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Changes in Protein Quality and Antioxidant Properties of Buckwheat Seeds and Groats Induced by Roasting

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This study focused on the evaluation of changes in protein quality and antioxidant properties of buckwheat seeds and groats induced by roasting. Changes in protein quality were indirectly measured by Maillard reaction chemical indicators such as furosine, FAST index, and browning. Characterization of antioxidant profiles of raw whole seeds, roasted whole seeds, raw groats, and roasted groats was undertaken by determining the extractable total phenolic compounds (TPC), extractable total flavonoids (TF) and individual flavonoids, lipophilic and hydrophilic peroxyl radical scavengers by $ORAC_{FL}$ assay, and scavengers of ABTS radical cations by TEAC assay. Roasting significantly decreased the total protein content of groats, whereas this parameter was not affected by the thermal treatment of whole seeds. The formation of MRPs was induced by the thermal treatment of both whole seeds and groats, thus suggesting deterioration of protein quality due to this chemical event. A significant degradation in natural antioxidants due to thermal processing was observed. Most of the peroxyl radical scavenging activity of samples was associated with hydrophilic compounds because L-$ORAC_{FL}$ values were on average 9% of the H-$ORAC_{FL}$ values. The H-$ORAC_{FL}$ values were positively correlated with extractable TPC contents (r = 0.51) and extractable TF contents (r = 0.93), whereas they showed a negative correlation with furosine (r = −0.87), FAST index (r = −0.85), and browning (r = −0.98) results.

KEYWORDS: Buckwheat; roasting; Maillard reaction; extractable total phenolics; extractable total flavonoids; hydrophilic antioxidants; lipophilic antioxidants; $ORAC_{FL}$; TEAC

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench L.) is a crop adapted to a cool and short growing season (70−90 days). Buckwheat is resistant to pathogenic damage, relatively inexpensive, and easy to grow. The cultivation of buckwheat has decreased over the past century. The main reason for this decline was self-incompatibility, which has led to breeding difficulties. In addition, buckwheat showed a poor response to the application of fertilizers compared to other crops. Common buckwheat (*F. esculentum* Moench) is the most commonly grown species, whereas two other species of buckwheat (*Fagopyrum tataricum* Gaertner and *Fagopyrum emarginatum*) have been cultivated on a smaller scale (*1*). Buckwheat is categorized as a pseudocereal, showing differences and similarities with cereals. The embryo in a buckwheat seed is located in the center of the endosperm, whereas the hull (pericarp) has a hard fibrous structure and surrounds the seed (*2*). Currently, buckwheat is considered to be a high nutritional value pseudocereal because of its high content in vitamins B_1 and B_2, lysine, protein with balanced amino acid composition (*3*), flavonoids (*4*, *5*), phytosterols (*6*), soluble carbohydrates, D-*chiro*-inositol, fagopyritols (*7*), and thiamin-binding proteins (*8*). Buckwheat is also rich in antioxidant compounds such as flavonoids (*4*, *5*), phenolic acids, tocopherols, reduced glutathione, inositol phosphates, and melatonin (*9*). This pseudocereal contains more rutin (quercetin-3-rutinoside) than most other plants. Rutin exhibits antioxidant, anti-inflammatory, and anticarcinogenic properties and is known as one of the most potent natural inhibitors of the formation of advanced glycation endproducts (AGE) (*10*). Buckwheat has also been described as a potential source of D-*chiro*-inositol with a lowering effect on serum glucose concentration in rats (*11*). Therefore, it is being considered as a health-promoting food with a tremendous range of applications for treating human diseases (*2*, *12*−*14*).

Buckwheat seeds are usually processed into flour. Raw whole seeds are dehulled before milling or the flour is sieved afterward. Dehulled raw whole seeds (raw groats) are principally used for human consumption as breakfast cereals or as processed flour for making pancakes, whereas in Central and Eastern Europe the roasted buckwheat groats are of increasing popularity (*15*). To obtain good-quality roasted groats, the dehulling process is preceded by raising the moisture content of the raw whole seeds followed by simultaneous steaming and heating. All of these serial steps form a unique technological process. The resulting roasted groats (roasted kasha) are ready for cooking and usually served as an alternative to potatoes and rice (*2*). The nonroasted groats

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(raw groats) are sometimes used in the United States as a breakfast cereal (*1*, *15*). Thermal processes may evoke changes to natural antioxidant buckwheat profiles (*9*, *16*, *17*). Because roasted buckwheat groats represent a very popular type of food in northern Europe, Asia, and the United States, the events occurring during their processing are of interest. During roasting, the Maillard reaction (MR) is a key chemical reaction involving free amino groups of lysine, peptides or proteins, and carbonyl groups of reducing sugars. MR is a complex chemical event based on a series of subsequent and parallel reactions that can be divided into three main stages for better comprehension: early, advanced, and final. At each stage, special chemical structures are formed (*18*). Estimation of the extent of protein damage caused by heating in the first stage of that reaction is often based on the indirect analysis of Amadori rearrangement products, such as furosine (*19*). At the advanced MR stage, the formation of fluorescence compounds as well as cross-linking products was observed in vitro (*20*). Advanced MRPs can be measured as the maximal fluorescence emission at 330−350 nm excitation wavelengths (*21*). The final products of MR are melanoidins responsible for the typical brown color of baked or roasted products that are commonly estimated by measuring the absorbance at 420 nm (*22*, *23*). The formation of MRPs is essential to provide positive notes to food products including flavor, color, texture, and shelf life. Additionally, the ability of MRPs to scavenge free radicals in vitro, which has been ascribed to structure-forming brown polymers such as pronyl-lysine, has been reported recently (*24*). However, extreme heating conditions may destroy MR antioxidants by pyrolysis (*25*). To date, no information is available related to the content of MRPs in the roasted buckwheat groats, and only a few data on the overall antioxidant activity of thermally processed buckwheat seeds and groats have been reported (*17*). The aim of this research was to evaluate how the thermal processing of buckwheat may affect its protein quality and antioxidant properties. A chemical characterization of raw and thermally processed buckwheat seeds and groats has been undertaken by means of analyses of furosine, FAST index, browning, and extractable TPC and TF, whereas lipophilic (L-ORAC$_{FL}$), hydrophilic (H-ORAC$_{FL}$), and Trolox equivalent antioxidant capacity (TEAC) have provided data on the antioxidant properties of the samples.

MATERIALS AND METHODS

Reagents. Quinine sulfate was from BDH (Poole, U.K.); SDS and sodium fluorescein were supplied by Fluka (Buchs, Switzerland). Furosine (2-furoylmethyl-lysine) was obtained from NeoMPS (Strasbourg, France). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2′-azobis(2-amidopropane) dihydrochloride (ABAP), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin (3,3′,4′,5,7-pentaxydroxyflavone), and rutin (quercetin-3-rutinoside) were from Sigma (Sigma Chemical Co., St. Louis, MO). Randomly methylated β-cyclodextrin (RMCD) was obtained from Cyclodextrin Technologies Dev., Inc. (High Springs, FL). Methanol, acetonitrile, formic acid, acetic acid (supragradient), sodium acetate, and β-mercaptoethanol were from Merck KGaA (Darmstadt, Germany). Orientin (3′,4′,5,7-tetrahydroxyflavone-8-glucoside), homoorientin (3′,4′,5,7-tetrahydroxyflavone-6-glucoside), vitexin (4′,5,7-trihydroxyflavone-8-glucoside), and isovitexin (4′,5,7-trihydroxyflavone-8-glucoside) standards (HPLC grade) were obtained from Extrasynthese Co. Inc. (Lyon, France). All other reagents of grade quality were supplied by POCh (Gliwice, Poland). Water was purified with a Milli-Q system (Millipore, Bedford, MA).

Buckwheat Material and Roasting. Raw whole buckwheat seeds (*F. esculentum* Moench var. Kora), roasted whole seeds, and roasted groats were provided by a local company located in northeastern Poland. Roasting was performed by initially raising the moisture content of the whole raw seeds to 22% of dry matter by simultaneously steaming (overheat water vapor at 588 kPa) and heating at 160 °C for 30 min. This step induced changes in hull structure, being softer and easier to remove. Afterward, the whole seeds were dried at 160 °C. The roasted whole seeds were dehulled, which yielded roasted groats as previously reported (*17*). All of these serial steps form a unique technological process. Raw groats were obtained indoors by manually dehulling raw whole buckwheat seeds. **Figure 1** shows a simplified flow diagram of the sample preparation procedures. The buckwheat material was freeze-dried, ground, and stored at −20 °C until analysis. The moisture contents of raw whole seeds, roasted whole seeds, raw groats, and roasted groats were 9.1, 8.2, 10.9, and 7.5% respectively.

Analysis of MRPs. *Furosine Assay*. The formation of early MRP (Amadori rearrangement products) was indirectly measured by the furosine RP-HPLC analysis (*26*). Prior to the chromatographic analysis, aliquots of samples containing 40−50 mg of protein were hydrolyzed with 8 mL of 8 M HCl at 110 °C for 23 h under anaerobic conditions. The hydrolysates were filtrated through Whatman no. 40 filter papers, and the filtrates (0.5 mL) were cleaned up by employing a Sep-Pak C_{18} cartridge (Waters, Milford, MA), pretreated with 5 mL of methanol and 10 mL of deionized water. Furosine was eluted from the cartridge with 3 mL of 3 M HCl, injected (50 μL) onto a C_8 Alltech furosine-dedicated column (250 × 4.6 mm; Alltech, Laarne, Belgium), and thermostated at 37 °C. Solvent A, 0.3% HPLC acetic acid in double-distilled water, and solvent B, 0.4% potassium chloride in solvent A, were employed as mobile phases. The elution was carried out following a linear gradient at a flow rate of 1.2 mL/min and detected at a 280 nm wavelength. Quantitative analysis was performed by the external standard method, using a commercial standard of pure furosine. Furosine recovery from buckwheat samples was determined by adding 8.28 μg of pure furosine to the acid hydrolysate of raw groats containing no furosine. A recovery value of 99.6 ± 1.7% was obtained. Data were mean values (n = 2) expressed as milligrams per 100 g of protein. Protein content was measured following AOAC method 979.09, and a nitrogen-to-protein conversion factor of 6.25 was used (*27*).

The furosine peak was identified by HPLC-MS analysis as described by del Castillo et al. (*28*). The analysis was performed at room temperature employing a Hewlett-Packard 1100 chromatograph, equipped with a diode array detector (DAD) coupled to a quadrupole HP 1100 mass detector operating in the electrospray ionization mode (API-ESI), under atmospheric pressure and positive polarity. Samples (75 μL) were injected onto the C_8 Alltech furosine-dedicated column. Furosine was eluted with acetic acid 2% (v/v) in double-distilled water under isocratic conditions at a flow rate of 0.7 mL/min. Mass spectrometry values of needle potential, gas temperature, drying gas, and nebulizer pressure were adjusted to 4000 V, 350 °C, 11 L/min, and 379 kPa, respectively. A fragmentor potential of 60 V was selected, and the scan range was 100−1500 uma.

Measurement of Fluorescent Advanced MRPs and Calculation of the FAST Index. Twenty milligram samples were mixed with 3 mL of 6% aqueous SDS, incubated for 30 min, stirred every 10 min for 30 s, and filtered through a cellulose paper filter (Whatman no. 40). The filtrates were employed for the determination of the FAST index according to the method of Birlouez-Aragon et al. (*21*) as

$$\mathrm{FAST_{index}} = 100[\mathrm{AMRPs_{FL}}/\mathrm{Trp_{FL}}]$$

where $\mathrm{AMRPs_{FL}}$ is fluorescence due to advanced MRPs measured at λ_{Ex} = 363 nm and λ_{Em} = 431 nm, whereas $\mathrm{Trp_{FL}}$ means tryptophan fluorescence at λ_{Ex} = 290 nm and λ_{Em} = 340 nm. Selection of the wavelengths for the detection of AMRPs was performed on the basis of fluorescence spectra of the samples. Wavelengths were chosen to achieve an optimal fluorescence measurement because it is highly dependent on the material to be analyzed. Readings were recorded in an RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan) with the slit width set at 2.5 nm. Samples were analyzed in triplicate, and FAST index data were expressed in percent (w/w).

Brown Pigments Assay. Samples were prepared following the same procedure previously described for fluorescent advanced MRPs. The formation of brown pigments was estimated as absorbance at 420 nm (*23*, *29*). The assay was performed in a Coulter DU 800 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). All of the measurements were made in triplicate. Results were expressed as arbitrary absorbance units.

Measurement of Extractable Total Phenolic Compounds (TPC) and Extractable Total Flavonoids (TF). *Sample Preparation*. Samples (175 mg) were extracted with 6.25 mL of 75 mM phosphate

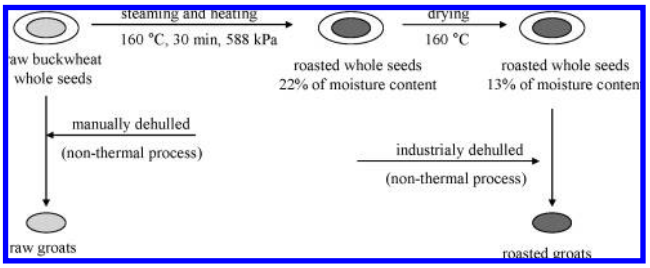

Figure 1. Simplified flow diagram of roasting of whole buckwheat seeds.

buffer, pH 7.4, incubated for 60 min with stirring every 10 min for 30 s and filtered by cellulose paper (Whatman no. 40) (*21*). The filtrates were used for the determination of the extractable TPC, extractable TF, and antioxidant capacity by the TEAC assay.

Determination of the Extractable TPC. TPC was assayed according to the method of Shahidi and Naczk (*30*). Briefly, 0.25 mL of 0.75 mM phosphate buffer extracts were mixed with 0.25 mL of Folin−Ciocalteu reagent/water (1:1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3), and 4 mL of water. The mixture was incubated at room temperature for 25 min and centrifuged at 2000*g* for 10 min. The absorbance of the clear supernatants was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu). The content of the extractable total phenolics in each sample was calculated by employing a standard curve of pure ferulic acid, and data were expressed as milligrams of ferulic acid equivalents (FAE) per gram of dry matter (dm).

Determination of Extractable TF. TF was determined by employing a colorimetric method (*31*). Two hundred microliters of a phosphate-buffered extract was diluted with 1.25 mL of distilled water. Then 75 μL of a 5% $NaNO_2$ solution was added, and the mixture was allowed to stand at room temperature. After 6 min, 150 μL of a 10% $AlCl_3 \cdot 6\,H_2O$ was added, and the mixture was allowed to stand for another 5 min. After that, 0.5 mL of 1 M NaOH was added. The solution was well mixed, and the absorbance was measured immediately at 510 nm using a spectrophotometer (UV-160 1PC, Shimadzu). A calibration curve of pure rutin in concentrations from 0.05 to 1.0 mg/mL was constructed. Results were expressed as milligrams of rutin equivalents per gram of dm.

Individual Flavonoid Profile. Flavonoids were extracted with the aqueous methanol and analyzed by RP-HPLC according to the method of Zielinska et al. (*17*). All chromatographic determinations were performed on a C18(2) Luna 5 μm column, 4.6 × 200 mm (Phenomenex, Torrance, CA), at 35 °C and a flow rate of 0.8 mL/min. The flavonoids were eluted in a gradient system employing aqueous 4% formic acid (solvent A) and acetonitrile containing 4% of formic acid (solvent B). Gradient was as follows: 12−22−70−12−12% B at gradient times t_G = 0−9−22−40−45−50 min. Identification and quantification of flavonoids in the samples were performed by injecting pure standard substances and plotting adequate calibration curves. Stock solutions of rutin (500 μM), orientin (517 μM), homoorientin (477 μM), vitexin (509 μM), and isovitexin (574 μM) in methanol were prepared and dissolved to obtain calibration curves ranging from 2.5 to 20 μM of each pure standard. Analyses were performed in triplicate.

Hydrophilic and Lipophilic $ORAC_{FL}$ Assays of Samples. The oxygen radical absorbance capacity ($ORAC_{FL}$) method as modified by Prior et al. (*32*) was employed to analyze both lipophilic and hydrophilic peroxyl scavengers. Freeze-dried buckwheat samples (0.1 g) were extracted twice with 2.5 mL of hexane and centrifuged at 5000*g* at 4 °C for 10 min. The lipophilic hexane fractions were combined and dried under nitrogen flow. The remaining residues were then extracted with 5 mL of acetone/water/acetic acid (70:29.5:0.5, v/v). The mixtures were homogenized by stirring in a magnetic plate stirrer for 30 s and sonicated for 2 min. This step was repeated, and after centrifugation (13000*g* at 4 °C for 10 min), the supernatants were collected and named as a hydrophilic fraction (H-$ORAC_{FL}$). For the lipophilic antioxidant assay (L-$ORAC_{FL}$), the dried hexane extract was dissolved in 250 μL of acetone and supplemented with 750 μL of 7% RMCD. The latter solution was also used as blank and dilution of Trolox standard in the L-$ORAC_{FL}$ assay. Prior to analysis, the samples were diluted at 1:20 (v/v) in the corresponding solvents, and then the reaction mixtures for determination of either H-$ORAC_{FL}$ or L-$ORAC_{FL}$ values were prepared by mixing 375 μL of diluted sample, 2.25 mL of 42 nM fluorescein, and 375 μL of 153 mM ABAP. The fluorescence readings were made at an excitation wavelength of 493 nm and an emission wavelength of 515 nm. H-$ORAC_{FL}$ or L-$ORAC_{FL}$ values were reported as Trolox equivalents. A standard curve was constructed by Trolox concentration versus net area under the FL decay curve and employed for calculation of the peroxyl radical scavenging properties of the samples. Data were expressed as micromoles of TE per gram of dm. Total $ORAC_{FL}$ values were calculated by combining L-$ORAC_{FL}$ and H-$ORAC_{FL}$ as previously described by Wu et al. (*33*).

TEAC Method. Trolox equivalent antioxidant capacity (TEAC) was evaluated as described by Re et al. (*34*). Absorbance values were measured at 734 nm using a spectrophotometer (UV-160 1PC, Shimadzu). TEAC values of the samples were calculated on the basis of the percentage inhibition of absorbance at 734 nm and were expressed as micromoles of TE per gram of dm.

Statistical Analysis Method. Data were subjected to a one-way analysis of variance (ANOVA) using the Fischer LSD test of the Statgraphic 5.0 program (Statistical Graphic, Rockville, MD) for Windows. The level of significance was set at 95%. A correlation test was performed to find out what kind of compounds greatly contribute to the overall antioxidant power of buckwheat products.

RESULTS

Protein Quality. Table 1 shows data on protein and MRPs found in buckwheat samples. Protein values detected in raw whole seeds and raw groats were significantly different ($p < 0.05$). Roasting significantly decreased the total protein content of groats, whereas this parameter was not affected by the thermal processing of whole seeds. A significant formation ($p < 0.05$) of MRPs (furosine, fluorescent AGEs, and melanoidins) was induced by roasting of both buckwheat whole seeds and groats. The contents of MRPs found in roasted seeds and groats were of the same order of magnitude ($p > 0.05$). Data on the relationship among the chemical indicators of protein quality hereby studied are shown in **Table 2**. A negative and statistically insignificant ($p > 0.05$) correlation between protein and MRP sets of data was observed, whereas there was a positive and mostly statistically significant ($p < 0.05$) correlation between MRPs data. No significant correlation ($p = 0.07$) was observed between the FAST index and browning.

Extractable TPC, Extractable TF, and Individual Flavonoid Profile. Table 3 provides extractable TPC and TF levels detected in raw and thermally processed buckwheat samples. The extractable TPC value of raw groats was about 12% higher than that corresponding to raw whole seeds ($p < 0.05$). There were no statistically significant differences ($p > 0.05$) between extractable TF values of raw seeds and groats. After roasting, contents of both chemical families of antioxidants (extractable TPC and TF) decreased significantly ($p < 0.05$).

Table 4 shows individual flavonoid contents of raw and roasted buckwheat groats. As can be observed, roasting caused a statistically significance decrease ($p < 0.05$) in all flavonoids naturally constituting groats. Rutin content of raw groats was about 6-fold higher than that found in roasted groats. Vitexin and isovitexin were present only in very low quantities, whereas homoorientin and orientin were not detected in the roasted groats.

Lipophilic (L-$ORAC_{FL}$) and Hydrophilic (H-$ORAC_{FL}$) Antioxidant Capacities. Table 5 shows L-$ORAC_{FL}$ and H-$ORAC_{FL}$ values of buckwheat whole seeds and groats before and after roasting. All of the samples scavenged peroxyl radicals. However, the anti-peroxyl radical power was significantly different among the samples ($p < 0.05$). L-$ORAC_{FL}$ values represented less than 9% of the H-$ORAC_{FL}$ values. Roasting induced a statistically significant decrease in L-$ORAC_{FL}$ (~45%) and H-$ORAC_{FL}$ (~54%) values, suggesting a depletion in both hydrophilic and hydrophobic buckwheat antioxidants. A positive and statistically

Table 1. Data on Protein, Furosine, and Fluorescent Advanced MRPs Determined by FAST Index and Development of Browning Measured by Absorbance at 420 nm in Raw and Roasted Buckwheat Samples[a]

material	protein (g/100 g of dm)	furosine (mg/100 g of protein)	FAST index	browning A_{420}
raw whole seeds	12.77 ± 0.13 a	nd	429.9 ± 49.7 a	0.24 ± 0.02 a
roasted whole seeds	12.43 ± 0.12 a	40.44 ± 0.76 a	2569.8 ± 354.4 b	0.32 ± 0.03 b
raw groats	15.61 ± 0.13 b	nd	56.5 ± 1.4 c	0.27 ± 0.01 a
roasted groats	13.41 ± 0.16 c	40.71 ± 1.37 a	2940.2 ± 556.6 b	0.34 ± 0.04 b

[a] Data are expressed as mean ± standard deviation (n = 3). Means in each column followed by different letters are significantly different ($p < 0.05$). nd, not detectable.

Table 2. Correlation between Chemical Indicators of Protein Quality

parent parameter	partner for correlation	correlation coefficients
protein content	furosine	−0.51 (p = 0.49)
	FAST index	−0.56 (p = 0.44)
	browning	−0.22 (p = 0.78)
furosine	FAST index	+0.99 (p = 0.01)
	browning	+0.95 (p = 0.05)
FAST index	browning	+0.93 (p = 0.07)

Table 3. Extractable Total Phenolic Compounds (TPC) and Extractable Total Flavonoids (TF) in Whole Buckwheat Seeds and Groats before and after Roasting[a]

material	extractable TPC (mg of FAE/g of dm)	extractable TF (mg of rutin equiv/g of dm)
raw whole seeds	4.44 ± 0.11 a	1.88 ± 0.02 a
roasted whole seeds	3.14 ± 0.12 b	1.58 ± 0.04 b
raw groats	4.98 ± 0.06 c	1.80 ± 0.02 a
roasted groats	3.93 ± 0.31 d	1.58 ± 0.06 b

[a] Data are expressed as mean ± standard deviation (n = 3). Means in each column followed by different letters are significantly different ($p < 0.05$).

Table 4. Concentration of Individual Flavonoids in Buckwheat Groats before and after Roasting[a]

compound	raw groats (μg/g of dm)	roasted groats (μg/g of dm)
homoorientin	18.6 ± 1.0	nd
orientin	12.0 ± 0.1	nd
vitexin	13.6 ± 1.6 a	3.8 ± 0.2 b
isovitexin	10.7 ± 0.4 a	1.7 ± 0.1 b
rutin	198.1 ± 3.3 a	34.1 ± 0.9 b
total	253.0 a	39.6 b

[a] Data are expressed as mean ± standard deviation (n = 3). Means in each row followed by different letters are significantly different ($p < 0.05$). nd, not detectable.

insignificant correlation ($p > 0.05$) was found between H-ORAC$_{FL}$ values and contents in extractable TPC ($r = 0.51$, $p = 0.49$) and extractable TF ($r = 0.93$, $p = 0.74$). In contrast, there was a mostly negative but not significant relationship between MRPs and overall antioxidant capacity values. Correlation coefficients between the H-ORAC$_{FL}$ values of buckwheat whole seeds and groats before and after roasting and furosine contents, FAST index, and browning were $r = -0.87$ ($p = 0.13$), $r = -0.85$ ($p = 0.15$), and $r = -0.98$ ($p = 0.02$), respectively. The same trend was noted for the correlation between the L-ORAC$_{FL}$ values and furosine contents, FAST index, and browning, and the correlation coefficients were $r = -0.78$ ($p = 0.22$), $r = -0.69$ ($p = 0.31$), and $r = -0.70$ ($p = 0.29$), respectively. Exceptionally and curiously, there was observed a negative statistically significant relationship between H-ORAC$_{FL}$ and the FAST index.

Trolox Equivalent Antioxidant Capacity (TEAC). TEAC values are provided in **Table 5**. Roasting resulted in a statistically significant reduction of ABTS radical cation scavenging activity of the samples. TEAC values were lower than those obtained by the ORAC$_{FL}$ assay. A positive relationship was observed between TEAC and H-ORAC$_{FL}$ values ($r = 0.68$, $p = 0.32$) and TEAC and L-ORAC$_{FL}$ values ($r = 0.59$, $p = 0.041$). However, the relationship between TEAC and H-ORAC$_{FL}$ values was not statistically significant, whereas that between TEAC and L-ORAC$_{FL}$ values was significant ($p < 0.05$). The TEAC values of the raw whole seeds, roasted whole seeds, raw groats, and roasted groats were 21, 35, 31, and 54% of total ORAC $_{FL}$ values calculated as H-ORAC$_{FL}$ + L-ORAC$_{FL}$. There were found positive and no statistically significant differences between TEAC values and extractable TPC content ($r = 0.88$, $p = 0.12$) and TEAC values and extractable TF content ($r = 0.82$, $p = 0.18$), respectively. Correlation coefficients between TEAC and furosine, FAST index, and browning were $r = -0.92$ ($p = 0.08$), $r = -0.96$ ($p = 0.04$), and $r = -0.78$ ($p = 0.21$), respectively. There was a negative and statistically significant relationship between TEAC values and furosine and TEAC values and FAST index, respectively.

DISCUSSION

MRPs, such as furosine, advanced MRPs, and melanoidins (brown pigments), are widely used as markers of the nutritional quality of foods (*35*). Furosine levels (**Table 1**) were similar to those reported in several thermally processed cereal foods, such as dried pasta (*36*), cookies, crackers, breakfast cereals (*37*), and baby cereals (*38*), and about 2−4-fold lower than those found in rye bread crust in our previous study (*39*). According to our data, differences in the morphological structure of the whole buckwheat seeds did not affect the rate of the Maillard reaction. Levels of MRPs in thermally processed samples were very similar ($p > 0.05$). Moreover, furosine data provided in this study indicated a decrease in the nutritional quality of buckwheat proteins due to roasting.

In the past few decades, the determination of fluorescence has been proposed as an effective procedure to assess the extent of the Maillard reactions (*41*). Fluorescence of raw whole buckwheat seeds and raw groats may be due to fluorescent compounds naturally constituting buckwheat seeds such as tryptophan. Data on the fluorescence of thermally processed samples agreed with those of furosine, thus suggesting the occurrence of the MR during roasting and its progress to advanced steps. Significant differences in the FAST index were observed among the samples (**Table 1**). After roasting, tryptophan fluorescence significantly decreased, indicating a reduction in protein quality of the processed food. On the other hand, dietary advanced MRPs may play an important role in the induction of chronic diseases associated with underlying inflammation, and only their excessive consumption may represent an independent factor for inappropriate chronic oxidant stress and inflammatory factor surges during healthy adult years (*18*).

Color formation is a typical characteristic of the MR that can be readily measured by reading the absorbance at wavelengths of

Table 5. Lipophilic (L-ORAC$_{FL}$), Hydrophilic (H-ORAC$_{FL}$), and Trolox Equivalent Antioxidant Capacity (TEAC) of Buckwheat Whole Seeds and Groats before and after Roasting[a]

material	L-ORAC$_{FL}$ (μmol of TE/g ofdm)	H-ORAC$_{FL}$ (μmol of TE/g of dm)	L-ORAC$_{FL}$ + H-ORAC$_{FL}$ (μmol of TE/g of dm)	TEAC (μmol of TE/g of dm)
raw whole seeds	5.77 ± 0.33 a	124.55 ± 7.18 a	130.31 ± 6.84 a	28.60 ± 0.81 a
roasted whole seeds	1.68 ± 0.16 b	70.53 ± 0.77 b	72.21 ± 0.93 b	25.57 ± 0.01 b
raw groats	4.83 ± 0.33 c	95.01 ± 5.09 c	99.70 ± 5.61 c	31.28 ± 0.09 c
roasted groats	4.06 ± 0.23 d	42.53 ± 1.46 d	46.09 ± 0.98 d	25.12 ± 0.56 b

[a] Data are expressed as mean ± standard deviation (n = 3). Means in each column followed by different letters are significantly different ($p < 0.05$).

360 and 420 nm (*29*). Color formation due to MR normally reaches its maximum at intermediate water activity (*42*). Changes in water activity taking place during roasting of whole buckwheat seeds may induce the generation of brown compounds. According to the buckwheat composition (*2*, *3*) the color development may be associated with the formation of "melanoprotein" (*43*). Indeed, an increase in absorbance readings at 420 nm was observed in roasted whole buckwheat seeds and roasted groats within the range of 33−36% when compared to the raw whole seeds and raw groats. However, further studies should be performed to eliminate the contribution of low molecular mass compounds to browning of roasted buckwheat samples. In a gluten-glucose system, color was attributed to compounds formed by glucose interaction with ammonia generated by deamination of glutamine residues (*44*). Color data agreed with those of furosine and fluorescence, providing more evidence for MR occurrence as well as its progress to advanced steps (**Table 2**).

Our findings are supported by the recent report by Christa et al. (*45*) on the changes in functional properties of starch originating from native and roasted (160 °C during 30 min) buckwheat groats. They indicate a decrease in starch content after roasting, which resulted in glucose liberation from starch. In this case, glucose released from the starch may be as substrate of the Maillard reaction, thus explaining the higher content of MRPs of roasted groats indicated in **Table 1** when compared to the raw groats.

Extractable TPC values were consistent with those recently reported by Zielinska et al. (*46*). However, small differences in extractable TPC between both sets of data related to whole seeds and groats may be ascribed to the effect of environmental factors on postharvest quality of common buckwheat. It was noted that extractable TPC and extractable TF were decreased by 29 and 16% in roasted whole buckwheat seeds, respectively, whereas fewer decreases in their content (by 21 and 12%) were observed in roasted groats (**Table 3**). The analysis of individual flavonoids clearly indicated a low stability of these buckwheat components under roasting conditions (**Table 4**). The flavonoid profile provided in the study was almost the same as that reported recently for buckwheat roasted groats by Zielinska et al. (*17*). Our findings are in agreement with those reported by Dietrych-Szostak and Oleszek (*5*) as well as Im et al. (*47*), who found that the most abundant flavonoids of buckwheat groats, named as rutin and isovitexin, were adversely affected by temperature and heating time.

Antioxidant Capacity. Data on the antioxidant capacity determined by either total ORAC$_{FL}$ or TEAC (**Table 5**) confirmed that all of the samples were able to scavenge peroxyl and ABTS radical cations, being better scavengers of the former than the latter. In this study, both lipophilic and hydrophilic peroxyl scavengers were determined by employing the ORAC$_{FL}$ assay (*32*, *33*). The ORAC assay has been used to study the antioxidant capacity of many food samples and has recently been adopted by the food industry as an adequate antioxidant index (*48*). Data on L-ORAC$_{FL}$ and H-ORAC$_{FL}$ clearly indicated that most of the peroxyl radical scavengers constituting buckwheat samples were hydrophilic compounds, which were significantly affected by roasting. The same trend of changes was observed by the ABTS assay. Our findings are in good agreement with our previous results before and after hydrothermal processing (*49*) and with the recent report on the extrusion cooking of buckwheat groats (*9*). Moreover, the antioxidant capacity of raw whole buckwheat seeds provided by the ORAC$_{FL}$ assay (H-ORAC$_{FL}$ + L-ORAC$_{FL}$) was at the same level as recently reported for the antioxidant capacity of buckwheat obtained by the direct extraction-independent procedure (*50*). The antioxidant capacities of the raw and roasted groats (H-ORAC$_{FL}$ + L-ORAC$_{FL}$) were about 4 and 2 times higher, respectively, than that of 19 breakfast cereals, breads, and snack food (*33*). This indicates that the roasted buckwheat groats still represent a significant source of antioxidants, which may support their potential as a natural functional food.

Conclusions. Both protein quality and overall antioxidant properties of buckwheat whole seeds and groats were significantly reduced during roasting. Further research should be conducted to determine the risk/benefits balance of food processing. Antioxidants surviving the roasting process might exert a beneficial role in human health.

ABBREVIATIONS USED

FAST index, fluorescence of advanced MRPs and soluble tryptophan; FCR, Folin−Ciocalteu reagent; extractable TPC, extractable total phenolic compounds; extractable TF, extractable total flavonoids; MRPs, Maillard reaction products; ORAC$_{FL}$, oxygen radical absorbance capacity analysis, which utilizes fluorescence as the fluorescence probe; RMCD, randomly methylated β-cyclodextrin; TEA, triethylamine; TEAC, Trolox equivalent antioxidant capacity.

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