

## POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN PSEUDOCEREALS AND THEIR PRODUCTS

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### ABSTRACT

Pseudocereals are important as gluten-free crops that could be utilized as functional foods. They contain proteins with high biological value and also bioactive compounds such as phenolic compounds, flavonoids, vitamins, and minerals that can possess positive health effects on the body. Three types of pseudocereals (amaranth, buckwheat, and quinoa) were evaluated for polyphenols and antioxidant activity. Spectrophotometric methods were used for the determination of free phenols amount with Folin-Ciocalteu reagent, and total antioxidant capacity (TAC) with DPPH and ABTS reagents. Free phenols, the predominant part of polyphenols, were in pseudocereals in the range from 12.4 to 678.1 mg GAE.100g<sup>-1</sup>. The highest content of FP was found in buckwheat products (146.8 – 678.1 mg GAE.100g<sup>-1</sup>); quinoa and amaranth products reached much lower values (up to 226.1 mg GAE.100g<sup>-1</sup>). Antioxidant activity was in an agreement with the FP amounts order, the highest TAC values were again for buckwheat products (167.3 – 473.9 and 876.9 – 3524.8 mg TE.100g<sup>-1</sup>), followed by quinoa (78.2 – 100.6 and 738.9 – 984.5 mg TE.100g<sup>-1</sup>) and amaranth ones (25.0 – 69.7 and 118.2 – 431.4 mg TE.100g<sup>-1</sup>). A high positive correlation between FP amount and TAC values was evaluated for analyzed pseudocereals. The highest content of free phenols and the best antioxidant potential showed buckwheat wholemeal flour, so buckwheat could be characterized as a great source of free phenols with high antioxidant activity.

**Keywords:** pseudocereal; free phenol; antioxidant activity; DPPH; ABTS

### INTRODUCTION

Pseudocereals are important gluten-free crops where belong especially amaranth, buckwheat, and quinoa. Their great nutrient properties and also suitability for the preparation of gluten-free foodstuffs (Alvarez-Jubete et al., 2010) predestinate them for the utilization as functional foods. They are known to have good nutritional value, specifically because of proteins with high biological value. It is due to the presence of essential amino acids (especially lysine and tryptophan) in a higher content (Kocková and Valík, 2011). Due to their starch content they are also sources of energy. They contain also natural antioxidants, high levels of flavonoids (e.g. rutin, hyperoside, vitexin, isovitexin, orientin, isoorientin, catechin and epicatechin gallate in buckwheat), vitamins and minerals (Salehi et al., 2018; Tomotake et al., 2007; Kiprovski et al., 2015).

Amaranth (*Amaranthus* spp.) is a rich source of proteins, with well-balanced amino acid composition and good bioavailability. It has higher lysine content than other cereal grains (López et al., 2019; Tovar-Pérez, Lugo-Radillo and Aguilera-Aguirre, 2019). Amaranth is known also due to some potential health benefits

(decreasing plasma cholesterol levels, reducing blood glucose levels and anemia) that have been conducted in experimental animal models (Caselato-Sousa and Amaya-Farfán, 2012). Its seeds contain a good amount of polyphenols such as flavonoids with quite high antioxidant activity (Vollmannová et al., 2013). To the important phenolics, there belong caffeic acid, ferulic acid and *p*-hydroxybenzoic acid (Klimczak, Małecka and Pacholek, 2002).

One of the most important pseudocereal sources for functional foods is common buckwheat (*Fagopyrum esculentum* Moench). To the functional substances in buckwheat belong flavonoids, phytosterols, fagopyrins, fagopyritols, phenolic compounds, resistant starch, dietary fiber, lignans, vitamins, minerals and antioxidants (Ahmed et al., 2014). Middling and bran buckwheat flours could be used to develop functional foods due to phenolic compounds presence. Phenolics are present there in the free and bound form. They are concentrated mainly in the outer layer (hull and bran) as the hull is removed before the milling of the buckwheat (Martín-García et al., 2019). The study of Li et al. (2013) showed that rather than buckwheat flours, hulls and brans are a better source

of antioxidants. The health-promoting properties of buckwheat are expressed due to the content of antioxidants such as phenolic acids, rutin (quercetin-3-rutinoside), and fagopyrin, and specific proteins (Öschläger et al., 2008; Sytar et al., 2016). To the other health benefits belong, similarly as for amaranth, plasma cholesterol level reduction, antidiabetic properties, and also anti-inflammatory effect and improvement of hypertension conditions (Giménez-Bastida and Zieliński, 2015).

Quinoa (*Chenopodium quinoa* Willd.) is a plant of the *Chenopodiaceae* family. It is also a gluten-free crop that is suitable for coeliac patients because it contains very little or no prolamin (Jancurová, Minarovičová and Dandár, 2009). It is exceptionally high in lysine that is not overly abundant in the vegetable kingdom. Quinoa seeds contain also phytohormones that have a good impact on human nutrition (Vega-Gálvez et al., 2010).

Processing of crops (procedures, extraction methods, used temperature, type of present compounds) can modify the polyphenol content of foods in several ways (Manach et al., 2004; Inglett et al., 2011).

This study aimed to assess differences in antioxidants of pseudocereals, concretely amaranth, buckwheat and quinoa, by comparison of free phenols content and total antioxidant capacity.

### Scientific hypothesis

The scientific hypothesis of this study was to examine the differences and relations between free phenolic content and antioxidant capacity measured by two methods (DPPH with IC50, and ABTS tests) in three types of pseudocereals (amaranth, buckwheat, and quinoa), and also differences between samples themselves.

## MATERIAL AND METHODOLOGY

### Pseudocereal samples

Three types of pseudocereals and their products (13 samples), bought from food markets of different origin, were analyzed. There were amaranth, buckwheat, and quinoa. Amaranth (4 samples of Indian, Hungarian, Czech and German origin; grains (AG), flour (AF), wholemeal flour (AWF) and particles (AP)); buckwheat (6 samples of Poland, Czech and Latvia origin; grains (BG1, BG2), flour (BF1, BF2), wholemeal flour (BWF) and groats (BGR)); and quinoa (3 samples of Peruvian and Bolivian origin; different types of grains (white QGW, red QGR, black QGB)).

### Determination of Free Phenolic Content

For the determination of free phenolics content (FP) in the pseudocereals modified spectrophotometric method with Folin-Ciocalteu reagent (Vollmannová et al., 2013) was used.

The extracts of pseudocereal samples were prepared from 1 g of homogenized pseudocereal sample and 25 mL of methanol (80% (v/v); Penta Chemicals, CZ) with stirring in a shaker for 8 h at room temperature. The extract was afterward filtered through a paper filter and used for the analyses of FP.

To extracts (1 mL) with 5 mL of distilled water, Folin-Ciocalteu reagent (2.5 mL; Penta Chemicals, CZ), was added and after agitation, it was left for 3 min in the dark

at room temperature. Then sodium carbonate solution (7.5 mL, 20% (w/v); Penta Chemicals, CZ) was added to a mixture and mixed again. The content was then filled up to 50 mL. After 2 h of the extract standing in the dark at room temperature the absorbance of samples was measured at wavelength 765 nm (Libra S6 Biochrom spectrometer, GB) against blank. As a standard a gallic acid was used. FP values were expressed as gallic acid equivalents (GAE), mg.100g<sup>-1</sup> sample. Determinations were made in triplicate.

### Determination of Antioxidant Activity

Antioxidant activity of pseudocereals was assessed as a total antioxidant capacity (TAC). It was evaluated by a modified spectrometric method with DPPH reagent (Vollmannová et al., 2013) and also with ABTS reagent (Škrovánková et al., 2018).

For both determinations there was used the same extraction process for analyzed samples. 1 g of homogenized pseudocereal sample was mixed with 25 mL methanol (80% (v/v); Penta Chemicals, CZ) with stirring in a shaker for 8 h at room temperature. The extract was afterward filtered through a paper filter and used for the analyses.

**DPPH method:** To pseudocereal extract (0.1 mL) a DPPH (1,1-diphenyl-2-picrylhydrazyl) solution in methanol (3.9 mL; 1 mM; Sigma Aldrich, CZ) was added. The mixture was shaken vigorously on a Vortex mixer in capped glass and left in the dark for 10 min. (room temperature). The absorbance of samples (A) and absorbance of control samples (AC) was measured on the spectrometer (Libra S6 Biochrom, GB) at  $\lambda = 515$  nm against a blank. The pseudocereal inactivations (I) were calculated from the decrease of absorbance (%) according to relation (1) and the results were then expressed, using the calibration curve of standard (trolox), as trolox equivalents (TE) in mg.100g<sup>-1</sup> sample. Average results were obtained from three parallel determinations.

$$I = \frac{AC-A}{AC} \cdot 100 \quad (1)$$

**IC50 method:** For the determination of 50% antioxidant inactivation, to scavenge 50% of DPPH free radicals, the most effective pseudocereal type, buckwheat, was used. For IC50 method there were prepared five diluted methanolic buckwheat extract solutions for each sample, in the range 1 – 10 mg.mL<sup>-1</sup>. The reaction mixtures were made the same way as for TAC (DPPH) determination. From the results of inactivation for these extract concentrations the IC50 values were quantified by linear regression.

**ABTS method:** To 50  $\mu$ L of pseudocereal extracts 4 mL of the reactive radical mixture composed of ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; Sigma Aldrich, CZ) (12 mL; 3.5 mM) with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (0.06 M; Lukes, CZ) and acetic buffer (pH 4.3), was added. The solution was shaken vigorously on a Vortex mixer and left to react without light exposure for 30 min at room temperature. Pseudocereal samples absorbance (A) and absorbance of control samples (AC) were then measured by a spectrometer (Libra S6 Biochrom, GB) at wavelength 734 nm against a blank. Inactivations (I) were

calculated from the decrease of absorbance (%) according to relation (1). Results of TAC (ABTS) were calculated from inactivation using a calibration curve with trolox as a standard. It was expressed as trolox equivalents (TE) in the  $\text{mg} \cdot 100\text{g}^{-1}$  sample. Average results were obtained from three parallel determinations.

### Statistical analysis

All data were expressed as mean values  $\pm$  standard deviation (SD), every determination was made in triplicate. Statistical analysis of the results was made by Statistica program, StatSoft version 9.0 (Dell, USA) using parametric test comparing mean values of two independent assortments (Student *t*-test). Differences at a 95% confidence level ( $p < 0.05$ ) were considered statistically significant. Correlations between the evaluated parameters were obtained using Pearson's correlation coefficient (*r*).

## RESULTS AND DISCUSSION

### Content of phenolics

Antioxidants that could donate electrons, such as polyphenols, vitamin C (ascorbic acid), and vitamin E (tocopherols), were evaluated by the method with Folin-Ciocalteu reagent.

As **Li et al. (2013)** found out predominant polyphenols in pseudocereals, such as buckwheat, are free phenolics (FP), accounted for about 94.07% of whole polyphenol content.

The amounts of FP in the pseudocereal samples in our study (Table 1) ranged from 12.4 to 678.1  $\text{mg GAE} \cdot 100\text{g}^{-1}$  with the average 208  $\text{mg GAE} \cdot 100\text{g}^{-1}$  pseudocereal sample. There were marked differences between individual pseudocereals ( $p < 0.05$ , Student *t*-test).

The highest content of FP was analyzed for the buckwheat products; quinoa and amaranth products reached much lower values. These findings are in agreement with **Alvarez-Jubete et al. (2010)** results for pseudocereal seeds determination. Also **Vollmannová et al. (2013)** and **Gorinstein et al. (2008)** detected for pseudocereals this order of polyphenols amount.

The average FP value for buckwheat seeds and products in our research was 357  $\text{mg GAE} \cdot 100\text{g}^{-1}$ , that is up to 3 times higher than in quinoa (average 141  $\text{mg GAE} \cdot 100\text{g}^{-1}$ ) and up to 10 times than in amaranth (average 34  $\text{mg GAE} \cdot 100\text{g}^{-1}$ ) seeds and their products. The highest amount was analyzed for buckwheat wholemeal flour followed by other types of buckwheat flours.

In **Alvarez-Jubete et al. (2010)** study polyphenol amount for buckwheat seeds was 323  $\text{mg GAE} \cdot 100\text{g}^{-1}$ , for amaranth 21.2 and for quinoa it was 71.7  $\text{mg GAE} \cdot 100\text{g}^{-1}$ . Buckwheat values of our determination were similar also to the contents of **Salehi et al. (2018)** study that were in the range from 265 to 430  $\text{mg}$  of caffeic acid equivalents per 100g.

In a study of **Gorinstein et al. (2007)**, the total phenol content in amaranth and quinoa reached similar values (40.5 – 43 and 91.2  $\text{mg GAE} \cdot 100\text{g}^{-1}$ , respectively) as in our study, for buckwheat it was much less, 60  $\text{mg GAE} \cdot 100\text{g}^{-1}$ . **Vollmannová et al. (2013)**, however, found in seeds of selected pseudocereals a higher amount of phenolics than evaluated in our study for buckwheat; quinoa, and amaranth seeds and their products.

### Antioxidant activity

To evaluate the antioxidant potential, the antioxidant activity of all selected pseudocereal seeds and their products was measured. Two methods, DPPH and ABTS tests were used. The results of total antioxidant capacity (TAC) are summarized in Table 1.

The TAC values for the DPPH method were in the range from 25 to 473.9 of trolox equivalents per 100 grams of pseudocereal sample with an average 166  $\text{mg TE} \cdot 100\text{g}^{-1}$ . Results of ABTS test were from 118.2 to 3524.8  $\text{mg TE} \cdot 100\text{g}^{-1}$ , the average 1280  $\text{mg TE} \cdot 100\text{g}^{-1}$ . For the results of both methods there were marked differences between pseudocereals ( $p < 0.05$ , Student *t*-test), similarly as for free phenols.

From the evaluation of the results it can be seen that the pseudocereal type with higher antioxidant values is buckwheat, followed by quinoa and amaranth. It is the same order as for FP values. These findings are in agreement with the values previously reported by **Alvarez-Jubete et al. (2010)** for pseudocereal seeds. The studies of **Gorinstein et al. (2007)**, **Gorinstein et al. (2008)** and **Vollmannová et al. (2013)** introduced for pseudocereals the same order for antioxidant capacity results.

Buckwheat samples for DPPH and ABTS test in our study reached 2 – 19 and 2 – 30 times higher values than in quinoa samples (average 92 and 860  $\text{mg TE} \cdot 100\text{g}^{-1}$ , respectively) and 2 – 6 and up to 5 times, respectively, than in amaranth (average 41 and 263  $\text{mg TE} \cdot 100\text{g}^{-1}$ ) seeds and their products. As for particular samples, the best product with the highest antioxidant activity, measured by both methods, was buckwheat wholemeal flour followed by other types of buckwheat flours and buckwheat grain (BG1).

A similar trend of buckwheat samples is shown for IC50 values in Figure 1. IC50 expresses the concentration of buckwheat extracts requisite for 50% inhibition, therefore the highest antioxidant capacity is showed by the lowest IC50 value. IC50 concentrations of buckwheat extracts were in the range 2.391 – 6.520  $\text{mg} \cdot \text{mL}^{-1}$ , the average 4.218  $\text{mg} \cdot \text{mL}^{-1}$ . Wholemeal flour (BWF) had the lowest IC50, nearly 3 times lower than the sample with the highest IC50 value (buckwheat grain BG2). So wholemeal flour has the highest antioxidant activity what could be seen also from DPPH and ABTS tests.

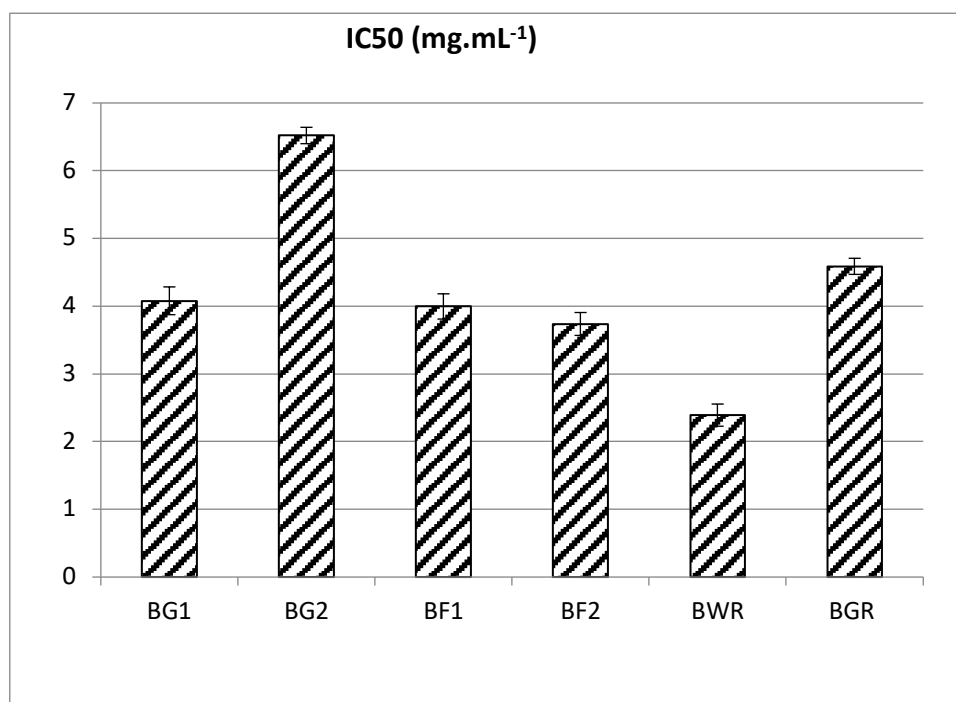
**Alvarez-Jubete et al. (2010)** reported analogous order of antioxidant capacity for pseudocereal seeds, determined by DPPH assay, the value of 620  $\text{mg TE} \cdot 100\text{g}^{-1}$  for buckwheat, 57.7 for quinoa, and 28.4  $\text{mg TE} \cdot 100\text{g}^{-1}$  for amaranth, respectively. In the study of **Salehi et al. (2018)** TAC results (DPPH test) in buckwheat seed samples varied from 268 to 628  $\text{mg TE} \cdot 100\text{g}^{-1}$ . **Zielińska et al. (2012)** determined TAC in buckwheat seeds 215  $\text{mg TE} \cdot 100\text{g}^{-1}$ .

Despite some variations in exact values of antioxidant potential in our research and other studies, they are comparable, and buckwheat could be reported as the greatest source of polyphenols with the highest antioxidant activity amongst pseudocereals and also some other cereals too (**Gorinstein et al., 2007; Gorinstein et al., 2008; Gallardo, Jiménez and García-Conesa, 2006; Zieliński and Kozłowska, 2000**).

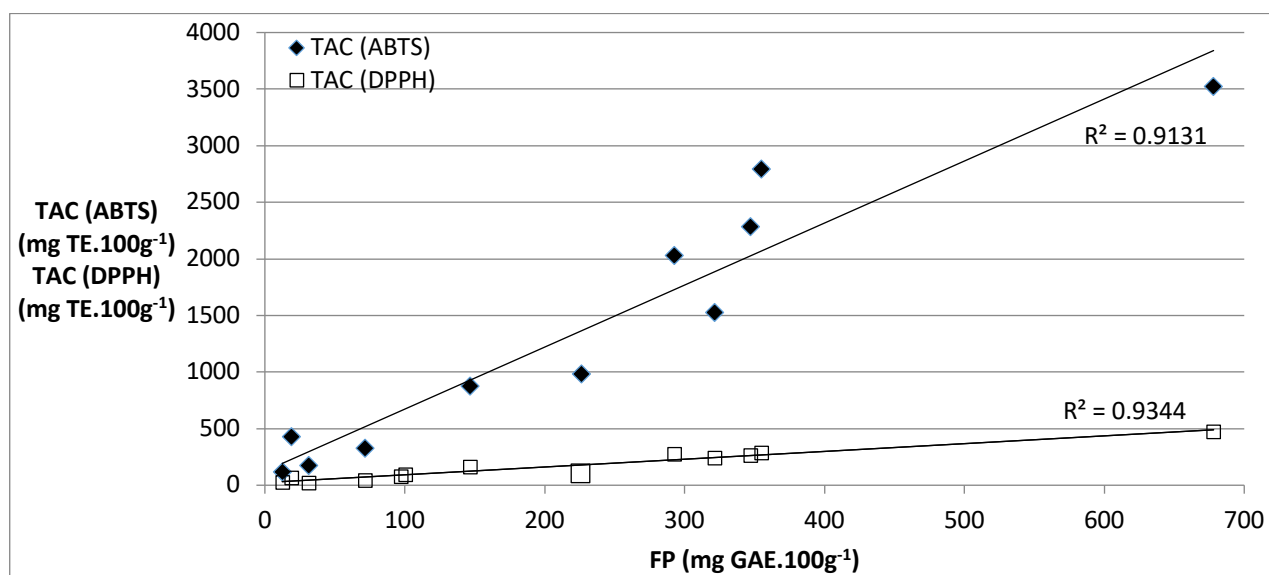
**Table 1** The content of free phenols (FP) and values of total antioxidant capacity (TAC) in pseudocereals.

Pseudocereal sample	FP (mg GAE.100g <sup>-1</sup> ±SD)	TAC (DPPH) (mg TE.100g <sup>-1</sup> ±SD)	TAC (ABTS) (mg TE.100g <sup>-1</sup> ±SD)
AG	12.4 ±0.7 <sup>a</sup>	26.4 ±1.3 <sup>a</sup>	118.2 ±8.3 <sup>a</sup>
AF	31.5 ±1.2 <sup>a</sup>	25.0 ±0.9 <sup>a</sup>	173.0 ±13.8 <sup>b</sup>
AWF	19.2 ±1.6 <sup>a</sup>	69.7 ±1.8 <sup>b</sup>	431.4 ±25.0 <sup>c</sup>
AP	71.6 ±3.5 <sup>c</sup>	44.2 ±3.5 <sup>c</sup>	327.9 ±17.9 <sup>d</sup>
BG1	292.5 ±10.8 <sup>d</sup>	280.0 ±11.5 <sup>d</sup>	2031.6 ±78.7 <sup>e</sup>
BG2	146.8 ±3.2 <sup>e</sup>	167.3 ±5.1 <sup>e</sup>	876.9 ±65.2 <sup>f</sup>
BF1	347.2 ±16.9 <sup>f</sup>	266.1 ±13.9 <sup>f</sup>	2287.4 ±90.4 <sup>g</sup>
BF2	354.9 ±18.0 <sup>f</sup>	291.6 ±14.2 <sup>d</sup>	2795.7 ±81.2 <sup>h</sup>
BWF	678.1 ±21.3 <sup>g</sup>	473.9 ±22.4 <sup>g</sup>	3524.8 ±121.6 <sup>i</sup>
BGR	321.4 ±10.1 <sup>f</sup>	242.0 ±9.1 <sup>h</sup>	1528.3 ±60.8 <sup>i</sup>
QGW	226.1 ±9.4 <sup>d</sup>	100.6 ±7.7 <sup>i</sup>	984.5 ±51.3 <sup>k</sup>
QGR	97.3 ±4.8 <sup>h</sup>	78.2 ±6.2 <sup>b</sup>	826.1 ±41.8 <sup>f</sup>
QGB	100.5 ±5.6 <sup>h</sup>	97.4 ±2.8 <sup>i</sup>	738.9 ±37.2 <sup>l</sup>

Note: Means within a column with at least one identical superscript are not significantly different by Student's t-test ( $p < 0.05$ ).



**Figure 1** IC50 values (DPPH test) (mg.mL<sup>-1</sup>) of buckwheat samples.



**Figure 2** Correlations between FP and TAC values (DPPH, ABTS tests) of pseudocereals.

Pseudocereal samples exhibited similar order of samples for FP and TAC values (DPPH and ABTS assays). The relationships between them are shown by the correlations in Figure 2. They are strongly related to a correlation factors  $r = 0.9666$  and  $0.9565$ , respectively. In the research of **Sun and Ho (2005)** there was also found a significant correlation (0.96) between polyphenols content and antioxidant activity (DPPH method), in buckwheat extract. In amaranth and quinoa extracts there were reported weak correlations between polyphenols content and antioxidant activity by **Nsimba, Kikuzaki and Konishi (2008)** study.

## CONCLUSION

Pseudocereals contain bioactive compounds such as phenolic compounds, flavonoids that can possess positive health effects on the body.

Amaranth, buckwheat, and quinoa were evaluated by spectrophotometric methods for the determination of free phenols amount and total antioxidant capacity. Free phenols in pseudocereals were in the range from 12.4 to 678.1 mg GAE.100g<sup>-1</sup>. The highest contents of FP were found in buckwheat products; quinoa and amaranth products reached much lower values (up to 226.1 mg GAE.100g<sup>-1</sup>). Evaluated antioxidant activity, the highest TAC values were determined again for buckwheat products (up to 473.9 (DPPH test) and 3524.8 (ABTS test) mg TE.100g<sup>-1</sup>), followed by quinoa (up to 100.6 and 984.5 mg TE.100g<sup>-1</sup>, respectively) and amaranth ones (up to 69.7 and 431.4 mg TE.100g<sup>-1</sup>, respectively). Antioxidant capacity values by two evaluation methods (DPPH, ABTS) are in agreement with polyphenols content order. The highest content of free phenols, and also the best antioxidant potential, showed buckwheat wholemeal flour. Our study is generally in agreement with the findings of previously reported researches focused on pseudocereals. Buckwheat therefore could be characterized as a great source of free phenols with high antioxidant activity and thus could be used as seed for production of high nutritional quality products, especially for people who do not could eat cereal products due to the gluten presence. Buckwheat seeds could be also added to other cereal products to heighten their nutritional quality.

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