

Cultivar Influence on Total Polyphenol and Rutin Contents and Total Antioxidant Capacity in Buckwheat, Amaranth, and Quinoa Seeds

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Abstract

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Five cultivars from each of the three types of pseudocereals, i.e. buckwheat, amaranth, and quinoa, were studied for total polyphenol and rutin contents as well as total antioxidant capacity of seeds. A spectrophotometric method was used for the determination of total polyphenol content (using the Folin-Ciocalteu reagent) and total antioxidant capacity (using DPPH). Rutin content in pseudocereal seeds was determined by HPLC. The determined total polyphenol content in seeds of buckwheat, amaranth, and quinoa cultivars was in the intervals of 15 874–71 359 mg/kg DM, 1381–2870 mg/kg DM, and 459–1839 mg/kg DM, respectively. Rutin content in buckwheat, amaranth, and quinoa seeds was in the intervals of 8722–17 125 mg/kg DM, 310–508 mg/kg DM, and 170–368 mg/kg DM, respectively. The presented results confirmed a statistically significant influence of cultivar on total polyphenol and rutin contents as well as on total antioxidant capacity of pseudocereal seeds.

Keywords: pseudocereals; phenolics; rutin

As a consequence of negative changes in the environment as well as in the lifestyle of modern civilisation, variability and number of so-called lifestyle diseases have been rapidly increasing. Among the factors contributing to this negative phenomenon are improper nutrition habits as well as improper composition of foods with excessive energetic intake, high consumption of lipids, saccharides and salt, and also insufficient intake of fibre and vitamins. A tendency to change this situation is the priority not only for physicians and experts in human nutrition by more intensive education of the population, but also for producers of foodstuffs who should offer products with declared higher contents of positively acting biological substances.

In recent years rediscovered pseudocereals like as buckwheat (*Fagopyrum esculentum* Moench), amaranth (*Amaranthus* L.) and quinoa (*Chenopodium quinoa* Willd.) have become very popular in the human nutrition. The research has been focused on these plants because of their potential to enable the development of functional foods (HOZOVÁ *et al.* 2007).

The renewed interest is due to their excellent nutrient properties and also to their suitability for the preparation of gluten-free foodstuffs (KRUPA-KOZAK *et al.* 2011). They are good sources of good-quality proteins, dietary fibre, lipids rich in unsaturated fatty acids, vitamins and several other protective compounds such as phenolic acids or

flavonoids with high antioxidant, anticarcinogenic and other positive activities (CHRISTA & SORAL-ŠMIETANA 2008; GROBELNIK MLAKAR *et al.* 2009; JANCUROVÁ *et al.* 2009). Amaranth is a rich source of polyphenols (flavonoids) with relative high antioxidant activity. Caffeic acid, p-hydroxybenzoic acid, and ferulic acid are the main phenolic compounds in amaranth grains (KLIMCZAK *et al.* 2002). BARBADELAROSA *et al.* (2009) also detected low levels of quercetin glycoside, rutin (4.0–10.1 µg/g dry matter – DM) in amaranth seeds. Quinoa seeds are also an abundant source of flavonoids. The main polyphenols present in quinoa are kaempferol and quercetin glycosides. According to PAŠKO *et al.* (2008) quinoa seeds contained at least flavonoids – mainly orientin (1076 mg/kg DM), vitexin (709 mg/kg DM) and rutin (360 mg/kg DM), but also morin (88,9 mg/kg DM) and traces of hesperidin (1.86 mg/kg DM) and neohesperidin (1.93 mg/kg DM).

The aim of this study was to compare polyphenol and rutin content as well as total antioxidant capacity of different selected buckwheat, amaranth and quinoa cultivars and to evaluate the cultivar influence on these investigated parameters of crop seeds.

MATERIAL AND METHODS

Plant material. Five cultivars from each of the three types of pseudocereals, i.e. buckwheat (Špačinská, Siva, Bamby, Aiva, Madawska), amaranth (Golden Giant, Rawa, Annapurna, Oscar Blanco, Koniz), and quinoa (Temuco, Quinoa, Yulai, Carmen, Ccankolla), were obtained from Plant Production Research Center in Piešťany, Slovak Republic. All cultivars are registered in the EU. The pseudocereal seeds were manually separated, dried at 105°C to constant weight (WTC Binder; Binder GmbH, Tuttlingen, Germany), and powdered (Fritsch Pulverisette, Idar-Oberstein, Germany).

Extract preparation. Seed extracts were prepared by adding 25 ml of 80% methanol (Sigma-Aldrich, St. Louis, USA) to 1 g of milled sample. The mixture was shaken at room temperature for 8 h at 250 rpm. Samples were then filtered through filter paper (130 g/m; Filtrak, Bärenstein, Germany) and kept at 8°C for further analysis.

Determination of total polyphenol content (TP). The total polyphenol content was estimated using the Folin-Ciocalteu reagent (Merck, Darm-

stadt, Germany) according to LACHMAN *et al.* (2003). Sample extract (0.05–1 ml to the expected polyphenol content), 2.5 ml Folin-Ciocalteu reagent and 3–5 ml H₂O were added to a 50-ml flask. After 3 min 7.5 ml of 20% Na₂CO₃ (Sigma-Aldrich, St. Louis, USA) were added to the flask and diluted to 50 ml with H₂O. The mixture was incubated at laboratory temperature for 2 h and the absorbance was measured at 765 nm with a Shimadzu 710 spectrophotometer (Shimadzu, Kyoto, Japan) against the blank sample. The total polyphenol content was expressed as gallic acid equivalents (GAE) in mg/kg DM. The linearity range for this assay was determined at 200–1000 mg/ml ($R^2 = 0.998$).

Determination of total antioxidant capacity (TAC). For the analysis of free radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used according to the protocol in BRAND-WILLIAMS *et al.* (1995). To obtain a stock solution, 0.025 g of DPPH (Sigma-Aldrich, St. Louis, USA) was diluted to 100 ml with methanol and kept in a cool and dark place. Immediately before the analysis, a 1:10 dilution of the stock was made with methanol. For the analysis, 3.9 ml of the DPPH working solution was added to a cuvette and the absorbance at 515 nm was measured (A_0) with a Shimadzu 710 spectrophotometer (Shimadzu, Kyoto, Japan). Subsequently, 0.1 ml of the extract was added to the cuvette with DPPH, and the absorbance was measured after 10 min (A_{10}).

An increasing amount of antioxidants present in the methanol extract of the sample reduced DPPH and faded the colour of the solution in a correlation proportional to the antioxidant concentration. The percentage of DPPH inhibition was measured according to the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_{10})/A_0] \times 100$$

The antioxidant capacity of the sample was then expressed as mmol Trolox equivalents (TE) per 1 kg DM.

Determination of rutin content. Sample extracts were filtered through a syringe filter unit (0.22 µm; Millipore, Billerica, USA). The filtrate was injected into a high performance liquid chromatography (HPLC) system that consisted of an Alliance 2695 chromatograph (Waters, Arlington, USA) and LiChroCART Purospher RP C18 column (5 µm, 250 ± 4.6 mm; Merck, Darmstadt, Germany) and DAD 2996 UV detector (Waters, Arlington, USA). The column temperature was 30°C. Gradient elu-

tion of the mobile phase was used with a flow rate of 1 ml/min. Solvent A was acetonitrile and solvent B was 0.1% phosphoric acid. The solvent gradient was as follows: the concentration of solvent A was 40% for the first 3 min, 5% for the next 5 min, and 5% for additional 2 minutes. The concentration of solvent B was 60% for the first 3 min, 95% for the next 5 min, and 95% for additional 2 minutes. The presence of rutin was detected at 365 nm, and the content was calculated on the basis of the calibration curve of rutin standards (Acros Organics, Waltham, USA) prepared in methanol (gradient elution grade; Sigma-Aldrich, St. Louis, USA). Results were expressed as mg/kg DM (dry matter). The linearity range for this assay was determined at 0.05–0.25 mg/ml ($R^2 = 0.9946$).

Statistical analysis. Statistica 8 (StatSoft, Inc., Tulsa, USA) was used for the statistical processing of results. One-way ANOVA was used. Mean comparisons between cultivars were done by Fisher's LSD (Least Significant Difference) test.

RESULTS AND DISCUSSION

In the group of cereals and pseudocereals buckwheat is one of the best sources of polyphenols with a high antioxidant capacity (Table 1). The determined total polyphenol content in seeds of the studied buckwheat cultivars was in the range from 15 874 mg/kg DM (cv. Špačinská) to 71 359 mg/kg DM (cv. Bamby).

ALVEREZ-JUBETE *et al.* (2010) determined a nearly tenfold lower total polyphenol content (3230 mg/kg DM) in buckwheat samples in comparison with our average (32 089 mg/kg DM). On the other hand, TAHIR and FAROOQ (1985) determined the total polyphenol content in the interval of 7700–16 600 mg/kg DM depending on the buckwheat variety. Our results correspond with the results of KREFT and GERM (2008), who found from 5000 mg/kg to 45 000 mg/kg of polyphenols in buckwheat seeds. According to KREFT *et al.* (2006) the high antioxidant capacity of buckwheat seeds is connected with high polyphenol content, especially high content of rutin.

Based on the data on total antioxidant capacity of buckwheat seeds the following order of the studied cultivars: Madawska > Bamby > Špačinská > Aiva > Siva can be created. Rutin content in our buckwheat samples was in the interval of 8722 mg/kg DM (cv. Madawska) to 17 125 mg/kg DM (cv.

Bamby). These results are higher than the results of ZIELIŃSKA *et al.* (2012a), who determined rutin in seeds of common and tartary buckwheat in the amount of 43 and 13 500 mg/kg DM, respectively. The analysis results show that there are highly significant differences in total polyphenol and rutin contents as well as in total antioxidant capacity between the studied buckwheat cultivars. The significant influence of cultivar on rutin content in buckwheat seeds was confirmed also by YAN *et al.* (2004). ZIELIŃSKA *et al.* (2010) confirmed that the contribution of rutin to the antioxidant capacity of selected buckwheat samples was low, and it was concluded that the attention paid to rutin as the main antioxidant in buckwheat can be overestimated. In another study ZIELIŃSKA *et al.* (2012b) found out that the rutin and flavone C-glucoside contents in the groats from common buckwheat cultivars and the interspecific hybrid were lower than those noted in the hulls. In contrast, groats and hulls from a tartary buckwheat accession contained a comparably high level of flavonoids.

In seeds of the studied amaranth cultivars (Table 1) the total polyphenol content was determined to be in the interval of 1381 mg/kg DM (cv. Rawa) to 2870 mg/kg DM (cv. Annapurna).

Our values of total polyphenol content are lower than those of ODUKOYA *et al.* (2007), who measured 3121.8–4063 mg/kg DM. On the other hand, OKARTER (2012) determined nearly tenfold lower values (238 mg/kg DM) in comparison with our results. Our results correspond to the results of GUZMAN-MALDONADO & PEREZ-LOPEZ (1998), who reported the total polyphenol content in amaranth seeds in the interval of 2000–4000 mg/kg DM. PAŠKO *et al.* (2009) analysed seeds of two amaranth cultivars and they determined similar values of total polyphenol content (2950 and 3000 mg GAE/kg DM, respectively).

On the other hand, DLAMINI *et al.* (2010) determined in amaranth seeds 17 600 mg GAE/kg DM. These values are comparable with our results. The order of the studied amaranth cultivars from the aspect of the total antioxidant capacity of seeds is as follows: Oscar Blanco > Annapurna > Rawa > Koniz > Golden Giant. SKWARYLO-BEDNARZ and KRZEPILKO (2009) also confirmed the influence of amaranth cultivar on the total antioxidant capacity of seeds.

In cv. Annapurna the highest rutin (508 mg/kg DM) as well as total polyphenol contents were

Table 1. Total polyphenol content (TP) (mg/kg DM), total antioxidant capacity (TAC) (mmol TE/kg DM), and rutin content (mg/kg DM) in investigated buckwheat, amaranth, and quinoa cultivars

	TP (mg GAE/kg DM)	TAC (mmol TE/kg DM)	Rutin (mg/kg DM)
Buckwheat			
Golden Giant	2548.75 ± 114.75 ^b	2.32 ± 0.49 ^a	481.25 ± 15.72 ^c
Rawa	1381.05 ± 77.68 ^a	3.00 ± 0.40 ^b	347.22 ± 20.81 ^b
Annapurna	2869.90 ± 74.29 ^b	3.90 ± 1.46 ^c	507.78 ± 17.11 ^d
Oscar Blanco	1634.10 ± 61.51 ^a	4.64 ± 0.57 ^d	309.97 ± 10.84 ^a
Koniz	1807.81 ± 128.68 ^a	2.46 ± 0.51 ^{ab}	483.88 ± 9.46 ^c
Amaranth			
Špačinská	15 873.71 ± 111.39 ^a	13.04 ± 0.16 ^c	12 277.75 ± 382.04 ^c
Siva	21 155.92 ± 108.32 ^b	10.95 ± 0.01 ^a	14 761.00 ± 502.53 ^d
Bamby	71 359.17 ± 325.62 ^d	20.21 ± 0.00 ^d	17 124.75 ± 283.99 ^e
Aiva	21 017.63 ± 92.74 ^b	11.40 ± 0.32 ^b	11 552.25 ± 708.44 ^b
Madawska	31 040.88 ± 169.76 ^c	23.66 ± 0.01 ^e	8 721.75 ± 176.08 ^a
Quinoa			
Temuco	1053.18 ± 106.05 ^b	7.38 ± 0.04 ^b	287.75 ± 9.51 ^b
Quinoa	1839.18 ± 45.67 ^d	2.60 ± 0.98 ^a	367.52 ± 22.96 ^d
Yulai	1069.30 ± 62.79 ^b	10.80 ± 0.06 ^c	283.25 ± 5.73 ^b
Carmen	459.36 ± 57.88 ^a	10.79 ± 0.08 ^c	326.26 ± 15.07 ^c
Ccankolla	1614.44 ± 88.27 ^c	12.40 ± 0.10 ^d	170.00 ± 9.11 ^a

Average values ± standard deviation marked with the same letter are not significantly different ($P < 0.05$)

determined. The lowest rutin content was determined in cv. Oscar Blanco (310 mg/kg DM). Our values correspond to the results of KALINOVA and DADAKOVA (2009), who determined the rutin content in amaranth seeds in the interval of 127–592 mg/kg DM. Similar conclusions were drawn by PAŠKO *et al.* (2009), who analysed two amaranth genotypes.

On the other hand, BARBADELAROSA *et al.* (2009) found no significant differences in rutin content among four different amaranth cultivars.

The analysis results show that there are significant differences in total polyphenol and rutin contents as well as in total antioxidant capacity among the studied amaranth cultivars.

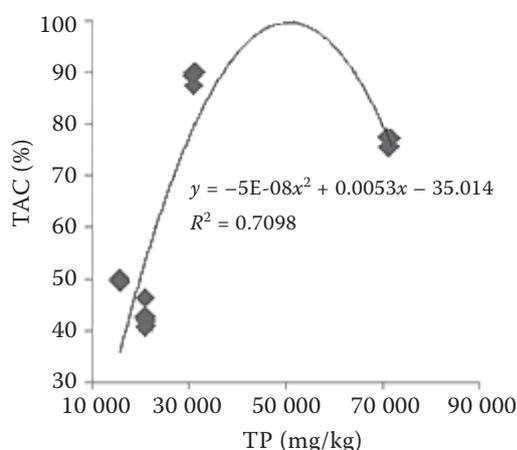


Figure 1. Relationship between total polyphenol (TP) and total antioxidant capacity (TAC) in buckwheat seeds

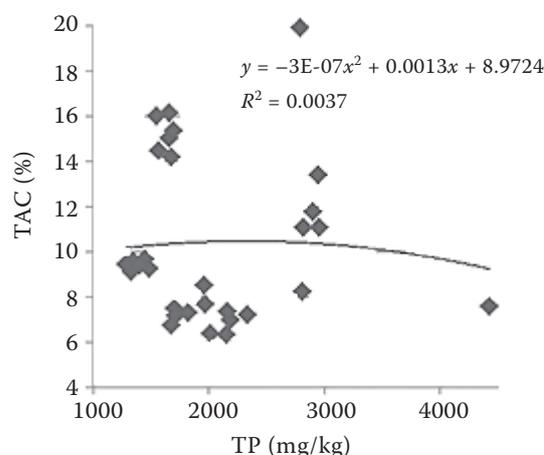


Figure 2. Relationship between total polyphenol (TP) and total antioxidant capacity (TAC) in amaranth seeds

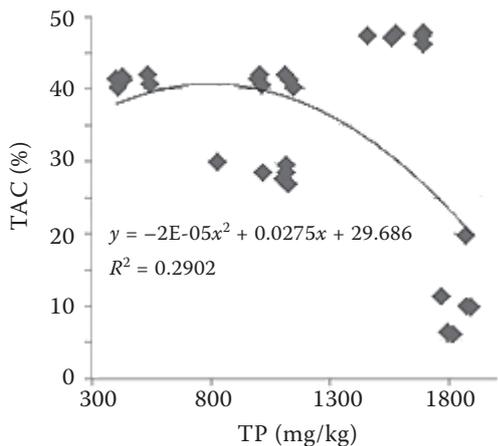


Figure 3. Relationship between total polyphenol (TP) and total antioxidant capacity (TAC) in quinoa seeds

Our values of the total polyphenol content in quinoa seeds (Table 1) were in the interval of 459 mg/kg DM (cv. Carmen) to 1839 mg/kg DM (cv. Quinoa). These results correspond to those of REPO-CARRASCO-VALENCIA (2011) and MIRANDA *et al.* (2010), who reported phenolics content in the range of 1420–1970 and 1590–2841 mg/kg DM, respectively. Similar results (717 mg/kg DM) were presented also by ALVAREZ-JUBETE *et al.* (2010). Based on values of the total antioxidant capacity of seeds the studied quinoa cultivars can be ranked as follows: Ccankolla > Yulai > Carmen > Temuco > Quinoa. Similar values of the total antioxidant capacity of quinoa seeds (38.84%) were presented also by PAŠKO *et al.* (2009).

The highest content of rutin (368 mg/kg DM) as well as polyphenols was determined in cv. Quinoa, while in this cultivar the lowest value of total antioxidant capacity was measured. In seeds of cv. Ccankolla the lowest rutin content of 170 mg/kg

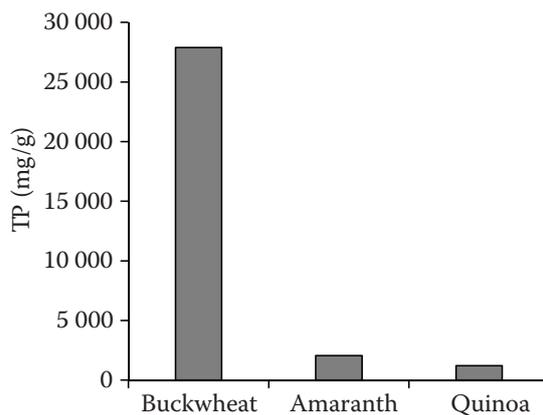


Figure 4. Average total polyphenol (TP) content

DM was determined. The determined values of rutin content in quinoa seeds are similar to those (360 mg/kg DM) determined by PAŠKO *et al.* (2008).

The analysis results show that there are significant differences in total polyphenol and rutin contents as well as in total antioxidant capacity among the studied quinoa cultivars.

Figure 1 shows a correlation between total polyphenol content and total antioxidant capacity of buckwheat seeds. Based on the correlation coefficient ($R = 0.843$), a strong statistical dependence between the studied parameters can be confirmed only in buckwheat seeds. Only a weak dependence ($R = 0.061$) between total polyphenol content and total antioxidant capacity of amaranth seeds was proved (Figure 2). Between total polyphenol content and total antioxidant capacity of quinoa seeds a medium dependence ($R = 0.538$) was confirmed (Figure 3).

Based on the data of average total polyphenol (Figure 4) and rutin (Figure 5) contents in seeds

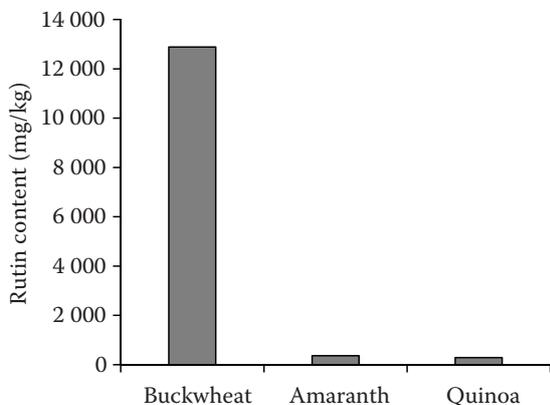


Figure 5. Average rutin content

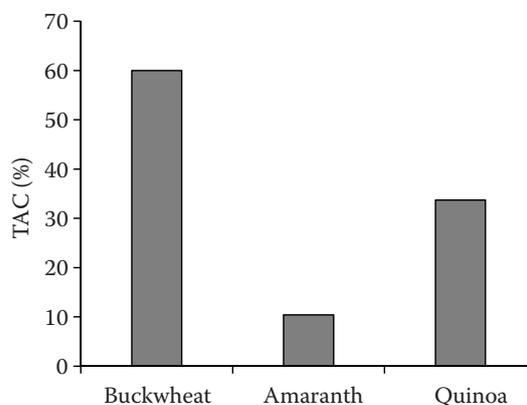


Figure 6. Average total antioxidant capacity (TAC)

of the studied buckwheat, amaranth and quinoa cultivars the order of the crops is as follows: buckwheat > amaranth > quinoa.

A different order is associated with average values of total antioxidant capacity (Figure 6) of the studied pseudocereal cultivar seeds: buckwheat > quinoa > amaranth.

CONCLUSION

Pseudocereals such as buckwheat, amaranth and quinoa are excellent sources of highly appreciated nutritional components as well as other bioactive compounds with benefits for the human health. Based on the average values of determined total polyphenol content in seeds of the studied buckwheat, amaranth and quinoa cultivars the order of the crops is as follows: buckwheat (32 089 mg GAE/kg DM) > amaranth (2048 mg GAE/kg DM) > quinoa (1207 mg GAE/kg DM). The same order is confirmed based on the average values of determined rutin content in seeds of pseudocereals: buckwheat (12 887 mg/kg DM) > amaranth (426 mg/kg DM) > quinoa (287 mg/kg DM). A different order is associated with average values of total antioxidant capacity: buckwheat (15.92 mmol TE/kg DM) > quinoa (8.79 mmol TE/kg DM) > amaranth (3.26 mmol TE/kg DM). The presented results confirmed a significant influence of cultivar on total polyphenol and rutin contents as well as on the total antioxidant capacity of pseudocereal seeds. The highest total polyphenol as well as rutin contents were determined in cvs Bamby (buckwheat), Annapurna (amaranth), and Quinoa (quinoa). The selection of the most suitable cultivars from the aspect of the highest presence of positively acting components is very important especially for functional food producers.

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