer extract)

PDME50%Met of ABTS decolorization assay where ABTS radical cation was produced by reacting ABTS with potassium persulfate (Table 2).

Extracts with concentration of 0.2 mg mL^{-1} (Fig. 1A, B) showed a high percentage of inhibition, nearly closed to BHA (0.04 mg mL^{-1}) and were placed in the following order: the highest was with BHA and then buckwheat and rice bran of about 9% in the PDME50%Met/HCl. Soybean, amaranth and quinoa exhibited inhibition from 5.5 to 5.7% (Fig. 1A). For free polyphenol extracts the kinetic curves were presented as follows: buckwheat and rice bran of 8%, quinoa of 4.9%, soybean and amaranth of 3.1% and Jasmin rice of 2.8% (Fig. 1B). The three kinetic curves of quinoa, soybean and amaranth were closed to each other in the end point of 6 min (Fig. 1).

As it was mentioned above the $ABTS^{\bullet+}$ was generated by incubating ABTS with potassium persulfate. Chemical compounds that inhibit the potassium persulfate activity may reduce the production of $ABTS^{\bullet+}$. This reduction results in a decrease of the total $ABTS^{\bullet+}$ in the system and contributes to the total $ABTS^{\bullet+}$ scavenging capacity. Other factors, such as stereoselectivity of the radicals or the solubility of extracts in different testing systems, may also affect the capacity of this extract to react and quench different radicals.

BHA, BTH and Trolox had similar reaction kinetic curves against β-carotene as determined by spectrophotometric

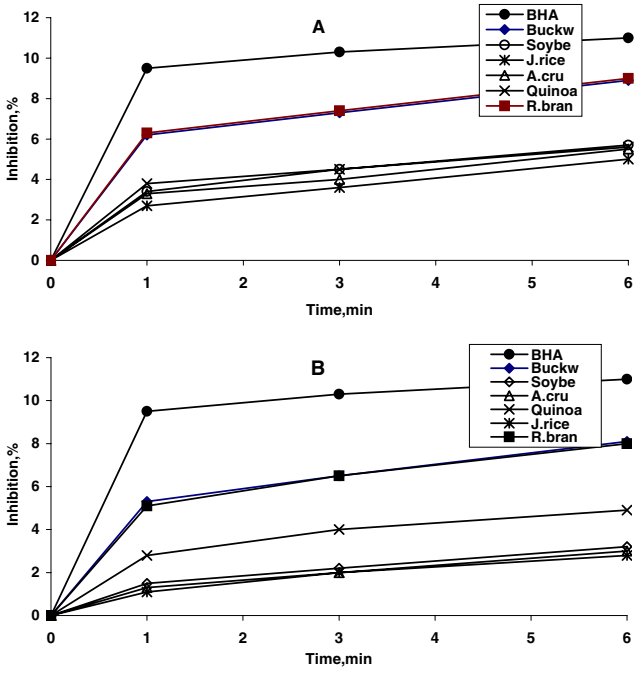

Fig. 1 Reaction kinetics of: (**A**), PDME50%Met/Ac; (**B**), PDME50% Met with $ABTS^{\bullet+}$ radical cation. *Abbreviations*: PDME50%Met/Ac, polyphenol dry matter extract with 50% methanol/HCl; Buckw, buckwheat; Soybe, soybean; A. cru, *Amaranth cruentus*; J. rice, Jasmin rice; R. bran, rice bran, in a concentration of 0.2 mg mL^{-1}; BHA, butylated hydroxyanisole, in a concentration of 0.04 mg mL^{-1}

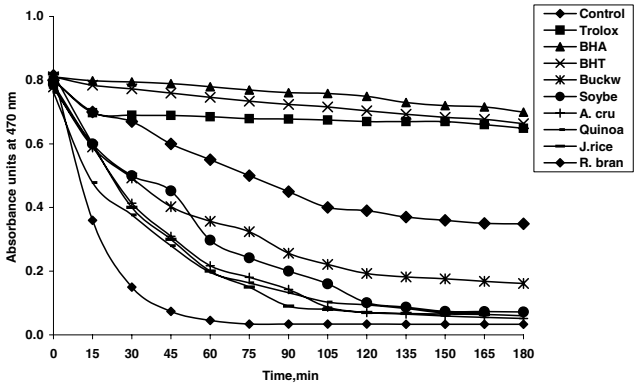

Fig. 2 Reaction kinetics of PDME50%Met/Ac with β-carotene. *Abbreviations*: PDME50%Met/Ac, polyphenol dry matter extract with 50% methanol/HCl; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; Buckw, buckwheat; Soybe, soybean; A. cru, *Amaranth cruentus*; J. rice, Jasmin rice; R. bran, rice bran

measurement (Fig. 2) and were very closed one to another. The investigated extracts of cereals and pseudocereals (PDME50%Met/HCl) showed the following order of antioxidant activity: rice bran > buckwheat > soybean > quinoa > amaranth > Jasmin rice. From the results of the present report, it can be concluded that similar data were obtained in our recent study on the same cereals and pseudocereals. The difference was only in the employed methods: similarities were found between the same samples of pseudocereals which could make them a substitution of each other as well as for cereals estimated by circular dichroism (CD) spectra and Fourier transform infrared (FT-IR) measurements [22]. According to UPGMA algorithm, the examined species and varieties could be clustered into two similarity groups. Soybean, quinoa, buckwheat and amaranth (as a genus) can be considered as phylogenic distant taxa [22]. In the present report, we have estimated for the first time the same plants by their bioactivity which is very important from the nutritional point.

Our results are in accordance with others that rice bran contains high amounts of beneficial antioxidants including tocopherols, tocotrienols, and oryzanols and also indicate that the long-grain rice bran averaged approximate to 15% more antioxidants [23] than the medium-grain rice bran which were lower than in the present report (Table 2). DPPH values (Table 2) were similar to other reports [24] and scavenged DPPH radicals, and scavenging activities of extracts were proportional to measured intensities of rice bran ($79.0 \pm 6.9\%$ of inhibition) and compared with that of gallic acid ($87.9 \pm 7.1\%$ of inhibition) which is a typical free radical scavenger.

The average data of all investigated samples of selected cereals and pseudocereals, their polyphenol and antioxidant composition are presented on Fig. 3.

The best correlation was between polyphenols in PDME50%Met/HCl and TEAC (Fig. 3B, $R^2 = 0.9515$), following by polyphenols in PDME50%Met/HCl and β-carotene (Fig. 3A, $R^2 = 0.9058$), then by polyphenols in PDME50%Met/HCl and DPPH (Fig. 3A, $R^2 = 0.8723$). Oppositely the correlation between polyphenols in PDME50%Met and DPPH (Fig. 3A, $R^2 = 0.1361$) was very low, indicating that the scavenging capacity of extracted polyphenols is based mostly on the amount of total polyphenols, not the free ones, extracted only with methanol. The flavonoids revealed as well high correlation with TEAC in PDME50%Met/HCl (Fig. 3C, $R^2 = 0.8989$) and slightly lower in PDME50%Met (Fig. 3D, $R^2 = 0.8826$). Anthocyanins in PDME50%Met/HCl showed $R^2 = 0.8707$ (Fig. 3C).

Our results for soybean (β-carotene $= 35.8 \pm 3.1\%$; and DPPH $= 33.0 \pm 3.1\%$, Table 2) were similar to others [25], who were screening the methanolic extracts of the pearled bean.

The properties of the solvents with different polarities used in this report significantly affect the yield, total phenolics and antioxidant activity and correspond with the reviewed results of buckwheat extracts [26]. It can be seen that the extracted all polyphenolic substances and corresponding activities varied with the solvent employed in this study (Tables 1 and 2).

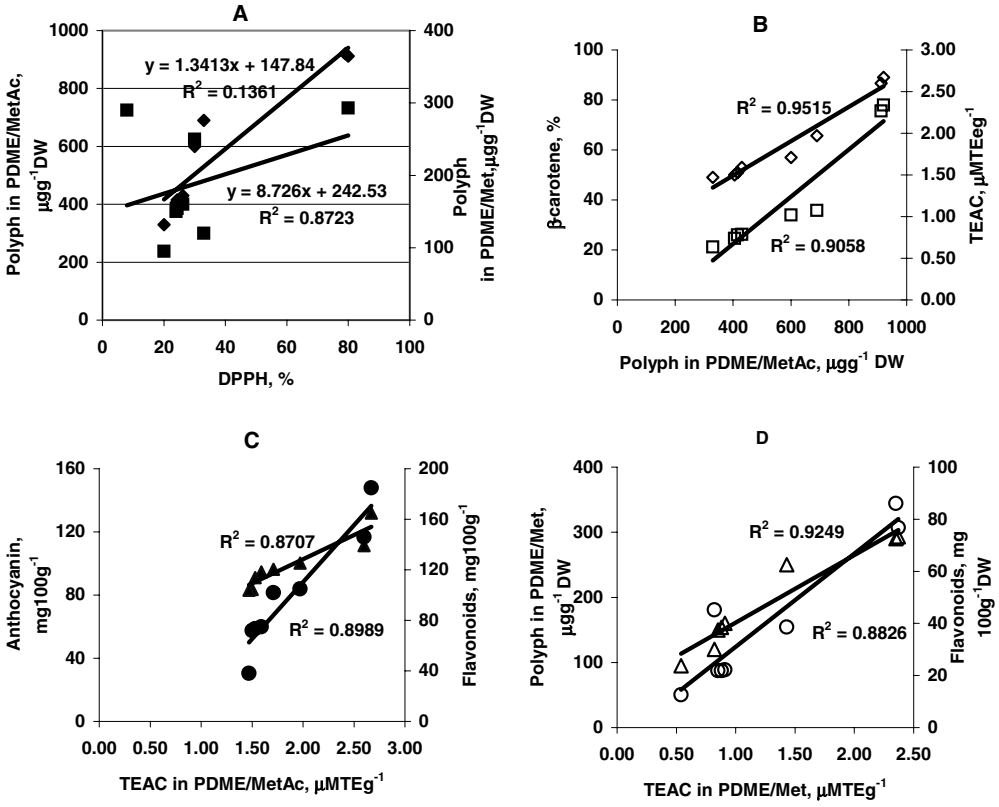

Fig. 3 Relationship, calculated by linear regression analysis for average data of all investigated samples of selected cereal and pseudocereal extracts between: **A** (■) DPPH (% of inhibition, X) to polyph in PDME/MetAc (μg g^{-1} DW, Y_1) and (♦) DPPH (% of inhibition, X) to polyph in PDME/Met (μg g^{-1} DW, Y_2); **B** (□) polyph in PDME/MetAc (μg g^{-1} DW, X) to β-carotene bleaching effect (% of bleaching, Y_1) and (◇) polyph in PDME/MetAc (μg g^{-1} DW, X) to TEAC (μMTE g^{-1} DW, Y_2); **C**, (▲) TEAC in PDME/MetAc (μMTE g^{-1} DW, X) to anthocyanins in PDME/MetAc (mg100 g^{-1} DW, Y_1) and (●) TEAC in PDME/MetAc (μMTE g^{-1} DW, X) to flavonoids in PDME/MetAc (mg100 g^{-1} DW, Y_2); **D**, (○) TEAC in PDME/Met (μMTEg^{-1} DW, X) to polyph in PDME/Met (mg100 g^{-1} DW, Y_1) and (△) TEAC in PDME/Met (μMTE g^{-1} DW, X) to flavonoids in PDME/Met (mg100 g^{-1} DW, Y_2). PDME/Met, polyphenol dry matter extract with 50% methanol/water; PDME/MetAc, polyphenol dry matter extract with 1.2 M HCl in 50% methanol/water. Polyphenols expressed as μg gallic acid g^{-1} dry weight (DW); TEAC, Trolox equivalent antioxidant coefficient; DPPH, 1,1- diphenyl-2-picrylhydrazyl (DPPH); β-carotene, β-carotene linoleate model system

Our results concerning the buckwheat antioxidant activity were similar to another report [26] where the buckwheat methanol extract showed the highest antioxidant activity coefficients (AAC) of 627 ± 40.0 at 200 mg L^{-1} by the β-carotene bleaching method. Opposite the acetone extract showed the highest total phenolics of 3.4 ± 0.1 g catechin equivalents 100 g^{-1} and the highest scavenging activity of $78.6 \pm 6.2\%$ at 0.1 mg mL^{-1} by the DPPH method. The buckwheat herb extract was compared to rutin which is the main constituent of this extract, regarding antioxidant and radical scavenging activity by DPPH assay and peroxidation of linolic acid [26]. In two assays the extract had significantly better antioxidant activity than pure rutin and seems to be more beneficial than the use of pure rutin. Our results report the buckwheat data: $75.6 \pm 6.7\%$ by the β-carotene bleaching and 80.0 ± 7.0 by the DPPH assays (Table 2), and rutin corresponded to 44.7% by the β-carotene and $51.6 \pm 4.9\%$ by the DPPH. This can be referred to the presence of minor phenolic compounds in the extract [27, 28]. Our results were mostly plant-derived antioxidants and therefore contained a complex mixture of constituents, like flavonoids, anthocyanins and polyphenols, which contribute to the overall activity and were in accordance with other reports [29, 30]. As it was discussed above, the obtained results of rice bran were in the same line as in other reports [23, 24, 31, 32]. The analysis of this cereal is important because of its high antioxidant activity and the potential role in the management of coronary risk factors based on the main components of rice bran [33]. The obtained antioxidant activity of rice bran in the present report in all applied antioxidant assays was relatively high and this corresponded with the data of [23, 24, 32], showing that rice bran has a potent radical-scavenging activity against DPPH radical (Table 2, DPPH, inhibition, % of 79.0 ± 6.9) and total phe-

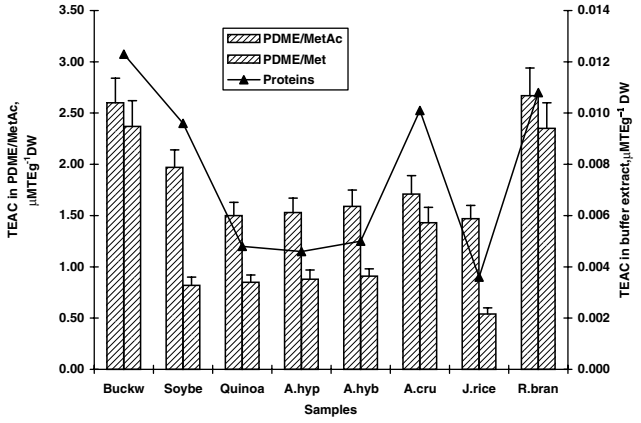

Fig. 4 Relationship between the extracts of investigated cereals and pseudocereals (Buckw, buckwheat; Soybe, soybean; Quinoa, quinoa; A. hyp, *Amaranthus hypochondriacus*; A. hyb, *Amaranthus hybridus*; A. cru, *Amaranthus cruentus*; J. rice, Jasmin rice; R. bran, rice bran, *X*), TEAC in PDME/MetAc, PDME/Met (μMTE g^{-1} DW, Y_1) and TEAC in buffer extract (μMTE g^{-1} DW, Y_2). PDME/Met, polyphenol dry matter extract with 50% methanol/water; PDME/MetAc, polyphenol dry matter extract with 1.2 M HCl in 50% methanol/water. Buffer extract, proteins extracted from dry matter with with 0.05 M Na_2HPO_4 buffer. TEAC, Trolox equivalent antioxidant coefficient

nolics (Table 1, (μg g^{-1} DW) of 920 ± 85.5). This activity is associated with some phenolic acids in the ethanol-soluble or methanol-soluble fractions. The significance of this finding is discussed from the viewpoint of the protective role of the rice bran against oxygen radical-induced chronic diseases.

The reviewed results of spectrophotometric measurements showed that the content of total antioxidants was between 16.0 and 11.4 mg 100 g^{-1} of fine flour of buckwheat and the important flavonoid rutin in buckwheat was 10.1–3.8 mg 100 g^{-1}. The antioxidant activity of buckwheat was higher than in other cereals in comparison with glutathione (by the DPPH for buckwheat $80.0 \pm 7.0\%$ and for the glutathione $53.1 \pm 6.1\%$). The correlation between the antioxidant activity and the total phenolic content was $R^2 = 0.81$ in comparison with our data of $R^2 = 0.9515$ (Fig. 3B). The correlation coefficients as well depend on the extraction procedure and it was a matching between the reviewed data and shown in the present report: the correlation coefficient between total phenolic compounds and total antioxidative activity of the extracts was -0.35 for water extracts and varied from 0.80 to 0.99 for 80% methanolic extracts of different plants [29]. From the reviewed and obtained results it can be indicated that the scavenging ability of polyphenol extracts were different with proposed assays. This can be explained on the basis of the mechanism of the antioxidant methods: inhibition of linoleic acid oxidation (β-carotene) involves hydrogen atom transfer reactions and DPPH, total phenols assay by Folin–Ciocalteu reagent and TEAC are based on electron-transfer reaction.

Proteins

For all investigated samples were obtained similar profiles (data not shown), which differ to a certain extent only in the shape of the second peak, which should contain low molecular substances (salts, vitamins, phenolic compounds and short chain protein fragments). The protein content of peaks 1 (protein-rich) in all samples was about 7–10 times higher than that of peaks 2. Both fractions, obtained from cereals and pseudocereals were freeze-dried and tested for their antioxidant potential. The results of the determination of the antioxidant capacity of proteins in the studied samples by TEAC assay are summarized in the Table 2, Fig. 4. The proteins were examined in three concentrations: 0.2, 0.4 and 0.8 mg mL^{-1}. The relationship between the antioxidant activities and the protein concentration showed direct increase. As can be seen, the highest content of proteins among the studied pseudocereals was in buckwheat and in allergic-free quinoa and amaranth. Amaranth (*A. hypochondriacus*) has higher protein concentration and superior protein quality than cereals. Lunasin which was found in amaranth albumin, globulin and prolamin can be an alternative source of bioactive peptides, having antihypertensive properties [34]. Amaranth protein concentrate has a hypotriglyceridemic effect, affects the metabolism of liver lipids, and increases parameters of antioxidant protection in male Wistar rats [6, 7]. Our results also in accordance with others [8], showing that the protein of quinoa is made up of large amounts of albumins and globulins in comparison with cereals such as rice and wheat.

The main antioxidants in pseudocereals are polyphenols, but also proteins play a role in the overall antioxidant activity, having antioxidants which are effective in inhibition of lipid peroxidation and acting as radical scavengers.

Our results have shown that cereals and pseudocereals have relatively high antioxidant status and therefore can be recommended for the use in balanced diets in the same scale as fruits and vegetables. This was shown as well in other investigations [1–5, 17], suggesting that Western diets based on highly palatable foods. Traditional diets or those typical of less developed countries based on the adding of pseudocereals as functional foods. In particular, some alternative crops (for example, buckwheat, oat, barley, spelt, rye, quinoa, amaranth) seem to be of great nutritional interest and to represent important recipes for healthier and typical regional food [2]. In order to achieve the best results, these natural products must have high antioxidant potential [7, 13]. It was shown in this study that the main contributor to the antioxidant potential of the natural products is polyphenols and others polyphenolic substances (anthocyanins and flavonoids). This statement corresponds with others [13, 25, 29, 30].

The results of total polyphenols and the antioxidant potentials in the studied cereals and pseudocereals support those

investigators, who have shown that high total polyphenol content increases antioxidant potential, and there is a linear correlation between phenolic content and antioxidant potential [28, 29].

Conclusion

The buckwheat and the allergic-free quinoa have the highest contents of total polyphenols and the highest antioxidant potential among the studied cereals and pseudocereals, respectively. The highest polyphenol content in cereals and pseudocereals could be achieved by extraction with 50% of methanol and HCl, following by 50% of methanol. It was found that the contents of total and free polyphenols, anthocyanins and flavonoids and the antioxidant potential among the studied samples was relatively high in pseudocereals. The use of four different assays: Trolox equivalent antioxidant activity (TEAC) with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)-ABTS$^{\bullet+}$/$K_2S_2O_8$; β-carotene linoleate model system; 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteu method secures reliable assessment of the antioxidant potentials of the investigated cereals and pseudocereals. High total polyphenol content increases antioxidant potential and there is a linear correlation between phenolics and antioxidant potential. Proteins take a small part in the overall antioxidant activity of investigated samples. The results of the investigation show that buckwheat following by quinoa and amaranth is the best choice among the studied pseudocereals as a substitute for cereals for common and atherosclerotic diets and sometimes in the allergic cases.

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