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Comparison of Spectrophotometric and Electrochemical Methods for the Evaluation of the Antioxidant Capacity of Buckwheat Products after Hydrothermal Treatment

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This paper reports the use of spectrophotometric and voltammetric methods for the determination of the antioxidant capacity of buckwheat and its products originated from a technological line of a buckwheat roasted groats producer. 80% methanol extracts from raw and roasted buckwheat and groats and hulls obtained from roasted buckwheat were used. The spectrophotometric methods included (1) free radical scavenging activities of the extracts against $ABTS^{•+}$ radical cation (TEAC) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH RSA) and (2) determination of reducing capacity by the means of Folin−Ciocalteu reagent (FCR) application. The radical scavenging activities of the extracts were also investigated using a voltammetric assay. Moreover, the flavonoids profiles of the studied materials were provided. Buckwheat roasting caused a decrease in TEAC, DPPH RSA, and FCR reducing capacity by 70%. The lowest TEAC, DPPH RSA, and FCR reducing capacities were noted for roasted groats. Both DPPH RSA and TEAC methods were highly positively correlated with the FCR reducing capacity assay ($r = 0.98$ and $r = 0.99$). Cyclic voltammograms of analyzed buckwheat extracts were useful for evaluation of the antioxidant capacity. The total charge below the anodic current waveform was correlated with the data obtained by TEAC ($r = 0.770$), DPPH RSA ($r = 0.88$), and FCR reducing capacity ($r = 0.81$). The changes in the antioxidant capacity of buckwheat and its products followed the changes in flavonoids composition. In particular, the concentration of flavonoids was related to measurements by cyclic voltammetry.

KEYWORDS: Whole buckwheat; hydrothermal treatment; roasted groats; hull; antioxidant capacity; spectrophotometric methods; cyclic voltammetry; flavonoids

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Möench L.) is a crop adapted to a cool, moist climate, short growing season (70−90 days) and requires negligible crop protection. Buckwheat after dehulling (pericarp removal) is primarily used for human consumption and at present is considered as a food of high nutritional value because of high vitamin B_1 and B_2 and lysine content and protein with a balanced amino acid composition (*1*). Moreover, buckwheat contains biologically active compounds with beneficial action on consumers' organisms, such as flavonoids (*2*, *3*) [catechins, rutin, quercetin, orientin, isoorientin, vitexin, isovitexin]; phytosterols (*4*) [mainly β-sitosterol, campesterol with traces of stigmasterol]; and a novel series of galactosyl cyclitols (*5*−*7*) including fagopyritols (A1, A2, A3, B1, B2, B3) and thiamin-binding proteins (*8*). Most buckwheat for human consumption is used as processed flour. In order to obtain buckwheat groats of consumption quality, the grains are dehulled. In Central and Eastern Europe, the dehulling process precedes simultaneously raising the grain moisture up to 22% dm and heating (steaming water vapor 6 atm at 160 °C for 30 min), and all processes are included in one technological line. The resulting roasted groat (roasted kasha) is ready for cooking and usually served like rice, and remaining hulls form the waste product. The nonroasted groats are sometimes used in the U.S. as a breakfast cereal (*9*).

Recently, Zieliński et al. (*10*). found after thermal treatment of buckwheat groats, using extrusion cooking, a significant decrease in inositol phosphates, reduced glutathione, tocopherols and tocotrienols, and melatonin content, except for a 2-fold higher content of free and released from ester bonds phenolic acids. Similar results in relation to the total phenolic compounds were reported by Sensoy et al. (*11*) after roasting of dark buckwheat flour. Moreover, Dietrych-Szostak and Oleszek (*3*) showed a drastic reduction of total flavonoids concentration in buckwheat after four thermal processing procedures routinely used for producing roasted groats.

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Recent research is focused on the development of buckwheat as a potential functional food material. Buckwheat appears to be a suitable component of food also from its antioxidant activity point of view, and this was a subject of many reports (*2*, *10*, *12*, *13*). However, the data compiled on the effect of thermal treatments on the antioxidant capacity of buckwheat and its products are limited. Different analytical methods have been applied to the measurement of the antioxidant capacity of the food sample. Among them, spectrophotometric methods such as Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorption capacity (ORAC), and ferric reducing antioxidant power (FRAP) assays have been the most commonly represented (*14*−*16*). More recently, a highly attractive, convenient, and sensitive voltammetric approach to study antioxidant properties has been reported (*17*−*19*). In a number of papers dealing with measurement of the antioxidant capacity, very rarely is a single method applied. When cyclic voltammetry (CV) is used, it is often compared to either spectrophotometric or HPLC methods (*20*). To the best of our knowledge, spectrophotometric and voltammetric methods have never been simultaneously applied to study the impact of thermal treatment on food antioxidant capacity. What is worthy of note regarding the work undertaken is that the obtained roasted groats represent the staple food originated from industry.

The objectives of the work were (1) to compare the antioxidant capacities of the buckwheat and its products originated from a technological line of a buckwheat roasted groats producer and (2) to compare widely differing spectrophotometric methods with the voltammetric method for their suitability to measure the antioxidant capacity when simultaneously applied to the same food samples.

MATERIALS AND METHODS

Reagents. Acetonitrile and methanol (HPLC-grade) were provided by Merck (Darmstad, Germany). Rutin (quercetin-3-rutinoside), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO). 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 Company Inc. (Lyon, France). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland. Water was purified with a Milli-Q-system (Millipore, Bedford, MA). All solutions prepared for HPLC were passed through a 0.45 μm nylon filter before use.

Raw Material and Hydrothermal Treatment. Buckwheat (var. Kora) was provided by the local industry from Northeast Poland. Whole buckwheat (untreated) was analyzed using the methods of the AOAC for protein, crude fat, starch, and ash content (*21*). Moreover, the hull content was determined as previously described (*22*).

The hydrothermal treatment included raising the moisture content of the whole grain to 22% of dry matter (% dm) followed by simultaneously steaming (water vapor 6 atm) and heating (at 160 °C for 30 min); after that the roasted groats and hulls were obtained by the dehulling process. Then, the following samples were used for the characterization of antioxidant properties: untreated buckwheat, hydrothermal treated buckwheat, roasted groats, and hulls. The material was lyophilized and ground using a laboratory mill.

Preparation of 80% Methanol Extracts. About 100 mg of dried and pulverized buckwheat samples was extracted with 1 mL of 80% methanol by a 30 s sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5000*g* at 4 °C). That step was repeated 5 times, and the supernatants were collected in a 5 mL flask. For the analyses of flavonoids and cyclic voltammetric experiments, 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 a 60 s sonication. Next, the mixture was vortexed for 60 s, again sonicated, and centrifuged for 5 min (5000*g*, 4 °C). That step was repeated on the residue with the next volume of 2.5 mL of the solvent. The supernatants were collected in a 5 mL flask. Finally, all extracts were kept at −80 °C prior to further analysis.

Total Antioxidant Capacity by Trolox Equivalent Antioxidant Capacity (TEAC) Assay. Trolox equivalent antioxidant capacity was determined following a procedure described by Re et al. (*23*) with a minor modification described below. For measurements, the $ABTS^{\bullet+}$ solution was diluted with 80% methanol, respectively, to the absorbance of 0.70 ± 0.02 at 734 nm. For the photometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 μL of the extracts or Trolox standards were mixed and measured immediately after 6 min at 734 nm at 30 °C using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). Appropriate solvent blanks were used in each assay. The Trolox equivalent antioxidant capacity of the 80% methanolic extracts was calculated, using the Trolox standard curve, on the basis of percentage inhibition of absorbance at 734 nm. Additionally, the Trolox equivalent antioxidant activity of rutin was determined in parallel with the Trolox standard curve.

DPPH Radical Scavenging Assay (DPPH RSA). $DPPH^{\bullet}$ scavenging activity was determined using a modified method of Brand-Williams et al. (*24*). In the DPPH RSA assay, antioxidants present in the sample reduce the free radical 2,2-diphenyl-1-picrylhydrazyl, which has an absorption maximum at 515 nm. The radical solution was prepared by dissolving 10 mg of $DPPH^{\bullet}$ in 25 mL of 80% methanol. At first, the absorbance of the disposable cuvette with 250 μL of the methanolic DPPH solution and 2.1 mL of 80% methanol was measured as a blank. Then, 100 μL of the 80% methanol extract was added to 250 μL of the methanolic DPPH solution and 2 mL of 80% methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 20 min. The decrease in absorbance of the resulting solution was monitored at 515 nm at 20 min using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The Trolox standard solutions (concentration 0.1−2.0 mM) in 80% methanol were assayed under the same conditions, and then the $DPPH^{\bullet}$ scavenging activity of the samples was expressed in terms of the Trolox equivalent antioxidant capacity on the basis of percentage inhibition of absorbance at 515 nm by standards.

FCR Reducing Capacity Assay. Determination of the FCR reducing capacity by the means of Folin−Ciocalteu reagent (FCR) application was carried out according to Shahidi and Naczk (*25*). Briefly, 0.25 mL of an 80% methanol extract was mixed with 0.25 mL of FCR previously diluted with distilled 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. Supernatant absorbance was measured at 725 nm in a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The data were calculated as milligrams of rutin equivalents on a dry matter basis.

Voltammetric Experiments. Cyclic voltammetric experiments were performed in 80% methanol extracts mixed with 0.2 M sodium acetate−acetic buffer (pH 4.5) at the ratio 1:1 (v/v) according to Cosio et al. (*19*). The sodium acetate−acetic buffer acted as supporting electrolyte for the voltamperometric measurements. The measurements were carried out using a conventional three electrode system: (a) a 3 mm diameter glassy carbon electrode as working electrode (BAS MF-2012), (b) a Ag/AgCl electrode as reference electrode, and (c) a platinum electrode as counter electrode. The voltammetric experiments were performed at room temperature using a voltammetric apparatus cell, to which analyzed buckwheat extract mixed with the supporting electrolyte was introduced. Exactly 100 μL of the extract and 100 μL of buffer were used. In order to avoid the diminishing of its sensitivity, the working electrode was carefully polished with 0.05 μm alumina paste and ultrasonically rinsed in deionized water at the end of each cycle. After washing, the electrode was then ready for further tests. The cyclic voltammograms were recorded by scanning the potential from −100 to +1300 mV. Cyclic voltammograms were acquired with a potentiostat/galvanostat KSP system at a scanning rate of 100 mV s^{-1}. For the test purpose, the total charge below the anodic wave curve of the voltammogram was measured. The method is actually based on the

Table 1. Antioxidant Capacity of the Untreated and Hydrothermally Treated Whole Buckwheat Evaluated by Spectrophotometric Methods[a]

material	TEAC (μmol Trolox/g dm)	DPPH RSA (μmol Trolox/g dm)	FCR reducing capacity (mg rutin equiv/g dm)
whole buckwheat	42.24 ± 0.28 a	27.20 ± 0.57 a	12.04 ± 0.04 a
treated whole buckwheat	12.59 ± 0.37 b	8.22 ± 0.44 b	3.50 ± 0.02 b
roasted groats	9.25 ± 0.50 c	5.48 ± 0.54 c	2.61 ± 0.02 c
hulls	13.53 ± 0.02 d	13.16 ± 0.37 d	4.62 ± 0.02 d

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

correlation between the total charge below the anodic wave of cyclic voltammograms and the antioxidant capacities of the sample and the reference substance. 80% methanol solutions of Trolox within the concentration range 0.1−1.25 mM were used, and the results were expressed as micromoles of Trolox per gram of dry matter (μmol Trolox/g dm).

Flavonoids Profile. The extracts prepared for the flavonoids analyses were submitted to HPLC analysis (Shimadzu, Kyoto, Japan), consisting of two pumps (LC−10 AD), a UV detector (SPD-10A) set at 330 nm, an autosampler set to 10 μL injection (SIL-10 AD_{VP}), a column oven (CTO-10 AS $_{VP}$), and a system controller (SIL-10 AD_{VP}). All chromatographic determinations were performed at 35 °C with the flow rate of 1 mL/min on a C18(2) Luna 5μ column, 4.6 mm × 250 mm (Phenomenex, Torrance, CA). The flavonoids were eluted in a gradient system composed of aqueous 0.05% formic acid (solvent A) and acetonitrile containing 4% formic acid (solvent B). The 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 iso-vitexin stock solutions were prepared in methanol at concentrations of 500, 517, 477, 509, and 574 μM, taking into account the purity of the standards. For quantitative analysis, calibration standards were prepared in duplicate at five concentrations within the range of 2.5−20 μM of each compound.

Statistical Analysis Method. The results are given as the means and the standard deviation of three independent experiments. Statistical analysis was performed using the Student *t*-test, and the significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Characterization of the Untreated Buckwheat. The contents of protein, crude fat, starch, and ash found in the untreated buckwheat were 16.9, 1.8, 53.9, and 2.4% dm, whereas the hulls contents showed about 23% of the buckwheat weight.

Trolox Equivalent Antioxidant Capacity (TEAC). The 80% methanol extracts of the raw whole grain, the hydrothermal processed whole grain, the roasted groat, and the hydrothermally treated hull were examined for their free radical scavenging activities against $ABTS^{•+}$ cation radical. The results were compared to the free radical scavenging activity of Trolox. The obtained data expressed as Trolox equivalent antioxidant capacity (TEAC) are presented in **Table 1**. The TEAC of the processed buckwheat whole grain was decreased by 70% when compared to that of the untreated whole grain. Moreover, the lowest TEAC was noted for roasted groats. It was decreased by 27%, whereas the TEAC of the hull increased by 7% after the process when compared to that of the hydrothermally treated whole grain. These results indicate that the 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 the groats. These findings are in good agreement with those published on bioactive compounds in the cereal grains before and after hydrothermal processing (*26*) and also confirm the recent report on the extrusion cooking of buckwheat groat (*10*). The TEAC of rutin determined by this assay showed a TEAC value equal to 2.4 mM Trolox. This value was identical to that reported by Rice-Evans et al. (*27*), and it was used to express the FCR reducing capacity as antioxidant capacity (μmol Trolox/g dm) to make further comparison of data obtained from different assays.

DPPH Radical Scavenging Activity (DPPH RSA). The 80% methanol extracts of the raw whole grain, the hydrothermal processed whole grain, the roasted groat, and the hydrothermally treated hull were also used for the determination their DPPH RSA. The obtained results compared to the DPPH RSA of Trolox are presented in **Table 1**. DPPH RSA of the hydrothermally processed whole grain was decreased approximately 70% when compared to that of the untreated whole buckwheat grain. This finding was in excellent agreement with the data obtained by the TEAC method. Moreover, the lowest radical DPPH scavenging activity was noted for buckwheat roasted groats, and it was decreased about 33% when compared to that of the whole grain after treatment, whereas the DPPH RSA of the hull increased by 60% after the process. The higher DPPH RSA of the hull obtained after dehulling the hydrothermally treated whole grain resulted from the weight proportion of the hull to the whole grain (one-fourth of the grain in weight) (*9*). These findings are in agreement with the recent report of Sensoy et al. (*11*), in which the antioxidant activity of buckwheat flours evaluated by the DPPH test was decreased after thermal treatment at 200 °C for 10 min. The results of the DPPH RSA for the buckwheat material were about twice lower where compared to the TEAC values. The data obtained by both DPPH RSA and TEAC assays were highly positively correlated (r = 0.97).

FCR Reducing Capacity. In this study, the reducing capacity of the investigated buckwheat extracts was measured. The Folin−Ciocalteu reagent (FCR) actually measures the reducing capacity of the sample (*28*), whereas the same assay based on the reaction of the FCR is usually recognized as the "total phenolic assay". The popularity of this latest method can mainly be attributed to its simplicity and speed of analysis. The major disadvantage of this assay is its low specificity, as the color reaction can occur with any oxidizable group, principally phenolic hydroxy. The FCR was initially intended for the analysis of proteins, taking advantage of the reagent's activity toward the protein tyrosine (containing a phenol group) residue. Many years later, Singleton and co-workers extended this assay to the analysis of total phenols in wine; since then the assay has found many applications (*29*). In this paper, in order to avoid misunderstanding of the actual meaning of "total phenolic contents", the "FCR reducing capacity" was used, as suggested by Huang et al. (*28*).

The FCR reducing capacity of the hydrothermally treated buckwheat whole grain was decreased by 71% when compared to that of the unprocessed whole grain. Moreover, the lowest FCR reducing capacity was noted for roasted groat, and it was decreased by 25% when compared to that for hydrothermally treated whole grain (**Table 1**). On the other hand, the FCR reducing capacity of the hull increased by 32% when compared to that of the hydrothermally processed whole grain. These findings were in accordance with those results provided by TEAC and DPPH RSA assays. Moreover, FCR reducing

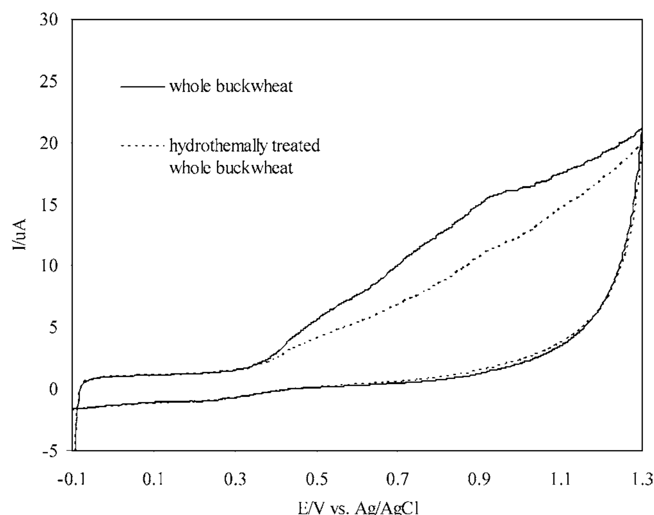

Figure 1. Cyclic voltammograms of analyzed buckwheat extracts originated from whole buckwheat and hydrothermally treated whole buckwheat. Operative conditions: concentration of each extract, 20 mg/mL 80% methanol; solution, 80% methanol mixed with 0.2 M sodium acetate–acetic buffer (pH 4.5) at the ratio 1:1 (v/v); scan rate, 100 mV s^{-1}.

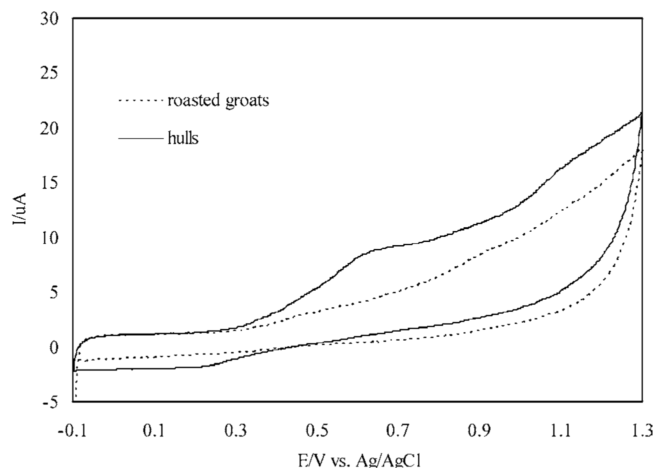

Figure 2. Cyclic voltammograms of analyzed buckwheat extracts originated from roasted groats and hulls obtained after hydrothermal treatment of whole buckwheat. Operative conditions: concentration of each extract, 20 mg/mL 80% methanol; solution, 80% methanol mixed with 0.2 M sodium acetate–acetic buffer (pH 4.5) at the ratio 1:1 (v/v); scan rate, 100 mV s^{-1}.

capacity data were highly positively correlated with both TEAC and DPPH RSA assays ($r = 0.98$ and $r = 0.99$). In this study, we converted the FCR data into antioxidant capacity using the experimentally determined rutin TEAC of 2.4 mM. In this case, the untreated whole buckwheat, the hydrothermally treated whole buckwheat, the roasted groats, and the hydrothermally treated hulls showed 47.28, 13.68, 10.26, and 18.16 μmol Trolox/g dm, and these values were higher by 12, 9, 11, and 34% than those obtained by the TEAC assay, respectively (**Table 1**). This finding clearly confirmed the overestimation of the results when the Folin−Ciocalteu reagent was used (*28*, *30*).

It should be pointed out that when the three above spectrophotometric methods were used for the characterization of the roasted buckwheat groat and the hydrothermally treated hull as the two products obtained after dehulling of the processed whole grain, an approximately twice higher antioxidant capacity of the hull was noted when compared to the groat. This makes it possible to use the hull in non-nutrition applications, whereas roasted groats should be more widely recommended in human nutrition.

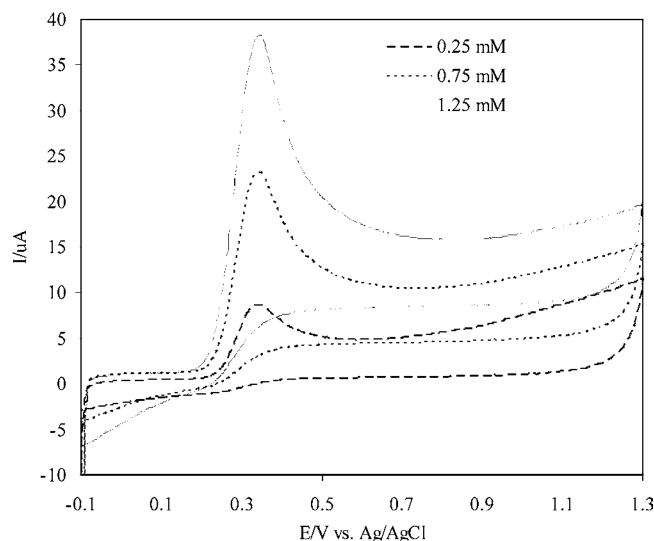

Figure 3. Cyclic voltammograms of Trolox concentration (0–1.25 mM) vs the total charge under the anodic wave. Operative conditions: concentration of each extract, 20 mg/mL 80% methanol; solution, 80% methanol mixed with 0.2 M sodium acetate–acetic buffer (pH 4.5) at the ratio 1:1 (v/v); scan rate, 100 mV s^{-1}.

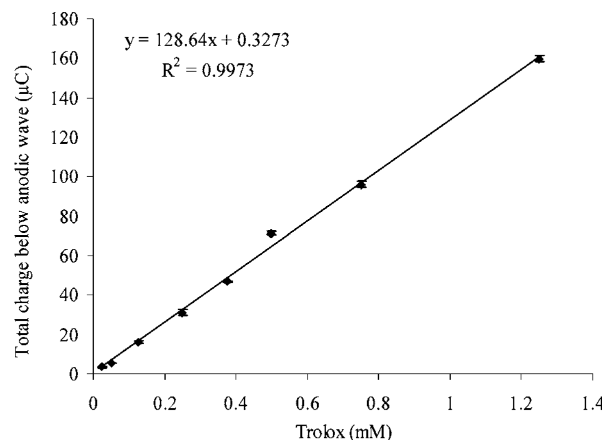

Figure 4. Dependency of the total charge under the anodic wave as a function of increasing concentration of Trolox (0.05–1.25 mM). The total charge below the anodic wave of the background signal (solvent + supporting electrolyte) was subtracted from the total charge obtained for each Trolox concentration.

Table 2. Antioxidant Capacity of the Untreated and Hydrothermally Treated Whole Buckwheat Provided by a Cyclic Voltammetric Method[a]

material	total charge below anodic wave (μC)	antioxidant capacity (μmol Trolox/g dm)
whole buckwheat	67.05 ± 4.23 a	5.19 ± 0.33 a
treated whole buckwheat	49.70 ± 3.40 b	3.84 ± 0.26 b
roasted groats	35.28 ± 2.43 c	2.72 ± 0.19 c
hulls	59.76 ± 5.44 d	4.62 ± 5.44 d

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

Antioxidant Capacity Derived from the Voltammetric Experiments. In recent years, cyclic voltammetry (CV) was shown to be a convenient methodology for evaluating the antioxidant capacity of human plasma, animal tissues, edible plants, wines, different types of tea, and coffee (*17*, *31*−*34*). A

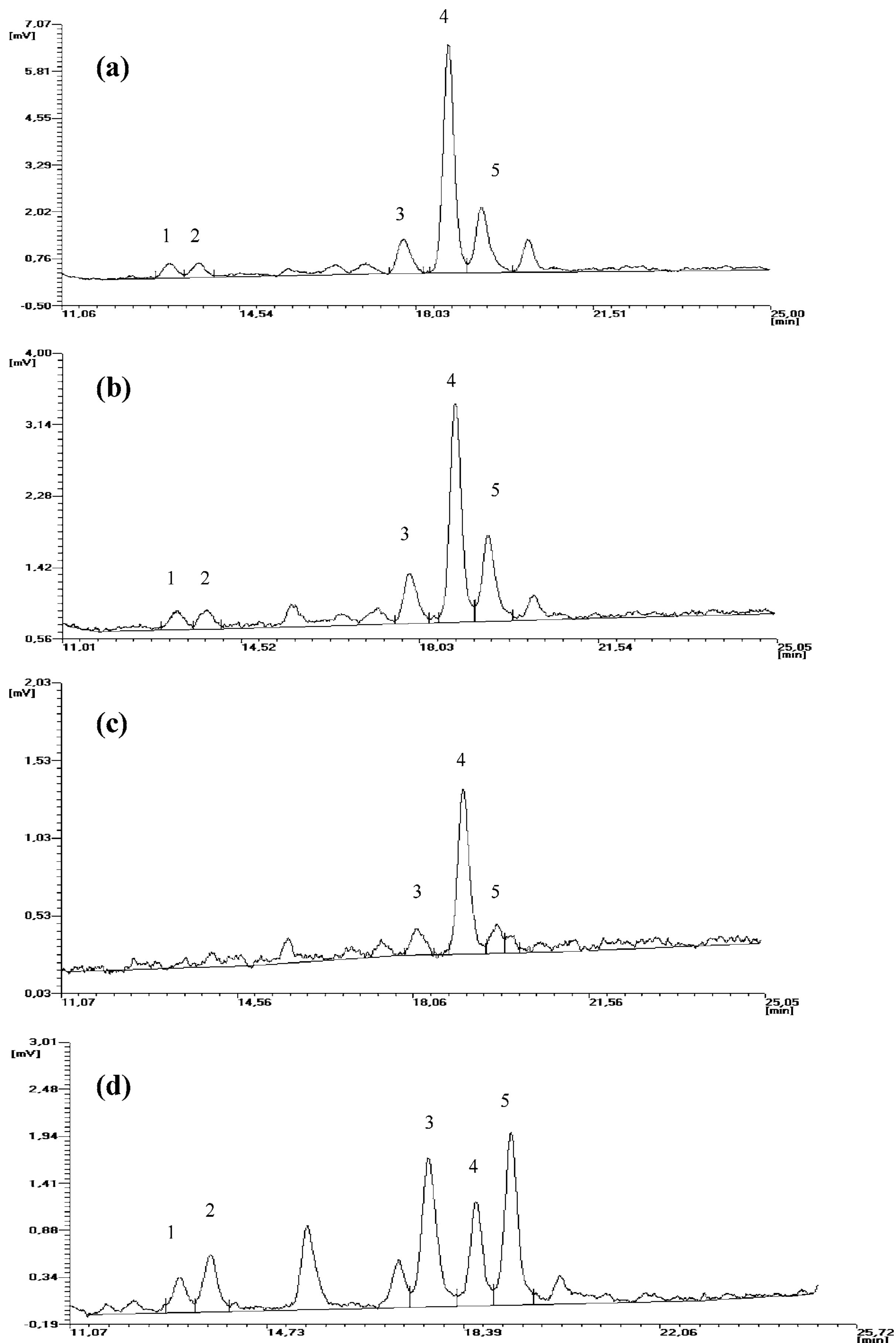

Figure 5. Typical HPLC chromatogram of buckwheat flavonoids recorded at 330 nm: (1) isoorientin; (2) orientin; (3) vitexin; (4) rutin; (5) isovitexin; (**a**) whole buckwheat; (**b**) treated whole buckwheat; (**c**) roasted groats; (**d**) hulls. The preparation of the extracts injected into an HPLC column was as described in the Material and Methods.

Table 3. Concentration of Flavonoids in Buckwheat before and after Hydrothermal Processing (μg/g dm)[a]

compd	whole buckwheat	treated whole buckwheat	roasted groats	hulls
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

[a] Roasted groats and hulls were obtained after dehulling of the treated whole buckwheat; see Materials and Methods; nd = not detected. Means in a row followed by the same letter are not significantly different ($p \leq 0.05$).

cyclic voltammogram (CV tracing) provides information describing the integrated antioxidant capacity without the specific determination of the contribution of each individual component. It is based on the analysis of the anodic current (AC) waveform, which is a function of the reductive potential of a given compound in the sample and/or a mixture of components. The total antioxidant capacity of the sample is a function combining two sets of parameters. The first is the biological oxidation potential, whereas the second is the intensity of the anodic AC current (I_a), reflecting the concentration of the components. Recently, it has been proposed that the area under the AC wave (S; related to the total charge) is a better parameter reflecting the antioxidant capacity of the sample (*17*).

The cyclic voltammograms of analyzed buckwheat extracts were recorded as shown in **Figure 1** (whole buckwheat before and after hydrothermal treatment) and **Figure 2** (roasted groats and hulls obtained from treated whole buckwheat). The observed anodic wave was broadened due to the response of several antioxidants with different oxidation potentials, including mainly flavonoids (*2*, *10*, *12*, *13*). In contrast, voltammograms obtained for the standard solutions of Trolox (0−1.25 mM) showed well resolved peaks and a shoulder in the potential region up to 1.1 V. A typical CV tracing of different Trolox concentrations is shown in **Figure 3**. The total charge under the anodic current (AC) waveform, provided using the CV computer software, was used to calculate the antioxidant capacity of the sample, based on the function AC versus concentration of Trolox (**Figure 4**), as was suggested by Chevion et al. (*17*) and Martinez et al. (*20*). The results obtained confirmed terms of quality the changes in antioxidant capacity of whole buckwheat due to the hydrothermal treatment provided by TEAC, DPPH RSA, and FCR reducing capacity. The antioxidant capacity of hydrothermally treated whole buckwheat was decreased by 26% when compared to that of the untreated whole grain. Moreover, the lowest capacity was noted for roasted buckwheat groat. In this case, the antioxidant capacity was decreased by 29%, whereas for the hull it increased by 20% after the process when compared to that for the hydrothermally treated whole grain (**Table 2**). Moreover, results from voltammetric experiments were correlated with those obtained by TEAC ($r = 0.77$), DPPH RSA ($r = 0.88$), and FCR reducing capacity ($r = 0.81$). Comparison of the CV with TEAC and DPPH RSA methods has shown that these two methods yielded considerably different chemical information. The TEAC assay gave almost three times higher and DPPH RSA gave more than two times higher antioxidant capacities when compared to those obtained by CV. In contrast, an excellent agreement was noted between data provided by FCR reducing capacity and antioxidant capacity obtained by cyclic voltammetric experiments.

Flavonoids Profile of Buckwheat before and after Hydrothermal Treatment. Typical HPLC chromatograms of buckwheat flavonoids recorded at 330 nm are shown in **Figure 5**. Column chromatography on a C18 support allowed us to separate five flavonoid compounds from untreated whole buckwheat grain (a), from the whole grain after hydrothermal treatment (b), and from the hydrothermal treated hulls (d). With respect to the roasted buckwheat groats, only three flavonoids were identified, as is shown in **Figure 5c**. The concentration of flavonoids in whole buckwheat after processing (160 °C, 30 min, water vapor pressure 6 atm) was decreased by 54% when compared to their concentration before treatment (**Table 3**). The obtained roasted 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) (*9*) and to the concentration of flavonoids found in treated whole grain. The rutin content was the highest, and it formed about 76, 71, 86, and 31% of all flavonoids content in untreated buckwheat, hydrothermal treated buckwheat, roasted groats, and treated hulls, respectively (**Table 3**). The concentration of flavonoids in untreated whole buckwheat and its products after hydrothermal treatment was highly correlated with the antioxidant capacity evaluated by TEAC, DPPH RSA, FCR reducing capacity, and voltammetric methods, and the respective correlation coefficients had values as follow: 0.95, 0.96, 0.96, and 0.90, respectively. Similarly, a positive correlation was also noted between antioxidant capacity and the concentration of each identified flavonoid compound. Our findings are in agreement with those reported by Dietrych-Szostak and Oleszek (*3*) and Im et al. (*35*), who found that the main buckwheat groats flavonoids, rutin and isovotexin, were affected by temperature and heating time adversely. The presence of other flavonoid compounds in buckwheat, such as catechins (*2*), quercetin (*3*), quercitrin (*12*), and kaempferol-3-rutinoside (*36*), was also reported; however, rutin is considered as the main one (*37*). Therefore, in this work, the TEAC of rutin was taken to calculate a pool of antioxidant capacity derived from total flavonoids (**Table 3**). In this case, the untreated whole buckwheat, the hydrothermal treated whole buckwheat, the roasted groats, and the hydrothermal treated hulls showed 9.12, 4.25, 1.45, and 4.21 μmol Trolox/g dm, and these values corresponded to those obtained by cyclic voltammetric experiments (**Table 2**). The calculation provided here was based on equal antioxidant activity of rutin and isooreintin, orientin, vitexin, and isovitexin, and further research on the antioxidant activity of these compounds is needed. Our findings are supported by recent reports on the characterization of natural antioxidants following their oxidation (*33*, *34*), thus providing a qualitative assessment of the antioxidant capacity of whole buckwheat and its products based on reducing strength.

Concluding Remarks. The antioxidant capacity of whole buckwheat and its products was affected by hydrothermal treatment. When spectrophotometric and cyclic voltammetry methods were used for the characterization of the roasted buckwheat groats and the hydrothermal treated hulls, an approximately twice higher antioxidant capacity of the hulls was

noted when compared to the groats. When comparing CV and spectrophotometric methods (TEAC, DPPH RSA, and FCR reducing capacity), FCR reducing capacity and CV experiments have both proven to be equally simple, low cost, and highly versatile methods appropriate for measuring the antioxidant capacity of whole buckwheat before and after hydrothermal treatment. Moreover, the concentration of flavonoids was related to the antioxidant capacity of buckwheat products measured by cyclic voltammetry.

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