Food Chemistry 115 (2009) 994–998

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth

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ARTICLE INFO

Article history:
Received 17 July 2008
Received in revised form 22 September 2008
Accepted 12 January 2009

Keywords:
Amaranth
Quinoa
Antioxidant
Seed
Sprout

ABSTRACT

Total antioxidant capacity, total phenolic contents (TP) and anthocyanins contents (ANT) were determined in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts. Antioxidant activity of the investigated seeds decreased in the following order: quinoa, amaranth v. Rawa, amaranth v. Aztek for FRAP and quinoa, amaranth v. Aztek, amaranth v. Rawa for both ABTS and DPPH. Sprouts activity depended on the length of their growth, and the peak values were reached on the fourth day in the case of amaranth and on the sixth day in the case of quinoa. The data obtained by the three methods showed significant correlation between TP content in seeds and sprouts. In sprouts grown in the daylight and in the darkness we observed some significant changes of TP, ANT and antioxidant activity. Amaranth and quinoa seeds and sprouts can be used in food, because it is a good source of ANT and TP with high antioxidant activity.

1. Introduction

In the last decade, the use of amaranth and quinoa has broadened not only in the common diet, but also in diet of people with celiac disease or allergies to typical cereals (Berti, Riso, Brusamolino, & Porrini, 2005). These pseudocereals seeds have high nutritional and functional values which are associated with the quality and quantity of their proteins, fats and antioxidant potential (Gorinstein et al., 2002; Gorinstein et al., 2007; Paśko, Bartoń, Fołta, & Gwizdz, 2007). A new way in nutrition, in recent years, is the consumption of sprouts – the atypical vegetable, which have received attention as functional foods, because of their nutritive value including amino acid, fibre, trace elements and vitamins as well as flavonoids, and phenolic acids (Paśko, Sajewicz, Gorinstein, & Zachwieja, 2008). Consumption of seeds and sprouts has become increasingly popular among people interested in improving and maintaining their health status by changing dietary habits. The seeds and sprouts are excellent examples of 'functional food' defined as lowering the risk of various diseases and/or exerting health promoting effects in addition to its nutritive value. However, most of the recently published papers are focused mainly on the studies of typical sprouts such as buckwheat, broccoli, mung bean, and soybean, which are already easily available on the market. The sprouts of amaranth and quinoa are "new" vegetables, which can be used in the nutrition of vegans and vegetarians and as a common diet too.

The aim of our study was to show the nutritional value of sprout as a good source of antioxidants. The model of plant-based diet, containing whole-grain products and vegetables as primary ingredients has become one of the most important guidelines for reducing the risk of diseases caused by the increased level of free radicals. Amaranth and quinoa sprouts can be used to flavour salads and sandwiches as well as additional components of bread. We decided to compare the antioxidant potential of seeds and sprouts of selected pseudocereals by the following methods: FRAP, ABTS and DPPH. We investigated also total polyphenols and anthocyanins content.

2. Materials and methods

2.1. Materials

Amaranth seeds (*Amarantus cruentus* varieties Aztek and Rawa) were cropped in eastern Poland, and quinoa seeds (*Chenopodium quinoa*) were imported from Bolivia.

De-ionised water 18 MΩcm was obtained from a Milli Ro & Q water purification system, (Millipore); methanol, acetone, acetonitrile, acetic acid, hydrochloric acid 36%, ferric chloride ($FeCl_3$), phosphate buffered saline (PBS) was from Merck, 2,2'-azinobis

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doi:10.1016/j.foodchem.2009.01.037

(3-ethylobenzothiazoline-6-sulphonate) (ABTS), triphenyltriazine (TPTZ) – 2,4,6-tris(2-pyridyl)–1,3,5-triazine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (trolox), sodium peroxydisulfate, Folin – Ciocalteu reagent, and gallic acid were purchased from Sigma. All reagents were of analytical grade.

2.2. Plant materials and growth conditions

Amaranth and quinoa seeds were immersed in water for 3 h and then put into a glass vessels. Sprouts were grown for 4, 6 or seven days after seeding (DAS) at fixed temperature of 20 ± 2 °C. They were watered every day. Half of the culture was stored in natural conditions (daylight), while the rest in the darkness all of the time. Sprouting was very difficult to carry out and, especially for quinoa, exhibited low efficiency level.

The duration of this period was based on the laboratory observation that 4–7 days proved to be optimal for growing this species of sprouts. In shorter periods sprouts do not develop sufficiently, in longer – they overgrow.

After 4, 6 and 7 days, sprouts were harvested and extracted immediately. Only the remaining portion of samples was stored at −80 °C until they were used for measurement of anthocyanins.

2.3. Extracts preparation

Powdered samples of seeds and blended sprouts (1 g) were extracted with 40 ml of solvent consisting of methanol, 0.16 M hydrochloric acid and water, mixed in proportion 8:1:1, respectively, for 2 h. The extracts were separated by decantation and the residues were extracted again with 40 ml of 70% acetone for 2 h. The initial methanol extracts were added to prepare a mixture which was subsequently decanted, centrifuged and stored in darkness in a freezer at a temperature of −20 °C.

2.4. Determination of FRAP activity

FRAP (Ferric Reducing Ability of Plasma) assay was conducted at 37 °C and pH 3.6. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction causes formation of intensive blue coloured ferrous-tripyridyl-s-triazine complex with absorbance maximum at 593 nm. Absorbance was measured after 8 min and was proportional to the combined ferric reducing/antioxidant power of the antioxidants in the extracts. The final results were expressed as mmol Fe^{2+} kg^{-1} DW (Benzie & Strain, 1996).

2.5. Determination of ABTS radical scavenging activity

ABTS radical scavenging measurements were performed according to Re et al., 1999 with modifications described previously (Bartoń, Fołta, & Zachwieja, 2005). ABTS radical cation was generated by the interaction of ABTS and $Na_2S_2O_8$. For measurement of sample scavenging activity, 2 ml of ABTS were added to the cuvettes containing the pre-diluted samples (0.15, 0.3, 0.45, 0.6, 1.0 ml extracts with addition of 0.85, 0.7, 0.55, 0.4, and 0 ml PBS respectively). The absorbance was measured after 6 minutes at the wavelength of 734 nm. The total antioxidant capacities (TAC) were estimated as trolox equivalents (TEAC) interpolation to 50% inhibition ($TEAC_{50}$).

2.6. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Yen and Chen (1995) with modification of Bartoń and Fołta (2006). For measurement of sample scavenging activity, 0.4 ml of methanolic acetate buffer was added to the cuvettes containing the increasing volumes of sample (e.g. 0, 0.1, 0.2, 0.3, 0.45, 0.6 ml) with adequate volumes of methanol to make total volume of 1 ml. One millilitre of DPPH stock solution (absorbance 1.3) was added to each cuvette, then absorbance was measured after 24 h. The absorbance of the resultant solution was determinated using a Jasco UV-530 spectrometr (Japan) at 514 nm. The total antioxidant capacities (TAC) were estimated as trolox equivalents (TEAC) by interpolation to 50% inhibition ($TEAC_{50}$) (Bartoń et al., 2005).

2.7. Determination of total phenolics

Total phenolics (TP) were determined colorimetrically using Folin–Ciocalteau reagent, as described by Emmons, Peterson, and Paul (1999), with modifications. Total phenolic assay was conducted by mixing 2.7 ml of de-ionised water, 0.3 ml of extracts, 0.3 ml 7% Na_2CO_3 and 0.15 ml Folin–Ciocalteu reagent. Absorbance of mixture was measured at 725 nm. A standard curve was prepared with gallic acid. Final results were given as gallic acid equivalents (GAE).

2.8. Determination of anthocyanins

Absorbance of extracts (1 g of the defatted sample was extracted with 1 ml of acetonitrile containing 4% acetic acid) was measured at 510 nm and 700 nm in buffers at pH 1.0 and 4.5, and calculated using following equation: $A = [(A_{510}-A_{700})_{pH1.0} - (A_{510}-A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidine-3-glucoside of 29 600. Results were expressed as milligrams of cyanidine-3-glucoside equivalent (CGE) 100 g^{-1} DW (Cheng & Breen, 1991).

2.9. Statistical analysis

Values are given as means ± SD of four measurements. Where appropriate, the data were tested by one-way ANOVA using Statistica 5.1 (StatSoft, Ic., 1997), followed by Tukey post hoc test. Pearson correlation coefficients and p-values were used to show correlations and their significance. Differences of $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Antioxidant activity of seeds

The investigated extracts of pseudocereals seeds showed (Table 1) the following order of antioxidant activity by the FRAP (mmol Fe^{2+} kg^{-1} DW) method: quinoa (4.97) > amaranth v. Rawa (3.73) > amaranth v. Aztek (3.37). These above data are similar to those reported by Nsimba, Kikuzaki, and Konisi (2008): however, they evaluated TAC by the FRAP method using different types of extracts. A comparison between FRAP of pseudocereals recalculated into adequate units and other grains and pseudocereals was based on data given by Halvorsen et al. (2002), whose work is genuinely essential in this field of research. Since their results are expressed in mmol Fe^{2+} kg^{-1} FW, we used aforementioned units as well, in order to facilitate the comparison. FRAP values of amaranth and quinoa seeds were lower than those of oat and higher than rice.

Also, by the ABTS method, the highest TAC (mmol trolox kg^{-1} DW) value was observed in quinoa seeds (27.2 ± 2). The lowest values are observed in amaranth v. Aztek and v. Rawa; 12.7 ± 1.1 and 11.4 ± 1.2 ($p < 0.05$), respectively, ABTS value was significantly higher in quinoa seeds as compared with amaranth seeds (Table 1). In comparison with literature data on pseudocereals and cereals, the present data about quinoa using the ABTS method were higher than TAC presented by Penarrieta, Alvarado, Akesson, and

Table 1
Antioxidant activity, anthocynanins and polyphenols content of investigated seeds*.

Seeds	FRAP	ABTS $TEAC_{50}$	DPPH $TEAC_{50}$	ANT	TP
Amaranthus cruentus v. Aztek	3.37 ± 0.40	12.71 ± 1.1	4.42 ± 0.5	103.6 ± 10.4	2.95 ± 0.07
Amaranthus cruentus v. Rawa	3.73 ± 0.20	11.42 ± 1.2	3.15 ± 0.3	90.83 ± 9.2	3.0 ± 0.42
Chenopodium quinoa	4.97 ± 0.15	27.19 ± 2.03	38.84 ± 1.63	120.4 ± 7.2	3.75 ± 0.05

* FRAP in mmol Fe^2 + kg^{-1} DW, ABTS and DPPH in mmol trolox kg^{-1} DW, anthocyanins – ANT mg CGE 100 g^{-1} DW, total polyphenols – TP mg GAE g^{-1} DW ± SD (n = 4).

Bergenstahl (2008) and TAC found in wheat (Yu, Haley, Perret, Harris, & Qian, 2002), (Gallardo, Jimenez, & Garcia-Conesa, 2006). Our results were close to data obtained for rye (Gallardo et al., 2006) and lower than barley, oat and buckwheat (Zieliński & Kozłowska, 2000). The ABTS value of amaranth seeds were lower than TAC values found in barley, oat and buckwheat (Zieliński & Kozłowska, 2000) and higher then wheat bran (Yu et al., 2002).

The TAC value by the DPPH method (mmol trolox kg^{-1} DW) was in the range of 3.15–38.84, the highest value was observed in quinoa seeds and the lowest scavenging activity in amaranth v. Rawa but the value of amaranth v. Aztek (4.42) was similar to v. Rawa (Table 1). DPPH value was significantly higher in quinoa seeds as compared with amaranth seeds, and Nsimba et al. (2008) observed a similar effect. The TAC (DPPH) values obtained in amaranth seeds were comparable to or lower than data obtained in different varieties of wheat (Yu et al., 2002). DPPH values were significantly lower than ABTS values for all seeds' samples of amaranth. In quinoa seeds we observed the opposite effect. There was a strong correlation between ABTS and DPPH (r = 0.98), which was also observed previously (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). We observed correlation between TP and TAC from all antioxidative activity methods used (all r > 0.97), which was also observed previously by Nsimba et al. (2008).

3.2. Antioxidant activity of sprouts

Sprouts activity depended on the length of their growth, and the peak value (in all methods used) was reached on the fourth day in the case of amaranth and on the sixth day in the case of quinoa.

The sprouts of amaranth v. Aztek demonstrated (Fig. 1) the highest antioxidant activity (AA) by the FRAP method (mmol Fe^{2+} kg^{-1} DW); the sprouts of amaranth v. Rawa AA had lower FRAP score (Fig. 2), and the quinoa sprouts had the lowest AA (Fig. 3). In each case, the AA of sprouts grown in daylight was higher than of those grown in the darkness. The differences in AA between sprouts grown in the daylight and in darkness were significant in amaranth sprouts. The TAC (FRAP) (mmol Fe^{2+} kg^{-1} FW) values obtained in pseudocereals sprouts (5–17.4, recalculated into adequate units) were comparable to or higher than data obtained in brussels sprouts (13.1) and in different green vegetables: spinach (11.1), broccoli (6.3), radish (4.2) (Halvorsen et al., 2002).

The TAC (ABTS) (mmol trolox kg^{-1} DW) values of investigated pseudocereals sprouts during germination obtained in amaranth v. Aztek (daylight; 133.1–222.1), darkness 99.5–172.5), amaranth v. Rawa (daylight; 112.9–151.3, darkness 78.8–176.1), quinoa (daylight; 116–136, darkness 92–116.9) were comparable to or higher than data obtained in germinated seeds of *Lupinus album* (Frias, Miranda, Doblado, & Vidal-Valverde, 2005). During 7-days germination TAC of amaranth v. Rawa sprouts decreased, and was highest in 4 DAS; this effect did not depend on conditions of tillage (Fig. 2). TAC of amaranth v. Aztek sprouts (daylight)

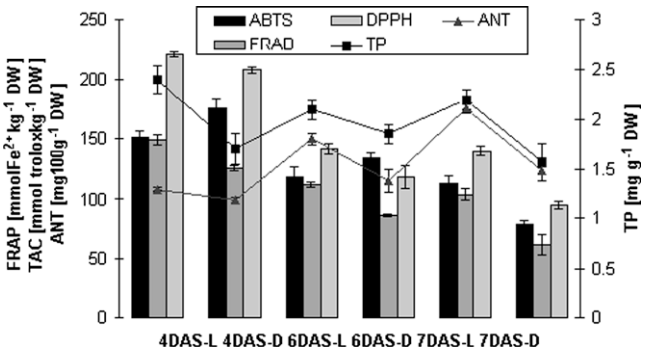

Fig. 2. FRAP, TAC, ANT, and TP for various groups of amaranth v. Rawa sprouts (legend: number DAS – day after seeding, daylight – L, darkness – D, left scale: FRAP in mmol Fe^{2+} kg^{-1} DW, TAC in mmol trolox kg^{-1} DW (TEAC) by DPPH and ABTS method, anthocyanins, ANT in mg CGE 100 g^{-1} DW, right scale: total polyphenols – TP in mg GAE g^{-1} DW).

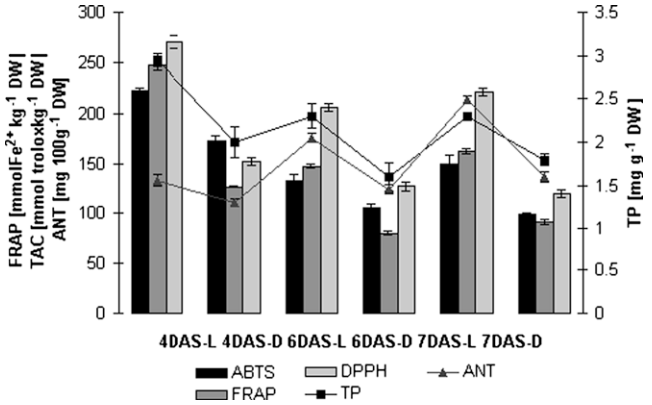

Fig. 1. FRAP, TAC, ANT, and TP for various groups of amaranth v. Aztek sprouts (legend: number DAS - day after seeding, daylight – L, darkness – D, left scale: FRAP in mmol Fe^{2+} kg^{-1} DW, TAC in mmol trolox kg^{-1} DW (TEAC) by DPPH and ABTS method, anthocyanins, ANT in mg CGE 100 g^{-1} DW, right scale: total polyphenols – TP in mg GAE g^{-1} DW).

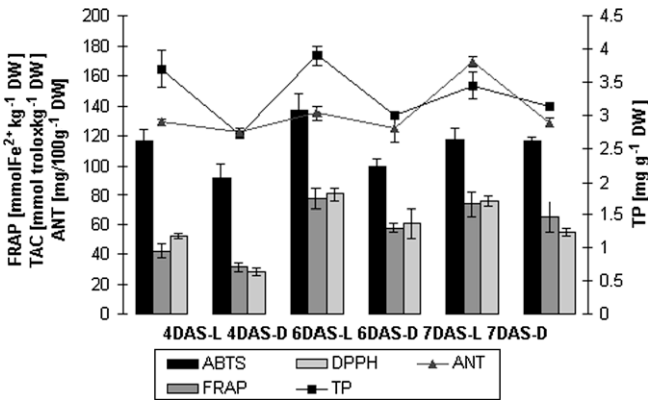

Fig. 3. FRAP, TAC, ANT, and TP for various groups of quinoa sprouts (legend: number DAS – day after seeding, daylight – L, darkness – D, left scale: FRAP in mmol Fe^{2+} kg^{-1} DW, TAC in mmol trolox kg^{-1} DW (TEAC) by DPPH and ABTS method, anthocyanins, ANT in mg CGE 100 g^{-1} DW, right scale: total polyphenols – TP in mg GAE g^{-1} DW).

(Fig. 1) was highest 4 DAS, while 6 DAS the ABTS value decreased significantly (40%). In contrast, the ABTS value decreased during 7-days germination in darkness. During 7-days germination TAC of quinoa sprouts (Fig. 3) was highest 6 DAS (daylight), and 7 DAS (darkness), but the differences were not statistically significant.

Following the evaluation of the antioxidant capacity of the investigated pseudocereals sprouts it was revealed that the treated samples had significantly lower DPPH free radical scavenging activity (mmol trolox kg^{-1} DW) than another sprouts: radish (2600) peas (3000) and red potato (3500) (Kim, Chen, Wang, & Choi, 2006).

The difference in ABTS and DPPH value between amaranth and quinoa sprouts was significant on all DAS (with except in 6-th and 7-th DAS), and it was not dependent on darkness or daylight conditions. Conditions of tillage influenced significantly antioxidant activity of sprouts only in amaranth v. Aztek in all DAS, as determined by ABTS and DPPH methods.

DPPH values were higher than ABTS values for all sprouts samples of amaranth. In quinoa sprout we observed the opposite effect, and DPPH values were significantly lower than DPPH values for the amaranth sprouts. The reason could be the pigments such as anthocyanins, which caused interference leading to underestimation of antioxidant activity when using the DPPH assay (Dykes, Rooney, Waniska, & Rooney, 2005) or content of other compounds. However, the method modification used (Bartoń & Fołta, 2006) is specially dedicated to coloured samples and such interferences are reduced. In publications it was found that when using light conditions during germination of selected cruciferous seeds (rape-seeds, white mustard, radish and small radish), the optimal germination time that would maximise the synthesis of vitamin E was found to be 4 DAS and 5 DAS. Under dark conditions, the optimal germination time was found to be 3 DAS for small radish and 4 DAS for radish and rape-seeds. After these periods, up to 7 DAS the vitamin E content reached a stable level (Zieliński & Kozłowska, 2003). In *Lens culinaris* sprouts after germination for 5 DAS brought a noticeable increase of vitamin C (Frias et al., 2002).

However, there is still a strong significant correlation in all sprouts between results of all methods ABTS and DPPH ($r = 0.87$) (Fig. 4a), FRAP vs. DPPH ($r = 0.98$) (Fig. 4b) and FRAP vs. ABTS ($r = 0.94$) (Fig. 4c). In daylight tillage we observed correlations: FRAP vs. DPPH ($r = 0.96$), ABTS vs. DPPH ($r = 0.92$) and FRAP vs. ABTS ($r = 0.95$). In darkness tillage we observed following correlations DPPH vs. ABTS ($r = 0.91$), FRAP vs. DPPH ($r = 0.92$).

3.3. Total polyphenols

The content of polyphenols (mg GAE g^{-1}) in evaluated pseudocereals (Table 1) was 3.75 ± 0.05, 2.95 ± 0.07, 3.0 ± 0.42 in quinoa, amaranth v. Rawa and amaranth v. Aztek, respectively. The amount of polyphenols in amaranth seeds as compared with quinoa seeds was significantly different. A strong correlation between total polyphenols content and antioxidant activity was observed (TP vs. ABTS, $r = 0.98$: TP vs. DPPH, $r = 0.98$), and these findings suggest that total polyphenols content is a good predictor of in vitro antioxidant activity. Şensoy, Rosen, Ho, & Karwe, 2006, observed lower content of total phenolics of buckwheat white raw flour than in amaranth and quinoa seeds. In black sorghum seeds (Awika, Rooney, & Waniska, 2004) observed higher content of total phenols. The results of total polyphenols content and antioxidant activity in the studied pseudocereals seeds support those authors, who have shown that total polyphenols content increases antioxidant activity in seeds and there is a linear correlation between total

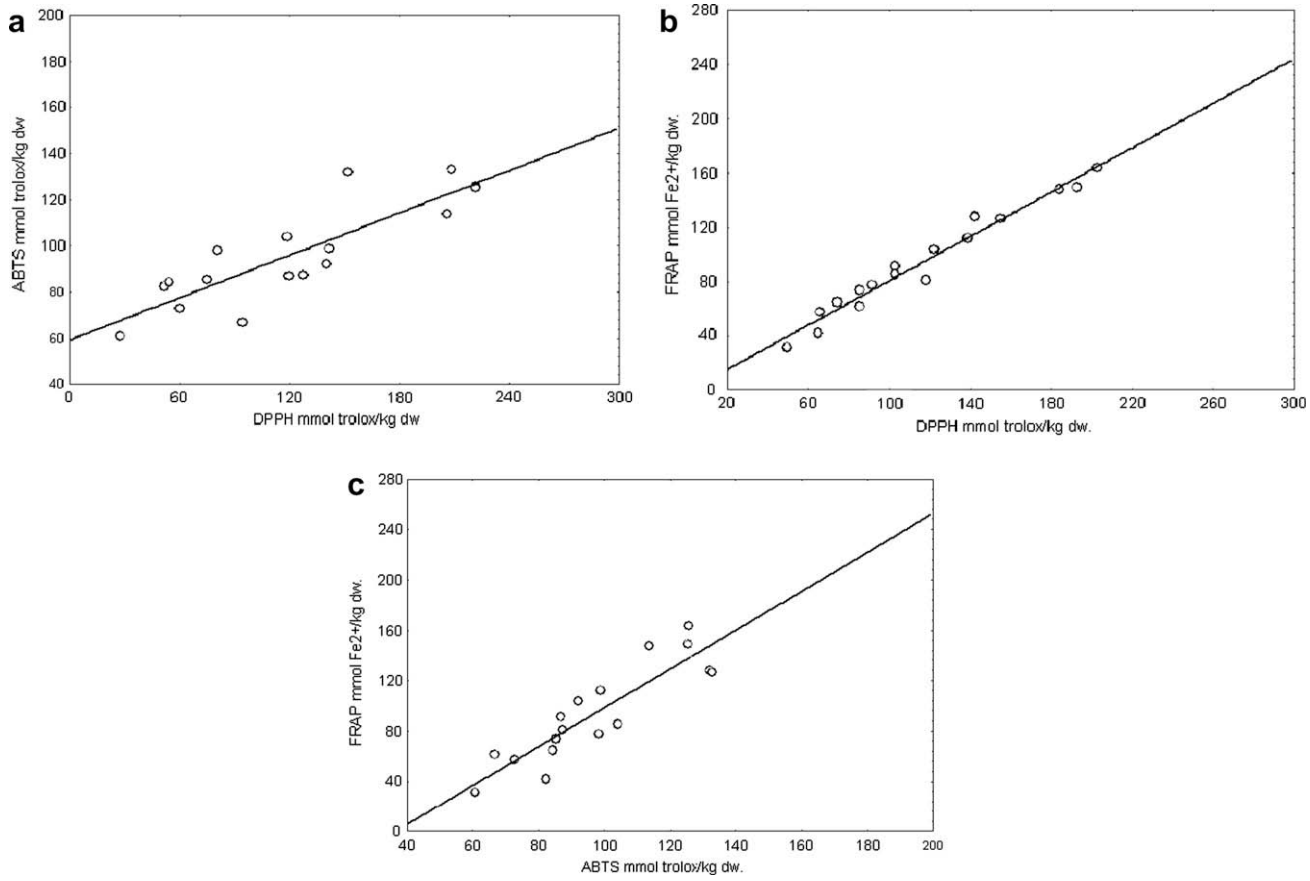

Fig. 4. Correlations between the parameters determined: (A) ABTS vs. DPPH in sprouts ($R^2 = 0.87$, $y = 0.387^{*}x + 51.27$), (B) FRAP vs. DPPH in sprouts ($R^2 = 0.98$, $y = 0.889^{*}x - 8.59$). C) FRAP vs. ABTS in sprouts. ($R^2 = 0.94$, $y = 1.598^{*}x - 61.07$).

phenols content and antioxidant activity (Gorinstein et al., 2007; Zieliński & Kozłowska, 2000).

The content of polyphenols in sprouts (Figs. 1–3) was higher in sprouts of quinoa as compared with amaranth sprouts, and the difference was significant. In sprouts of amaranth and quinoa the content of polyphenols slightly dropped during sprouting. Generally, the content of polyphenols was higher in the sprout in the light than in the darkness, but this difference was not significant. The polyphenols content of four types of amaranth species leaves locally known as spinach (Amin, Norazaidah, & Hainida, 2006) were higher than amaranth and quinoa sprouts.

3.4. Anthocyanins

The content of anthocyanins (ANT) in seeds was shown in Table 1. Our results concerning the amaranth and quinoa anthocyanins contents in seeds were similar to those reported elsewhere (Gorinstein et al., 2007). Compared to the few data on ANT in another cereals and pseudocereals available in the literature, our ANT values were higher than jasmine rice, Amaranthus hybridus, soybean (Gorinstein et al., 2007) and black sorghum (Awika et al., 2004). ANT values of amaranth and quinoa seeds were lower than those of rice bran (Gorinstein et al., 2007).

The results indicated that an increase of anthocyanins in sprouts (Figs. 1–3) was due to the sprouting day and effect of light. The sprouts grown in lightness were purplish red colour, especially amaranth's, but sprouts grown in darkness were yellowish colour. The sprouts that were grown in daylight had higher content of anthocyanins than sprouts grown in darkness, but no significant differences were found in quinoa sprouts. In 6th and 7th DAS we found significant differences in content of anthocyanins in amaranth sprouts, and this effect was not depend on amaranth varieties, but in Aztek sprouts the content of anthocyanins was higher than Rawa. Similar effects was observed by Kim et al. (2007) in buckwheat sprouts. In general, most dark grown plants accumulated fewer anthocyanins as compared to light grown plants, and this effect is controlled by multiple regulatory genes and induced by various factors for example light (Taylor & Briggs, 1990). Dube, Bharti, & Laloraya, 1992 showed that another factors such as ionic stress or environmental factors (Sene, Dore, & Gallet, 2001) may inhibit anthocyanin synthesis in sorghum. It is important to conduct additional studies to determine which specific environmental factors influence the anthocyanin contents of amaranth and quinoa seeds and sprouts, this will help maximise ANT production.

4. Conclusions

Our results have proved that pseudocereal seeds and sprouts show relatively high antioxidant activity. Taking that into consideration, quinoa seems to be the better substitute for traditional cereals than amaranth, as long as aforementioned seeds are concerned. The results of our investigation have shown that sprouts have a significantly higher antioxidant activity than seeds, which may be a result of difference in the content of polyphenols, anthocyanins and other compounds. Alternative crops and sprouts described above can be used in traditional diet as a beneficial source of food with very high nutritional value.

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