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ANTIOXIDANT CAPACITY OF THERMALLY-TREATED BUCKWHEAT

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This paper reports the use of an *in vitro* chemiluminescent method, $ORAC_{FL}$ and SOD-like activity assays for the evaluation of antioxidant capacity of the whole buckwheat and its products after hydrothermal treatment. Phosphate buffer (0.75 mmol/L, pH 7.4) and 80% methanol (v/v) were used for the preparation of extracts originated from untreated buckwheat, hydrothermally-processed whole buckwheat, and obtained light groat and hull from the treated whole buckwheat. The antioxidative capacities of water- (ACW) and lipid-soluble (ACL) compounds were investigated by a facile chemiluminescence assay using a Photochem® device. The superoxide dismutase-like activity (SOD-like activity) was evaluated as free radical scavenging activities of the extracts against superoxide anion radicals (O_2^-) whereas Oxygen Radical Antioxidant Capacity ($ORAC_{FL}$) of the extracts was determined with the spectrofluorimetric assay. Moreover, the content of flavonoids in untreated buckwheat and its products after hydrothermal treatment was provided.

The antioxidant capacity of the whole buckwheat before hydrothermal treatment evaluated with the chemiluminescence assay was formed mainly by lipid soluble antioxidants (ACL; 88.8 μmol Trolox/g d.m.) and only in part by the water soluble compounds (ACW; 5.1 μmol Trolox/g d.m.). The hydrothermal treatment of buckwheat whole grains caused a decrease in ACW and ACL by approximately 58% and 17%, respectively. The changes in the antioxidant capacity of untreated buckwheat and its products obtained after hydrothermal treatment were confirmed by the application of SOD and $ORAC_{FL}$ methods. Antioxidant capacity of buckwheat material was related to changes in flavonoids composition provided by HPLC analysis.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Mőench L.) is a crop adapted to cool, moist climate, short growing season (70–90 days) and negligible crop protection. The buckwheat seed is actually a fruit, an achene which, when dehulled (pericarp removed), is referred to as groat. Buckwheat is primarily used for human consumption and it is at present considered as a food component of high nutritional value [Li & Zhang, 2001; Krkoskova & Mrazova, 2004].

In human diet buckwheat is usually used as processed flour or in pancake mixes [Bonafaccia *et al*., 2003; Im *et al*., 2003]. The Japanese use buckwheat flour largely for noodles and curds, in addition its pericarp is used for pillow stuffing materials. To obtain buckwheat groats of consumption quality, the grain needs to be dehulled. This is being done with raising the moisture content of the grain to 22% of dry matter followed by heating. The resulting brown seeds referred to as light buckwheat groat are ready for cooking or can be roasted to produce dark brown groats. Moreover, in eastern Europe, light groats called kasha and roasted groat called roasted kasha are cooked and served like rice. The groats are sometimes used in the U.S. as a breakfast cereal [Edwardson, 1996].

Recently researchers have focused on the development of buckwheat as a potential functional food material [Manthey *et al*., 2004]. Buckwheat appears to be a suitable component of food products from the point of view of its antioxidant capacity. Several authors studied its antioxidative properties [Watanabe, 1998; Przybylski, 1998; Holasaova *et al.,* 2002; Zieliński *et al.,* 2006]. However, the data compiled on the effect of thermal treatments on the antioxidant capacity of buckwheat grain are limited. In numerous papers dealing with measurement of the antioxidant capacity, very rarely, a single method is applied [Prior *et al*., 2005]. To the best of our knowledge, never chemiluminescent, $ORAC_{FL}$ nor SOD-like activity methods have been applied for studying the antioxidant capacity of buckwheat material, and what is worthily in the work undertaken, the obtained buckwheat light groat represents the food staple originated from local industry and then available for consumers in markets. Therefore, the aim of this work was to compare the antioxidant capacity of whole buckwheat before and after hydrothermal treatment evaluated with the above listed methods and then to show a correlation between antioxidant capacity of thermally-treated buckwheat products and flavonoids content.

MATERIALS AND METHODS

Reagents. Acetonitrile and methanol (HPLC-grade) were provided by Merck (Darmstad, Germany). The ACL and ACW kits for PCL assay were obtained from Analytik

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Jena (Leipzig, Germany). Superoxide Dismutase kit (Cat No SD 125) was obtained from Randox Laboratories Ltd (United Kingdom), whereas fluorescein sodium was supplied by Fluka. Rutin (quercetin-3-rutinoside), 2,2'-azobis (2-amidopropane) hydrochloride (AAPH) and 6-hydroxy-2,5,7,8--tetramethylchroman-2-carboxylic acid (Trolox) were from Sigma (St. Louis, USA). Orientin (3',4',5,7-tetrahydroxyflavone-8-glucoside), homoorientin (3',4',5,7-tetrahydroxy-flavone-6-glucoside), vitexin (4',5,7-trihydroxyflavone-8-glucoside) and isovitexin (4',5,7-trihydroxyflavone-8glucoside) standards (HPLC-grade) were obtained from Extrasynthese Company Inc. (Lyon, France). All other reagents of reagent--grade quality were from POCh, Gliwice, Poland. Water was purified with a Mili-Q-system (Milipore, Bedford, USA). All solutions prepared for HPLC were passed through a 0.45 μm nylon filter before use.

Raw material and hydrothermal treatment. Buckwheat (*Fogopyrum esculentum*, variety Kora) was provided by the local industry from North-East Poland. The hydrothermal treatment included raising the moisture content of the whole grain to 22% of dry matter followed by simultaneous steaming (water vapour 6 atm) and heating at 160°C for 30 min, after that the light buckwheat groat and hulls were obtained in the dehulling process. Next, the following samples were used for the characterization of antioxidant properties: whole buckwheat grain, hydrothermallytreated whole grain, light groat and hydrothermallytreated hull. The material was lyophilized and ground using a laboratory mill.

Preparation of 80% methanol extracts for PCL and SOD-like activity assay. About 100 mg of dried and pulverized buckwheat samples was extracted with 1 mL of 80% methanol (v/v) by 30 s sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5 000 × g at 4°C). That step was repeated 5 times, supernatant was collected into a 5-mL flask and kept at -80°C until analysed.

Preparation of phosphate buffered extracts for SOD--like activity assay. About 100 mg of dried and pulverized buckwheat samples was extracted with 1 mL of 75 mmol/L phosphate buffer (pH 7.4) by 30 s sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5 000 × g at 4°C). That step was repeated 5 times, supernatant was collected into 5-mL flask and it was used for direct evaluation of SOD-like activity.

Determination of the antioxidative capacities of water (ACW) and lipid-soluble (ACL) compounds by the photochemiluminescence (PCL) assay. The PCL method, based on the methodology of Popov & Lewin [1999], was used to measure the antioxidant activity of buckwheat extracts with a Photochem apparatus (Analytik Jena, Leipzig, Germany) against superoxide anion radicals generated from luminol, a photosensitizer, when exposed to UV light. The antioxidant activity of buckwheat 80% methanol extracts was measured using both 'ACW' and 'ACL' kits provided by the manufacturer designed to measure the antioxidant activity of hydrophilic and lipophillic compounds, respectively. Lag time (s) for the ACW assay, obtained from the PCLsoft® control and analysis software was used as the radical-scavenging activity and the antioxidant capacity estimated by comparison with a Trolox standard curve and expressed as μmol Trolox/g on dry matter basis of a sample. For ACW and ACL assay the extracts were diluted 1:10 (v/v) and 1:40 (v/v) with 80% methanol, respectively, and 20 μL of the diluted extract was sufficiently to correspond within the standard curves. The antioxidant index was obtained by dividing the antioxidant capacity by lag time multiplied by 1000 (antioxidant capacity/lag time × 1000). Antioxidant capacity using the ACL kit was monitored for 180 s and expressed as μmol Trolox/g sample. The extracts were centrifuged (5 min at 16,000 × *g*) prior to analysis. Antioxidant assay was carried out in triplicate for each sample.

Determination of SOD-like activity of the buckwheat extracts. SOD-like activities were quantified in buckwheat phosphate buffered and 80% methanol extracts using a superoxide dismutase kit (RANSOD). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4--nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase scavenging activity of the investigated extracts was then measured by the degree of inhibition of this reaction at 505 nm by using UV/VIS spectrophotometer. The superoxide dismutase with an activity of 5.4 U/mL was used as a standard. The percent of reaction inhibition was plotted against $\log_{10}$ of different SOD activities (SOD/mL) giving a standard curve and then SOD activity of the sample was calculated onto SOD unit/mL of the investigated extracts. The results were finally expressed as SOD unit/g of d.m. Assays were performed at 37°C using a UV-VIS spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu, Japan). The test required 50 μL of sample, with a read time of 3 min.

Oxygen Radical Antioxidant Capacity assay. Prior to $ORAC_{FL}$ assay, 175 mg of samples were extracted with 6.25 mL of 75 mmol/L phosphate buffer pH 7.4 or 80% methanol, incubated for 30 min with stirring every 10 min for 30 s and filtrated by cellulose paper (Whatman n° 40) [Birlouez--Aragon *et al*., 2001]. Filtrates were used for further analysis. $ORAC_{FL}$ assay was carried out according to del Castillo *et al.* [2005]. This assay measures the ability of antioxidants present in the sample to inhibit the loss of fluorescein induced by radicals generated by AAPH. The stock solution of fluorescein (100 μmol/L) was prepared in 75 mmol/L phosphate buffer (pH 7.4). A fresh working solution (42 nmol/L) was prepared daily. The stock solution of AAPH (153 mmol/L) and quality control solution (25 μmol/L Trolox) were prepared in 75 mmol/L phosphate buffer (pH=7.4) and they were kept on ice bath to avoid degradation. Fluorescence readings were recorded at λ_{Ex}= 493 nm and λ_{Em}= 515 nm in a Perkin-Elmer LS 50 B Luminescence Spectrometer (Massachusetts, USA) with slit width at 2.5 nm. Results were expressed as μmol Trolox/g d.m.

Flavonoids profile. For the analyses of flavonoids, extractions of buckwheat material were carried out as follows: about 500 mg of dried and pulverized buckwheat samples was extracted with 2.5 mL of 80% methanol by 60 s sonica-

tion. Next, the mixture was vortexed for 60 s, again sonicated and centrifuged for 5 min (5 000 × *g* at 4°C). That step was repeated on the residue with next volume of 2.5 mL of the solvent. Supernatants were collected in 5-mL flask. Finally, all extracts were kept at -80°C prior to further analysis. The extracts were submitted to HPLC analysis (Shimadzu, Kyoto, Japan), consisting of two pumps (LC—10 AD), UV detector (SPD-10A) set at 330 nm, autosampler set to 10 μL injection (SIL-10 AD_{VP}), column oven (CTO-10 AS_{VP}) and system controller (SIL-10 AD_{VP}). The oven's temperature was set at 35°C. All chromatographic determinations were performed on a C18(2) Luna 5 μ column, 4.6 × 250 mm (Phenomenex, Torrance, CA, USA) with the flow rate of 1 mL/min. The flavonoids were eluted in a gradient system composed of water containg 0.05% of formic acid (solvent A) and acetonitrile containg 4% of formic acid (solvent B). Gradients were as follows: 15-15-22-70-15-15% B at gradient time, t_G=0-7-22--25-35-40 min. Rutin, orientin, homoorientin, vitexin and isovitexin stock solutions were prepared in methanol at concentrations of 500, 517, 477, 509 and 574 μmol/L, taking into account the purity of the standards. For quantitative analysis, calibration standards were prepared in duplicate at five concentrations, 2.5–20 μmol/L, for these five flavonoids.

Statistical analysis. Data were subjected to one-way analysis of variance (ANOVA) using the Fischer LSD test (at a significance level of $p<0.05$) with the Statistica 7.1.30.0 (Statsoft Inc., USA) for Windows using a PC-Pentium. The "goodness of fit" of the linear regression analysis through each set of data was indicted by the regression coefficient (r^2) calculated with the use of Microsoft Excel Sheet.

RESULTS AND DISCUSSION

Antioxidative capacities of water (ACW) and lipid--soluble (ACL) compounds

The antioxidant capacities of buckwheat extracts based on the material obtained before and after hydrothermal treatment were compared against one another using the Photochem® device and the ACW and ACL kits supplied by Analytik Jena AG. The Photochem® device is the first system than can qunatify the antioxidant capacity of water- and lipid-soluble substances. It combines the very fast photochemical excitation of radical generation with the highly sensitive luminometric detection. In this study, the 80% methanol extracts were taken for ACW and ACL measurements with this system, and the results are expressed as Trolox equivalents. The same system was considered by Oomah *et al.* [2006] when different genotypes of lupin seeds were evaluated for their antioxidant capacity by means of ACW and ACL measurements. Because of the high sensitivity of the PCL of luminol, only nanomolar concentrations of non-enzymatic antioxidant substances are required to delay the observance of PCL. The antioxidative capacities of water (ACW) and lipid-soluble (ACL) compounds of buckwheat extracts are compiled in Table 1. It was found that antioxidant capacity of untreated buckwheat grain was formed only in part by the water soluble compounds (5.1 μmol Trolox/g d.m.). In contrast, the 80% methanol-soluble antioxidants formed about 95% of antioxidant capacity of the untreated grain (88.8 μmol Trolox/g d.m.). The hydrothermal treatment of buckwheat grains caused a decrease in ACW and ACL by approximately 58% and 17%, respectively, thus indicating different resistance of water and 80% methanol-soluble antioxidants against the hydrothermal treatment. The ACW of the light buckwheat groat decreased by 59% whereas the ACW of the hull increased by 67% when compared to the hydrothermally processed whole grain. Similar changes, however to a smaller extent, were found for ACL values of the light buckwheat groat and thermally-treated hull. In this respect, a decrease in ACL of groat was about 4% and an increase in ACL of the hull was about 13%. These results indicate that 80% methanol-soluble antioxidants responsible for the antioxidant properties of buckwheat whole grain are mainly distributed within the hull and then in smaller amounts in light groat. The provided findings are in agreement with the research on bioactive compounds in the cereal grains before and after hydrothermal processing [Zieliński *et al.,* 2001], and also confirm the recent report on the extrusion cooking of buckwheat groat [Zieliński *et al*., 2006]. In our study, an additional parameter of radical scavenging ability, *i.e.* antioxidant index, was calculated as reported by Oomah *et al.* [2006]. The photochemiluminescence (PCL) antioxidant assay generated two indicators: lag time (L in seconds), a parameter of delay in the photochemical generation of superoxide radical anion, and antioxidative capacities of water (ACW) and lipid-soluble (ACL) compounds [Prior *et al.,* 2005]. In this case, the antioxidant index was obtained by dividing the ACW by lag time multiplied by 1000 (antioxidant capacity/lag time × 1000). The values of antioxidant index reflected the changes after hydrothermal treatment. What is more important, using this parameter we were able to compare our data to those reported on different genotype of lupin seeds. In this case, the antioxidant index of light buckwheat groat was almost twenty times higher than that of lupin seeds [Oomah *et al.,* 2006].

TABLE 1. Antioxidant index and antioxidative capacities of water (ACW) and lipid-soluble (ACL) compounds of the untreated and hydrothermally--treated whole buckwheat material.

Material	ACW (μmol Trolox/g d.m.)	Lag time (s)	Antioxidant index	ACL (μmol Trolox/g d.m.)
Untreated whole buckwheat	5.09±0.65^a	17.7±1.8^a	286.4±7.7^a	88.81±3.77^a
Hydrothermally-treated whole buckwheat	2.11±0.11^b	11.9±0.4^b	177.9±2.7^b	74.11±1.19^b
Light groat	0.86±0.02^c	6.3±0.1^c	137.6±1.7^c	70.90±0.77^b
Hydrothermally treated hull	3.52±0.05^d	15.5±0.2^d	228.0±1.8^d	83.41±3.74^a

Data expressed as mean ± standard deviation (n=3). Means in a column followed by the same letter are not significantly different (p 0.05).
Antioxidant index = ACW/lag time x 1000.

Superoxide anion radical scavenging capacity

The capacity of buckwheat samples to scavenge superoxide anion radical was provided using the xanthine-xanthine oxidase system and the results were reported as SOD unit/g of d.m. The untreated whole buckwheat samples demonstrated significant capacity against $O_2^{\bullet-}$ (Table 2). This capacity was higher for the phosphate buffered extracts when compared to 80% methanol extracts. The SOD-like activity of the thermally-treated buckwheat whole grain was decreased by 77% (phosphate buffer extracts) and by 46% (80% methanol extracts) when compared to the untreated whole grain. Moreover, after the hydrothermal treatment the lowest superoxide anion radical scavenging capacity was noted for light buckwheat groats and hulls. The ability to scavenge $O_2^{\bullet-}$ radicals by buckwheat samples was in the order of untreated whole buckwheat > treated whole buckwheat > hulls light > groats, for both solvents used for the extraction of compounds responsible for the SOD-like activity formation. The results provided by phosphate and 80% methanol extracts were highly correlated (r=0.97). The SOD-like activity expresses the cumulative action of antioxidants to scavenge superoxide anion radicals by non-enzymatic and by superoxide dismutase action. The latest catalyze the dismutation of two superoxide radical anions into hydrogen peroxide and oxygen and its essential role is connected with removing damaging Reactive Oxygen Species (ROS) from the cellular environment [Attar *et al.,* 2006]. In this case, determination of SOD-like activity in phosphate buffered buckwheat extracts represents rather scavenging of $O_2^{\bullet-}$ radicals by enzymatic action whereas those provided by 80% methanol extracts describe the scavenging activity of low molecular weight antioxidants against superoxide radicals. Therefore, it was noted that the SOD-like activity of buckwheat material after thermal treatment was significantly reduced based on phosphate buffered extracts whereas that formed by antioxidants extracted with 80% methanol was still present despite of the reduction described earlier (Table 2). The ability of buckwheat-based products to scavenge superoxide anion radical, especially by low molecular weight antioxidants, can be important in human nutrition. For example, in humans the loss or dysfunction of SOD may trigger the ROS-mediated pathologies like coronary heat disease, atherosclerosis, and diabetes [Fattman *et al*., 2003]. However, little is still known about the SOD-like activity in food products which could prevent the formation of superoxide radical in the gastrointestinal tract. In this study the antioxidant activity of buckwheat extracts was measured against superoxide anion radicals generated from two different systems: from luminol, a photosensitizer, when exposed to UV light and by the xanthine-xanthine oxidase system. Therefore, the results from a chemiluminescence assay obtained for buckwheat material were correlated with those obtained in the SOD-like activity assay. It was found that higher correlation was provided for 80% methanol extracts (PCL ACL *vs*. SOD-like activity; r=0.88) when compared to that provided by phosphate buffered extracts (PCL ACW *vs*. SOD; r=0.80).

TABLE 2. The SOD-like activity of the raw and hydrothermally-treated whole buckwheat material.

Material	SOD-like activity (U/g d.m.)	
	Extracted by phosphate buffer	Extracted by 80% methanol
Whole buckwheat	70.50±0.71[a]	52.25±3.89[a]
Treated whole buckwheat	16.25±1.06[b]	28.25±0.35[b]
Light groats	9.00±1.41[c]	25.20±2.47[b]
Hulls	9.50±1.41[c]	31.73±2.92[b]

Data expressed as mean ± standard deviation (n=3). Means in a column followed by the same letter are not significantly different (p 0.05).

Antioxidant capacity measured with the $ORAC_{FL}$ method

$ORAC_{FL}$ data are shown in Table 3. Results demonstrated that all buckwheat samples scavenged peroxyl radicals generated by thermolysis of AAPH. The highest scavenging activity of peroxyl radicals was found in whole buckwheat before thermal treatment. This activity was mainly connected with antioxidants extracted with 80% methanol (94.7 μmol Trolox/g d.m.) and to a lower extent with those extracted with phosphate buffer (42.6 μmol Trolox/g d.m.). In contrast, the lowest antioxidant capacity was noted for light buckwheat groat and hulls, two products obtained after hydrothermal treatment of whole buckwheat. Moreover, data suggest that compounds responsible for scavenging the peroxyl radicals after thermal treatment were present both in phosphate buffer and 80% methanol extracts. However, those solubilised in 80% methanol were rather thermolabile compounds. Indeed, this is the case of flavonoids which were reported to be present in both groat and hull. When results provided by the $ORAC_{FL}$ method were compared to those obtained in the chemiluminesce assay, the correlation coefficient r=0.88 was noted. It was also suggested that both methods provided different chemical information in respect of the compounds present in the thermally-treated buckwheat material since the PCL ACL results were about twice higher when compared to those data obtained by $ORAC_{FL}$. This finding indicates different scavenging activity of antioxidants remaining in the thermally-treated buckwheat material against superoxide and peroxyl radicals.

TABLE 3. Antioxidant capacity of the raw and hydrothermally-treated whole buckwheat material provided by $ORAC_{FL}$ method.

Material	Antioxidant capacity (μmol Trolox/ g d.m.)	
	Extracted by phosphate buffer	Extracted by 80% methanol
Whole buckwheat	42.62±0.66[a]	94.73±0.21[a]
Treated whole buckwheat	36.02±2.15[b]	36.62±4.45[b]
Light groats	35.24±1.15[b]	27.87±1.72[c]
Hulls	30.40±0.46[c]	43.54±2.24[b]

Data expressed as mean ± standard deviation (n=3). Means in a column followed by the same letter are not significantly different (p 0.05).

Flavonoids profile of whole buckwheat before and after hydrothermal treatment

Column chromatography on a C18 support allowed us to separate five flavonoid compounds from whole buckwheat before and after hydrothermal treatment (Table 4). In respect of the light buckwheat groat, only three flavonoids were identified, namely vitexin, rutin and isovitexin. The con-

TABLE 4. Concentration of flavonoids in whole buckwheat before and after hydrothermal processing (μg/g d.m.)[1].

Compound	Untreated whole buckwheat	Hydrothermally-treated whole buckwheat	Light groat	Hydrothermally-treated hull
Isoorientin	8.49±0.42[a]	4.49±0.22[b]	nd	8.43±0.41[c]
Orientin	8.57±0.34[a]	4.91±0.20[b]	nd	14.27±0.57[c]
Vitexin	17.82±0.80[a]	10.42±0.47[b]	3.55±0.16[c]	30.37±1.37[d]
Rutin	176.51±5.30[a]	76.36±2.29[b]	31.46±0.94[c]	33.05±0.99[d]
Isovitexin	21.56±0.21[a]	11.69±0.12[b]	1.60±0.02[c]	20.39±0.21[d]
Total	232.99±1.41[a]	107.87±0.66[b]	36.60±0.37[c]	106.51±0.71[d]

[1] Light groat and hydrothermally-treated hull obtained after dehulling the thermally processed of the whole buckwheat; see Materials and Methods; nd – not detected.; Means in a row followed by the same letter are not significantly different (p 0.05).

centration of flavonoids in whole buckwheat after processing (160°C, 30 min, pressure 6 atm) was decreased by 54% when compared to their concentration before treatment (Table 4). The obtained light buckwheat groat contained about three times less flavonoids when compared to processed hulls, and these concentrations corresponded to the weight proportion of the hull to the whole buckwheat grain (one-fourth of the grain in weight) [Edwardson, 1996] and the concentration of flavonoids found in treated whole grain. The main flavonoid compound was rutin which constituted about 76, 71, 86 and 31% of all flavonoids content in untreated buckwheat, hydrothermallytreated buckwheat, light groat and treated hull, respectively (Table 4). It was also reported on the presence of other flavonoids compounds in buckwheat such as catechins [Watanabe, 1998], quercetin [Dietrych-Szostak & Oleszek, 1999], quercitrin [Przybylski, 1998], kaempferol-3--rutinoside [Tian *et al.,* 2002]; however rutin is considered as the main one [Kreft *et al.,* 2006]. The concentration of flavonoids in untreated buckwheat grain and buckwheat products after treatment was highly correlated with antioxidant capacity evaluated by PCL ACW and PCL ACL, and the respective correlation coefficients had values of 0.94 and 0.88, respectively. However, the highest correlation was noted for the SOD-like activity and results provided by $ORAC_{FL}$ when 80% methanol was used as a solvent. In this case, the respective correlation coefficient had the same value of r=0.97. Similarly, a positive correlation was noted between antioxidant capacity and concentration of each identified flavonoid compound. The values of correlation coefficients are collated in Table 5. Our findings are in agreement to those reported by Dietrych-Szostak & Oleszek [1999], and Im *et al*. [2003] who found that the main buckwheat groat flavonoids - rutin and isovotexin, were affected by temperature and heating time adversely.

TABLE 5. The correlation coefficients (*r*) between methods used for the determination of antioxidant capacity and flavonoids content of the untreated and hydrothermally-treated whole buckwheat material: (A) phosphate buffered extracts, (B) 80% methanol (v/v) extracts.

(A) Phosphate buffered extracts	SOD	$ORAC_{FL}$	Flavonoids content
PCL ACW	0.80	0.46	0.94
SOD	-	0.89	0.93
$ORAC_{FL}$		-	0.72
(B) 80% methanol extracts	SOD	$ORAC_{FL}$	Flavonoids content
PCL ACW	0.88	0.88	0.88
SOD	-	1.00	0.97
$ORAC_{FL}$		-	0.97

CONCLUSIONS

In conclusion, the research on scavenging activity of the whole buckwheat before and after hydrothermal treatment against superoxide and peroxyl radicals was fully applicable for the evaluation of the antioxidant capacity. Changes in the antioxidant capacity of untreated buckwheat and its products obtained after hydrothermal treatment were related to the changes in flavonoids composition.

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AKTYWNOŚĆ ANTYOKSYDACYJNA NASION GRYKI PO OBRÓBCE TERMICZNEJ

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W pracy badano zawartość flawonoidów i pojemność przeciwutleniającą nasion gryki po obróbce hydrotermicznej. Do oceny pojemności antyoksydacyjnej zastosowano dwie metody oparte na badaniu zdolności ekstraktów buforowych i metanolowych do wymiatania anionorodników ponadtlenkowych wytwarzanych w dwóch różnych układach fizyko-chemicznych oraz metodę wymiatania rodników ponadtlenkowych.

Badania metodą chemiluminescencji wykazały, że pojemność antyoksydacyjna nieprzetworzonej gryki była głównie kształtowana przez związki rozpuszczalne w 80% metanolu (ACL), natomiast znacznie mniejszy udział wnosiły związki rozpuszczalne w wodzie (ACW). Zastosowany proces hydrotermiczny obniżał ACW o 58% oraz ACL o 17% (tab. 1). Spadek pojemności antyoksydacyjnej stwierdzono także na podstawie wymiatania anionorodników ponadtlenkowych generowanych w układzie ksantyna-oksydaza ksantynowa (tab. 2) oraz rodników peroksylowych wytwarzanych podczas termicznego rozpadu azozwiązków (tab. 3). Otrzymana w procesie kasza gryczana jasna charakteryzowała się niższą pojemnością antyoksydacyjną w stosunku do hydrotermicznie przetworzonej gryki, podczas gdy pojemność przeciwutleniająca łuski gryczanej była podwyższona. Stwierdzone zmiany pojemności antyoksydacyjnej były dodatnio skorelowane z zawartością flawonoidów, w tym głównie z zawartością rutyny (tab. 4). Zastosowane w pracy metody analityczne do oceny pojemności przeciwutleniającej były wzajemnie dodatnio skorelowane (tab. 5).