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EVALUATION OF ANTIOXIDANT ACTIVITY OF AMARANTH (*AMARANTHUS CRUENTUS)* GRAIN AND BY-PRODUCTS (FLOUR, POPPING, CEREAL)

OCENA AKTYWNOŚCI ANTYOKSYDACYJNEJ NASION SZARŁATU WYNIOSŁEGO *(AMARANTHUS CRUENTUS)* ORAZ PRODUKTÓW SPOŻYWCZYCH Z NIEGO OTRZYMANYCH (MĄKA, POPPING, PŁATKI)

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The objective of our study was evaluation antioxidant activity of Amaranthus cruentus grain and by-products (flour, cereals and popping). The evaluation was performed by FRAP, DPPH and ABTS methods. FRAP and ABTS assays gave comparable results, DPPH method gave lower values.

Key words: *Amaranthus cruentus***,** seed, antioxidant activity, FRAP, ABTS, DPPH
Słowa kluczowe: *Amaranthus cruentus,* nasiona, aktywność antyoksydacyjna*,* FRAP, ABTS, DPPH

INTRODUCTION

The amaranth seed (*Amaranthus cruentus* from the family *Amaranthaceae*) is native to South America but for the last few years it has been known also in Poland. The amaranth grain is prized due to its amino-acid composition [10], superior to conventional grains. Besides, Amaranth seed contains considerable amounts of mineral salts, vitamins and fibre [9], oil rich in unsaturated fatty acids [15] and squalene [12], polyphenols, anthocyanins and flavonoids [7], tocopherols and tocotrienols [13]. The amaranth seed positively affects treatment of hypercholesterolemia in animal studies [8], or nutrition of people with celiac disease[16], although this is disputable since there are no clinical research plainly affirming influence amaranth products on their state of health.

Apart from high nutritional value of its grain, antioxidant activity of different Amaranth species (*Amarantus cruentus* and *Amaranthus blitum* [1] *Amaranthus hypochondriacus* [7], *Amaranthus caudatus* [6]) seed is known. Red amaranth is classified as peudocereal. Two va-

rieties are grown in Poland: Aztek and Rawa. Antioxidative properties of these cultivars' grain have not been analized yet. The objective of research presented in this paper was comparison on antioxidant activities of red amaranth seed (varieties Aztek and Rawa) and by-products. Positive results would contribute to popularization this hardly known but valuable pseudo-grain in Poland.

MATERIALS AND METHODS

Plant materials

Amaranth seed was purchased from „Szarłat" company in Łomża. Flour, popping and cereals were commerial („Szarłat"). Cultivar Aztek was raised in Tomaszów Lubelski and cultivar Rawa in Cyców (harvest 2004/2005).

Chemicals

De-ionized water 18 Mohm.cm obtained from Milli Ro & Q double purification system(Millipore), methanol, acetone, hydrochloric acid 36%, ferric chloride ($FeCl_3$) produced by POCh, phosphate buffered saline (PBS) was from Merck, 3-ethylobenzothiazoline-6-sulphonate (ABTS), triphenyltriazine (TPTZ) - 2,4,6-tris(2-pyridyl)–1,3,5-triazine, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and trolox (Sigma).

Laboratory equipment

UV–530 spectrophotometer UV-VIS (Jasco, Japan), disposable plastic cuvettes with 1 cm path length, thermostatic dryer (±0,1°C), centrifuge.

Humidity of samples determination

Moisture content was determined by drying in temperature of 105°C to a constant weight.

Extracts preparation

Powdered samples weight 1 g were extracted with 40 ml of solvent mixture 1 (methanol and 0,08 molar aqueous solution of hydrochloric acid, mixed in volume proportion 8:2, respectively) for 2 hours. Extract was roughly separated by decantation (or centrifugation) and solid residue was extracted with 40 ml of mixture 2 (acetone – water 7:3) for 2 hours. The methanolic extract 1 was combined with mixture 2 and whole was decanted, centrifuged, freezed and stored in darkness in temperature of –22 DC.

Seed soaking

The samples of amaranth grain (weight 1±0,001 g) poured distilled water was soaked for 24 hours in refrigerator (ca. +10 DC). Before extraction water was drained off.

Assessment of antioxidant activity by FRAP method

FRAP (Ferric Reducing Ability of Plasma) assay was developed by *Benzie & Strain* [5]. Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction causes forming ferrous-tripyridyltriazine complex with absorbance maximum at 593 nm. The assay adapted for analysis of food [3] gave highly reproducible results.

Assessment of antioxidant activity by ABTS and DPPH method

ABTS radical scavenging measurements were performed according to Re [14] and modifications published previously [3]. DPPH method was applied according to [3] and new approach for spectral background correction [4]. The mixtures of radical reagent and sample at increasing concentration were thermostated at 30±0.1 DC, than absorbance was measured after 6 minutes (ABTS) or 24 hours (DPPH) at the wavelength 734 nm and 514 nm respectively. The total antioxidant capacities (TAC) were estimated as trolox equivalents (TEAC) both by extrapolation to zero sample concentration ($TEAC_0$) and interpolation to 50% inhibition ($TEAC_{50}$) [3].

Statistical analysis

The results was analysed using Statistica 5.1 Pl software (StatSoft, Poland). The t-Student or *Kolmogorov-Smirnov* test was applied for statistical evaluations. Results were considered significant at the $p < 0.05$ level.

Abbreviations

FW - fresh weight, DW - dry weight, v. Aztek (Rawa) - varieties Aztek (Rawa)

RESULTS AND DISCUSSION

Absorbance readings (corresponding to antioxidant capacity) in FRAP method were taken after 8, 15, 30 and 60 minutes of incubation in temperature 30 DC, in ABTS method after 6 minutes and in DPPH method after 24 hours to achieve stationary state. Results are reported in table 1 for FRAP method and in table 2 for DPPH and ABTS method.

Among amaranth by-products the highest antioxidant properties characterized cereal (the least processed product in this group), lower antioxidant activity has flour and the lowest – popping. Statistically significant differences were observed between flour and popping as well as between cereal and popping. Little antioxidant activity of popping compared with other examined products arises from the method of its production – seed is treated with air stream of temperature up to 260 DC for a while. FRAP value increased for all extracts in consecutive measurements. It is presumably a consequence of antioxidants diversity or/and secondary processes. FRAP60 to FRAP8 ratio (mean ca. 1.8) showed that after 60 minutes FRAP value increment came to 80% of FRAP8 value. Thus fast reducers prevail in this plant material.

Comparing FRAP values for grain of varieties Rawa and Aztek it was found that the first one had higher antioxidant activity. There was practically no difference between FRAP values for these varieties after 60 min. incubation as well as FRAP after 8 min., hence reacting compounds were similar nature and the only dissimilarity is in the amount of fast reacting antioxidants.

Furthermore, in order to find whether soaking amaranth grain before boiling (as it is recommended for consumers) decreases antioxidant properties of the nourishment, antioxidant activity of amaranth seed soaked for 24 hours in water was estimated. As shown in table I soaking brought about essential decline of antioxidant activity, regardless of analyzed parameter. The differences were significant for the measurement after 8 minutes. Decrease of antioxidant activity of soaked seed, especially at the beginning of the test, indicated that the most active antioxidants could be dissolved in water and washed off, thus are probably located near the cover of grain.

Table I Antioxidative activity of investigated samples determined by FRAP assay*

Parameter (Incubation)	FRAP8 (8 min)	FRAP15 (15 min)	FRAP30 (30 min)	FRAP60 (60 min)	Difference **	Ratio ***
Material (*Amaranthus cruentus*)						
Flour	4.07±0.18	4.76±0.13	5.94±0.14	7.40±0.10	3.33	1.81
Popping	3.52±0.12	4.24±0.06	5.44±0.33	6.48±0.09	2.96	1.84
Cereal	4.33±0.12	5.05±0.17	6.29±0.26	7.99±0.28	3.66	1.84
Seeds (dry)						
v. Aztek	3.36±0.40	4.24±0.76	5.25±1.22	6.40±1.49	3.04	1.90
v. Rawa	3.73±0.18	5.17±0.77	6.95±1.47	7.89±1.81	4.16	2.11
Sedds (soaked)						
v. Aztek	1.86±0.22	2.81±0.50	3.90±1.00	4.81±1.11	2.95	2.58
v. Rawa	3.20±0.21	4.29±0.50	5.55±1.00	6.37±1.09	3.17	1.99

* mean FRAP values (in mmol Fe^{2+}/kg DW) from four (n=4) estimations ±SD – standard deviantion; ** Difference: FRAP60 - FRAP8, *** Ratio: FRAP60 / FRAP8.

Antioxidant activity parameters reported in table I and II showed convergent reaction profiles. Data obtained from $ABTS_{50}$ method was about twice higher than results obtained from FRAP method but it could be explained by the number of electrons involved: 1 in FRAP assay and 2 in ABTS assay with trolox calibration. However, the highest values were estimated by extrapolation to zero sample concentration ($ABTS_0$) and this technique includes the greatest portion of reactive substances in comparison to the other methods. TAC estimated in DPPH method was lower for tested extracts, what was probably caused by incomplete involvement in reaction of active in other methods compounds. A similar relationship was observed for buckwheat grain [2].

Table II. Antioxidative activity of investigated products estimated by ABTS and DPPH methods*

Material	ABTS (6 min)		DPPH (24 h)	
Amaranthus cruentus:	$TEAC_0$	$TEAC_{50}$	$TEAC_0$	$TEAC_{50}$
Flour	16.89±1.86	9.18±0.87	4.25±0.65	3.77±0.14
Popping	11.43±1.08	6.91±0.53	6.05±0.35	4.63±0.15
Cereal	19.63±1.38	11.31±0.63	5.46±0.39	4.58±0.13
Seeds(dry)				
v. Aztek	12.84±0.92	6.69±0.34	4.42±0.48	2.94±0.02
v. Rawa	11.61±0.65	6.01±0.27	3.15±0.24	3.13±0.05
Seeds(soaked)				
v. Aztek	13.81±0.99	6.90±0.37	2.71±0.88	1.56±0.13
v. Rawa	8.73±0.45	4.23±0.17	2.22±0.44	2.48±0.11

* in mmol Trolox/ kg DW ±SD – standard deviation (N=4-5); $TEAC_0$ – Trolox Equivalent Antioxidant Capacity extrapolated to zero sample concentration; $TEAC_{50}$ - Trolox Equivalent Antioxidant Capacity interpolated to 50% inhibition (IC_{50} equivalent).

The parameters estimated in this study by three methods were positively correlated as show data in table III. These results indicated that all above methods can be considered equivalent in evaluation of the antioxidant activities of investigated products. Moreover, observed linear correlation between parameters from the above methods suggested lack of selectivity of these methods. It should be emphasized, that some literature announced lack of such correlations, what may be explained by differences between details of the methods of estimation. Application of direct literature method of estimation of TEAC values for the investigated samples [14] proved a lack or revealed less significant correlations.

Table III. Pearson product correlations between TAC values of investigated materials estimated by ABTS, DPPH and FRAP methods*

Parameter	$TEAC_0$[ABTS]	FRAP8	FRAP15	FRAP30	FRAP60
$TEAC_0$[DPPH]	0.920(0.027)	0.883(0.047)	-	-	-
FRAP8	0.988(0.002)	-	0.981(0.003)	0.961(0.009)	0.998(0.000)
FRAP15	-	-	-	0.996(0.000)	0.975(0.005)
FRAP30	-	-	-	-	0.954(0.012)

* r^2 (p-level), N=5 ; $TEAC_0$ – Trolox Equivalent Antioxidant Capacity extrapolated to zero sample concentration; FRAP8, 15, 30, 60 – reducing power expressed in mM Fe(+2)/kg DW for 8, 15, 30, 60 minutes incubation

A comparison between FRAP of amaranth recalculated into adequate units and other grains and pseudograins is based on data given by *Halvorsen* [11]. FRAP values of amaranth seed (3,0 v. Aztek and 3,4 v. Rawa mmol Fe^{2+}/kg FW) were lower than oat (5,9 mmol Fe^{2+}/kg FW) and higher than rice (1,7 mmol Fe^{2+}/kg F.W.). Antioxidant activity of amaranth flour (3,7 mmol Fe^{2+}/kg FW) was similar to activity of whole grain flours such as sorghum flour (3,0 mmol Fe^{2+}/kg FW) and wheat flour (3,3 mmol Fe^{2+}/kg FW). Flours of maize, barley and buckwheat (6,0; 10,9 and 12,3 mmol Fe^{2+}/kg FW respectively) revealed higher activity and rice flour (2,3 mmol Fe^{2+}/kg FW) - lower activity than amaranth flour.

CONCLUSIONS

Valuation of antioxidative properties of *Amaranthus cruentus* seed and by-products (flour, cereal, popping) leads us to conclude that the highest antioxidant activity is characteristic to cereal, then - to flour and the lowest - to popping. The cultivar Rawa has higher antioxidant capacity than Aztek. Furthermore, we found that grain soaking causes decrease of antioxidant activity of amaranth food.

Antioxidant properties of amaranth seed are comparable to other grains and pseudograins but the main advantage is more balanced amino acid composition (close to FAO/WHO standard) as other researchers reported, therefore amaranth products should be popularized in Polish diet.

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Summary

The objective of our study was evaluation antioxidant activity of *Amaranthus cruentus* grain and by-products (flour, cereals and popping). The evaluation was performed by FRAP, DPPH and ABTS methods. FRAP and ABTS assays gave comparable results, DPPH method gave lower values. Among by-products cereal had the highest activity as the least processed product. Additionally, antioxidant capacities of two cultivars of amaranth (varieties Aztek and Rawa) were compared and the influence of grain soaking on antioxidant properties was taken into account. It was found, that soaking decreased antioxidant activity of amaranth seed.

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Streszczenie

Przedstawiono ocenę aktywności przeciwutleniającej nasion dwóch odmian szarłatu *Amaranthus cruentus* v. Aztek i v. Rawa oraz produktów spożywczych z niego otrzymanych (mąka, popping, płatki) w oparciu o metodę FRAP i zmodyfikowane metody ABTS i DPPH. Oznaczone wartości parametrów

były skorelowane liniowo. Stwierdzono, że nasiona v. Rawa posiadały wyższą aktywność niż v. Aztek, wśród produktów spożywczych najwyższą zdolność antyoksydacyjną posiadały płatki zaś najniższą popping. Namaczanie nasion powodowało obniżenie ich aktywności antyoksydacyjnej.

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