We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Antioxidant Properties of Dark Wheat Bread with Exogenous Addition of Buckwheat Flour

Dorota Szawara-Nowak, Danuta Zielińska,
Henryk Zieliński and Małgorzata Wronkowska

Additional information is available at the end of the chapter

Abstract

In this study, the antioxidative/reducing activity of buckwheat-enhanced dark wheat breads (BEDWBs), based on the substitution of dark wheat flour (DWF) with buckwheat flour (BF) or flour from roasted buckwheat groats (BFR) at levels of 10, 20, 30 and 50% (w/w), was investigated. The antioxidative activity was measured against the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS$^{\bullet+}$), the 2,2-diphen-yl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) and the superoxide anion radical (O$_2^{-\bullet}$) by photochemiluminescence (PCL), reducing power by Fe(III) reduction and directly by cyclic voltammetry (CV) technique. The Fe(II) chelating capacity was also provided. The substitution of dark wheat bread with white and roasted buckwheat flour up to 50% (w/w) resulted in higher scavenging capacity against free radicals. The chelating and reducing power were above threefold higher as compared to a reference dark wheat bread. The improved antioxidant properties of buckwheat-enhanced dark wheat breads were due to the incorporation of buckwheat flour polyphenols. The high correlation noted between the total phenolic content and antioxidant capacity suggested that these assays may be used to characterize the cereal products enriched by buckwheat flours. Overall, buckwheat-enhanced dark wheat bread could be applied as food with more efficient antioxidant properties.

Keywords: buckwheat flours, dark wheat flour, breads, antioxidant/reducing capacity, chelating activity, reducing power

1. Introduction

Development of products which positively affect the consumer health is an important aspect followed by the food industry. This assignment may be realized when process of the industry is concentrated on the natural antioxidants. Antioxidants present in food can protect against lipid and protein autoxidation. It is very important to quantify the antioxidant properties of different types of food. In addition, a special attention is devoted to the processing methods to maintain the beneficial antioxidant properties of food [1].

Presently, wheat flour is widely used in bread making; however, other types of flour are also used. Rye and spelt types of flour are preferred due to the content of micro- and macronutrients and fibre [2, 3]. Recently, the potential usage of buckwheat flour as a functional component in food has been demonstrated [4]. Buckwheat (*Fagopyrum esculentum* Moench), commonly, cultivated in Russia and China, is added to the other cereal grains because of likenesses in usage [5]. Buckwheat is a rich source of nutrients (lysine, vitamins B, carbohydrates) [6] and antioxidants such as vitamin E, glutathione, phytic acid [7], phenolic acids and flavonoids—mainly rutin, with anti-inflammatory, anti-carcinogenic and anti-glycation properties [4, 8, 9]. Buckwheat polyphenols can function as antioxidants in one or more possible ways: as reducing agents, as compounds that scavenge free radicals, as chelating agents of metals that catalyse oxidation reactions and thus limiting their ability to initiate free radical chain reactions or by inhibiting oxidative enzymes such lipoxygenases [10–13]. However, processing conditions may considerably affect biological activity of polyphenols. There are many studies regarding negative effects of thermal processing on the phenolic compounds including flavonoids. The type of heat transfer and processing conditions are the major factors responsible for the observed decrease in the flavonoid content in food [7, 14–16]. Having all these evidences, buckwheat mill products seem to be an attractive ingredient in the bakery industry [17–20]. The recent evidences have shown that the intake of bread with addition of buckwheat flour in the recipe resulted in a positive increase of antioxidant potential in humans [17]. This finding, due to the quality properties of buckwheat bread as described by Lin et al. [21], can make it favourable for developing a healthy diet. Recently, a number of food products containing buckwheat has been investigated such as buckwheat-enhanced ginger nutty cakes [18], buckwheat enriched wheat bread [17, 20, 21] and buckwheat cakes [18, 19, 22]. Therefore, due to the nutritional value and beneficial effects on human health, buckwheat and partially buckwheat-based products form a pool of potential functional food [4, 10].

For the overall characterization of a new food product, more often the antioxidative capacity is used. There is a variety of analytical methods to assess the antioxidant capacity of food. However, there is no single standard method that would be used to determine the antioxidant capacity of the complex matrix and give consistent, unquestionable results in confrontation with other analytical methods. Therefore, it is advisable to use more than one method. A part of analytical methods is based on scavenging of non-natural free radicals, other deal with lipid peroxidation chemical markers. These methods need a little preparation, small amounts of reagents and are quick [13].

Many antioxidant capacity approaches have been suggested to assess antioxidant properties of food products and to clarify their relationships with antioxidants. Among them, ABTS, DPPH, reducing power assay and metal chelating activity are used for the assessment of antioxidant capacity of food [1, 23–25]. The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) is a spectrophotometric method based on inhibition of green colour in the presence of an antioxidant. DPPH (2,2-diphenyl-1-picrylhydrazyl) is based on the assumption that antioxidants are the hydrogen donors. This spectrophotometric method uses the DPPH radical, which changes from purple to yellow in the attendance of antioxidant compounds. However, ABTS is soluble in water and in alcoholic solutions, but DPPH is soluble only in organic solvents. The photochemiluminescence (PCL) method is based on the scavenging activity against the superoxide anion radical. The chelation of Fe(II) ions may cause significant antioxidative effects by delaying metal-catalysed oxidation [26, 27]. The reducing power assay involves the formation of coloured complexes, in the presence of antioxidants, with potassium ferricyanide, trichloroacetic acid and ferric chloride. The increase of absorbance of the reaction mixture is related to the reducing power of the samples. Currently, a mixture of the methods should be used to evaluate the antioxidant capacity of food *in vitro* to cover all aspects of antioxidant effectiveness [1, 12, 25]. Therefore, the aim of this study was to characterize the dark wheat bread with exogenous buckwheat addition as a source of antioxidative activity for humans.

2. Materials and methods

2.1. Chemicals and reagents

n-Hexane and methanol (HPLC-grade) were provided by Merck (Darmstadt, Germany). 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid (AA) and sodium dodecyl sulfate (SDS) were from Sigma Chemical Co. (St. Louis, MO, USA). PCL ACW (antioxidant capacity of water-soluble substances) and PCL ACL (antioxidant capacity of lipid-soluble substances) kits were from Analytik Jena AG (Jena, Germany). Monobasic potassium phosphate, dibasic potassium phosphate, ethylenediaminetetraacetic acid (EDTA), ferric chloride and ferrous chloride were purchased from POCh (Gliwice, Poland). Water was purified with a Mili-Q-system (Millipore, Bedford, USA).

2.2. Preparation of buckwheat-enhanced dark wheat breads

Dark wheat flour (DWF) and buckwheat (variety Kora) flour were purchased from a healthy food store in Olsztyn, Poland. The flour from roasted buckwheat groats (BFR) was procured from a local company in Poland. The dry matter in BF, BFR and DWF was 87.6, 89.7 and 87.0%, whereas protein content was 10.6, 14.3 and 8.1%, respectively. BF or BFR was used to replace DWF at levels of 10, 20, 30 and 50% (w/w). Buckwheat-enhanced dark wheat breads (BEDWBs) and reference dark wheat bread (DWB) were baked in a laboratory bakery. **Table 1** shows the

buckwheat-enhanced dark wheat breads formulation and baking conditions. Three pieces of each type of bread was baked. Samples were freeze-dried, milled and sieved through of 0.6 mm, and then were stored at –20°C before using for analysis.

Ingredient and conditions	Addition of buckwheat flours (%)				
	0	10	20	30	50
Dark wheat flour (g)	350	315	280	245	175
Buckwheat flour (g)	–	35	70	105	175
Roasted buckwheat flour (g)	–	35	70	105	175
Water (mL)	228	228	228	228	228
Salt (g)	3.5	3.5	3.5	3.5	3.5
Yeast (g)	10.5	10.5	10.5	10.5	10.5
Fermentation					
Temperature (°C)/time (min)	37/90	37/90	37/90	37/90	37/90
Pieces of dough (g)	250	250	250	250	250
Proofing (75% rh)					
Temperature (°C)/time (min)	37/25	37/25	37/25	37/25	37/25
Baking					
Temperature (°C)/time (min)	250/30	250/30	250/30	250/30	250/30

Table 1. Buckwheat and reference dark wheat breads formulation and baking conditions.

2.3. Preparation of bread crude extracts for measurement of antioxidant capacity by ABTS and DPPH assays, and reducing capacity by cyclic voltammetry (CV)

Bread samples (0.25 g) were extracted in triplicate at 25°C with 5 mL of 67% aqueous methanol using Thermomixer comfort (Eppendorf, Germany) by shaking at 1400 rpm for 60 min. Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R centrifuge, Eppendorf, Germany). After that, the 67% methanol extracts were directly used to determine the antioxidant capacity.

2.4. Preparation of hydrophilic and lipophilic bread extracts for measurement of antioxidant capacity by photochemiluminescence assay

Hydrophilic extracts: About 0.1 g of bread samples were extracted in triplicate for 3 min with 1 mL of deionized water using Genie-2 type vortex (Scientific Industries, USA). Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R, Eppendorf, Germany) and the fresh supernatants were used to determination of antioxidant activity formed by water-soluble antioxidants (ACW). Lipophilic extracts: About 0.1 g of bread samples were extracted in triplicate for 3 min with an n-hexane and methanol (1:4 v/v) using Genie-2 type vortex (Scientific Industries, USA). Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R, Eppendorf, Germany) and the fresh supernatants were used to determine the antioxidant capacity of lipid-soluble antioxidants (ACL).

2.5. Antioxidant capacity measured by ABTS, DPPH and PCL assays

2.5.1. ABTS assay

For the determination of the antioxidant activity was using the method described by Re et al. [28]. The ABTS$^{\bullet+}$ stock solution was diluted with 67% methanol to the absorbance of 0.70 ± 0.02 at 734 nm. Appropriate, solvent blank was used in each assay. The Trolox standard curve was determined in the range of 0.1–2.5 mM. The measurements were performed by a spectropho‐tometer UV-160 1PC with CPS-controller (Shimadzu, Japan). The antioxidant capacity was expressed in μmol Trolox/g of bread dry matter (DM).

2.5.2. DPPH assay

DPPH$^{\bullet}$ scavenging activity was determined as described previously in details [29]. The Trolox standard solutions were prepared in 67% methanol in the range of 0.1–2.5 mM. The measure‐ments were performed by a spectrophotometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). The antioxidant capacity was expressed in μmol Trolox/g of bread dry matter.

2.5.3. Antioxidant capacity measured by photochemiluminescence (PCL) assay

The PCL assay was carried out using the method according to Popov and Lewin [30]. This method consists in determining the superoxide anion radicals generated by luminol (under UV light) in the presence of antioxidants. The antioxidant capacity of buckwheat bread extract was determined using the analytical kits, which are designed to determine the antioxidant activity of the hydrophilic (ACW) and lipophilic (ACL) compounds, as reported previously by Zielińska et al. [29]. Measurements were performed with a Photochem® apparatus (Analytik Jena, Leipzig, Germany). PCL values are showed as a sum of ACW and ACL. The antioxidant capacity was expressed in μmol Trolox/g of bread dry matter.

2.6. Metal chelating activity of buckwheat-enhanced dark wheat breads

Bread samples (0.1 g) were extracted in triplicate with 1 mL of 0.2 M phosphate buffered saline (PBS, pH 7.4), which contains 1% (m/v) SDS for 30 s using VC 750 type sonicator (SONICS, USA) followed by vigorously shaking for 30 s using Genie-2 type vortex (Scientific Industries, USA). That stage was repeated three times. Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R, Eppendorf, Germany). The supernatants were directly used to determine Fe(II) chelating power of breads. Chelating power was measured using the method of Wang et al. [31]. To the reaction tube was added 0.25 mL of 1 mM FeSO$_4$, 0.25 mL of fresh bread extract, 1 mL of PBS (pH 7.4) with 1% (m/v) SDS, 1 mL of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), 0.4 mL of 10% NH$_2$OH · HCl and 2.1 mL of ethanol. The mixture was shaken and left at room temperature for 20 min. The absorbance at 522 nm was determined and used to evaluate Fe^{+2} chelating activity using ethylenediaminetetraacetate (EDTA) as a standard. The standard curve was constructed within the range of 0.125–2.0 mM of EDTA. The Fe(II) chelating capacity of samples was measured in triplicate using a temperature-controlled spectropho‐

tometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). Results were expressed as μmol EDTA equivalents/g DM.

2.7. Measurement of reducing power of buckwheat-enhanced dark wheat breads

Bread samples (0.25 g) were extracted in triplicate at 25°C with 5 mL of 67% aqueous methanol using Thermomixer comfort (Eppendorf, Germany) by shaking at 1400 rpm for 60 min. Next, samples were centrifuged for 5 min (16,100 × g, 4°C) (5415 R centrifuge, Eppendorf, Germany). After that, extracts were dried at 40°C using a rotary evaporator. Then samples were dissolved in 5 mL of phosphate buffer (0.2 M, pH 6.6) and were used immediately for the measurement of reducing power of bread extracts. The reducing power was determined by Oyaizu [32] with minor modification according to Liyana-Pathirana and Shahidi [33]. The assay mixture contained 1 mL of sample, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, incubated at 50°C for 20 min. Then, 2.5 mL of 10% TCA was added to the mixture and centrifuged for 5 min (2000 × g, 4°C). Exactly 2.5 mL of the extract of sample was mixed with 2.5 mL water and 0.5 mL of 0.1% $FeCL_3$ and was measured at 700 nm using a spectrophotometer UV-160 1PC with CPS-Controller (Shimadzu, Japan). A standard curve was prepared using ascorbic acid within the range of 0.015–0.5 mM and the reducing power was expressed as μmol ascorbic acid equivalents/g DM.

2.8. Measurement of reducing capacity of buckwheat-enhanced dark wheat breads by cyclic voltammetry

The cyclic voltammetry experiments were performed in 67% methanol bread extracts mixed with 0.1 M sodium acetate–acetic buffer (pH 4.5) at ratio 1:1 (v/v) according to Zielińska et al. [29]. The sodium acetate–acetic buffer acted as a supporting electrolyte for cyclic voltammetry measurements. A micro-electrochemical cell (with the total volume of 200 μL), made all of Teflon, was used during the course of this experiment. Three electrodes: a glassy carbon (GC) working electrode (BAS MF-2012, 3 mM diameter), an Ag/AgCl (3.5 M KCl) reference and a Pt (0.5 mM diameter coiled Pt wire) counter electrode constituted the cell. Working electrode was hand-polished with 0.05 μm alumina-water paste (BAS CF-1050), using BAS (MF-1040) polishing cloth and then rinsed with ultra-pure water and methanol. The cyclic voltammetry experiment was performed in the range of 100–1100 mV at a potential sweep-rate of 100 mV s⁻¹ at room temperature using a potentiostat/galvanostat G 750 (Gamry Ins., USA). The total charge below the anodic wave curve of the voltammogram was calculated. The cyclic voltammograms of Trolox solutions over the concentration range of 0.05–2.5 mM was determined. The reducing capacity of buckwheat-rich wheat breads was expressed in terms of μmol Trolox/g DM.

2.9. Statistical analysis

Results of the chemical analyses are illustrated as mean values and the standard deviation of three independent measurements. The obtained results were analysed with one-way ANOVA. Fisher Least Significant Difference (LSD) test at a significance level of $p < 0.05$ was performed

for post-hoc comparison. The Statistica ver. 5.0 software was used (General Convention and Statistica, StatSoft, USA, 1995).

3. Results

Free radical scavenging activity of food extracts should be determined by using different techniques to evaluate the antioxidant capacity of food *in vitro* to cover all aspects of antioxidant effectiveness. Recently, we provided evidences for the main differences in bioactive compounds content as well as in antioxidant properties of two types of buckwheat flours, e.g. BF and BFR when compared to DWF [34]. The estimated values of antioxidant capacity of flours based on the relative abilities of 67% methanol crude extracts to scavenge the ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radicals in comparison to Trolox showed the following order: BF > BFR > DWF. Moreover, chelating and reducing power of two types of buckwheat flour showed a comparable level, being higher than determined for DWF. A well-illustrated difference in the reducing capacity of buckwheat flours and dark wheat flour is presented on **Figure 1** when cyclic voltammetry technique was applied.
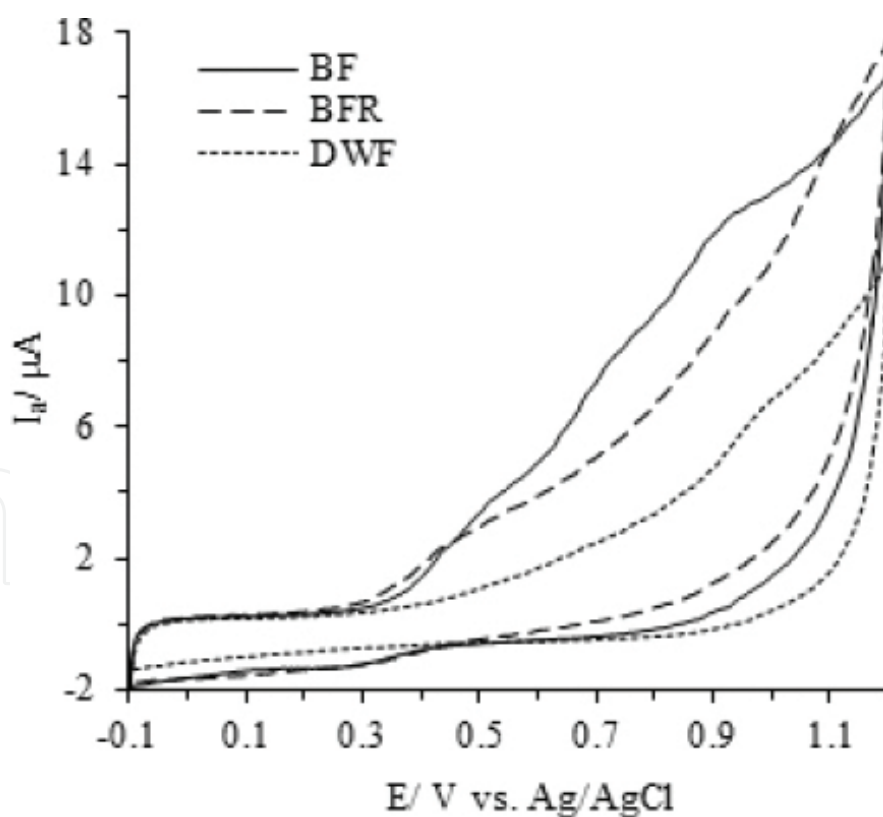

Figure 1. Cyclic voltammograms of buckwheat flour (BF), flour from roasted buckwheat groats (BFR) and dark wheat flour (DWF). Measurements were performed with 67% methanol extracts (100 mg/mL) mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v); scan rate 100 mV s^{-1}. The higher total charge under anodic current wave indicates a higher reducing capacity of the investigated flour extracts.
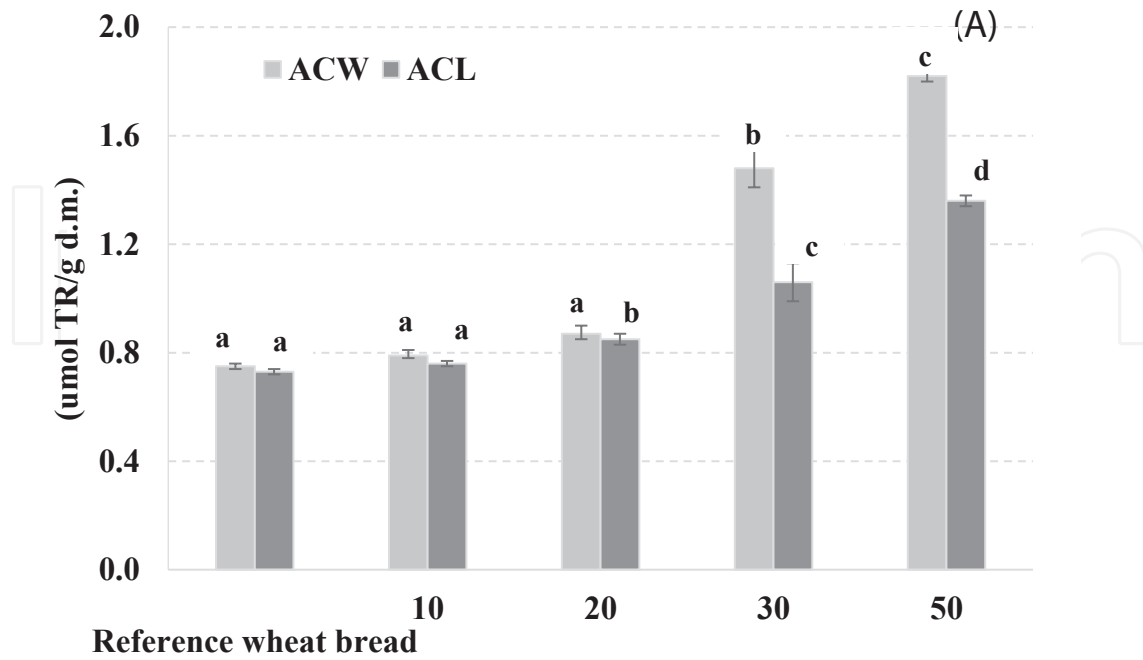
3.1. Antioxidant capacity of buckwheat-enhanced dark wheat breads as measured against free radicals

The antioxidant activity of buckwheat-enhanced dark wheat breads determined by ABTS, DPPH and PCL assays is shown in **Table 2**. The PCL values show the sum of antioxidant capacity of the hydrophilic (ACW) and lipophilic (ACL) fractions of bread (**Figure 2**). The rank of scavenging effect of reference DWB extract was 5.24 ± 0.24 µmol Trolox/g DM (DPPH assay) > 4.31 ± 0.07 µmol Trolox/g DM (ABTS assay) > 1.48 ± 0.01 µmol Trolox/g DM (PCL assay). The addition of BF or BFR in the range of 10, 20, 30 and 50% in the bread formula caused a significant ($p < 0.05$) increase in antioxidant capacity as compared to the reference DWB. The highest scavenging activity was found in BEDWBs with addition of 50% of BF (for ABTS assay 15.02 ± 0.90 µmol Trolox/g DM, for DPPH assay 8.36 ± 0.12 µmol Trolox/g DM and 3.18 ± 0.07 µmol Trolox/g DM for PCL assay). A similar rank of values was noted in BEDWBs after substitution of DWF by BFR at 50% level (13.99 ± 0.05 µmol Trolox/g DM, 9.20 ± 0.17 µmol Trolox/g DM and 4.43 ± 0.06 µmol Trolox/g DM, respectively). The increased substitution level of DWF by BF or BFR resulted in higher ACL values as compared to ACW (**Figure 2**).

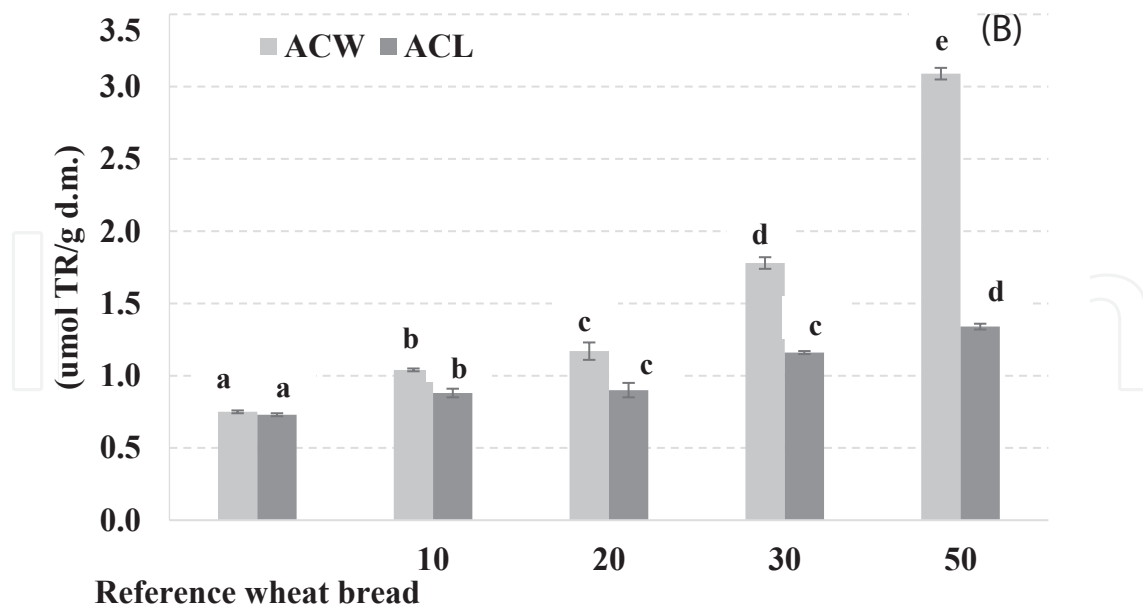| Type of bread	% of buckwheat flours	Antioxidant capacity (µmol Trolox/g d.m.)		
		ABTS	DPPH	PCL
Dark wheat bread (DWB)	0	4.31 ± 0.07a	5.24 ± 0.24a	1.48 ± 0.01a
Buckwheat-enhanced dark wheat breads	10	8.07 ± 0.15b	5.82 ± 0.07b	1.55 ± 0.01b
(BEDWBs) with BF	20	10.06 ± 0.24c	7.48 ± 0.15c	1.72 ± 0.03b
	30	10.82 ± 0.78c	8.24 ± 0.04d	2.54 ± 0.16d
	50	15.02 ± 0.90e	8.36 ± 0.12d	3.18 ± 0.07e
Buckwheat-enhanced dark wheat breads	10	7.13 ± 0.22b	5.99 ± 0.08b	1.92 ± 0.04b
(BEDWBs) with BFR	20	8.83 ± 0.05c	7.68 ± 0.12c	2.07 ± 0.11c
	30	10.03 ± 0.23c	9.00 ± 0.17d	2.94 ± 0.04d
	50	13.99 ± 0.05d	9.20 ± 0.17d	4.43 ± 0.06e

Values are means of three determinations ± standard deviation. Values within column followed by the same letter are not significantly different at 95% confidence level. PCL values show the sum of ACW and ACL values.

Table 2. Antioxidant capacity of bread samples determined against ABTS, DPPH and PCL assays.

Figure 2. Antioxidant capacity of buckwheat-enriched dark wheat breads formed by hydrophilic (ACW) and lipophilic (ACL) antioxidants. (A) DWF was substituted by BF. (B) DWF was substituted by BFR.

3.2. Reducing power and capacity of buckwheat-enhanced dark wheat breads

Table 3 illustrates the reducing power of BEDWBs as determined by the potassium ferricyanide method. The reducing power of BEDWBs was higher ($p < 0.05$) than noted for DWB. It was found that substitution of DWF by BF or BFR at levels of 10, 20, 30 and 50% w/w on the total flour basis caused an increase of the reducing power of BEDWBs. The highest level of DWF substitution (50%) by BF of BFR resulted in 2.5-fold increase of the reducing power of breads as compared to the reference DWB.

Type of bread	Substitution level (%)	Fe(II) chelating capacity	Reducing capacity by CV method	Reducing power by Fe (III) reduction
Dark wheat bread (DWB)	0	9.89 ± 0.01a	1.86 ± 0.11ab	1.76 ± 0.00a
Buckwheat-enhanced dark wheat breads (BEDWBs) with BF	10	10.81 ± 0.12b	1.79 ± 0.12a	2.56 ± 0.03b
	20	10.86 ± 0.09b	3.24 ± 0.21c	3.34 ± 0.09c
	30	13.14 ± 0.32c	3.35 ± 0.22c	3.59 ± 0.05c
	50	13.71 ± 0.05d	4.05 ± 0.27d	4.40 ± 0.26d
Buckwheat-enhanced dark wheat breads (BEDWBs) with BFR	10	12.32 ± 0.15bc	2.43 ± 0.16b	3.37 ± 0.29c
	20	13.48 ± 0.09c	2.40 ± 0.16b	3.93 ± 0.01c
	30	13.78 ± 0.34d	3.57 ± 0.24c	4.62 ± 0.01d
	50	13.97 ± 0.20d	4.92 ± 0.31d	5.34 ± 0.06e

Values are means of three determinations ± standard deviation. Values within column followed by the same letter are not significantly different at 95% confidence level.

Table 3. Reducing power (μmol ascorbic acid equivalents/g DM), reducing capacity (μmol Trolox/g DM) and Fe(II) chelating capacity (μmol EDTA equivalents/g DM) of buckwheat-enhanced dark wheat breads.

A special focus was put on the cyclic voltammetry (CV) experiments as a novel technique. The cyclic voltammograms of 67% MeOH extracts from breads were recorded as it is shown on **Figure 3**. The reducing capacity of BEDWBs was higher ($p < 0.05$) than noted for DWB. The reducing capacity of BEDWBs provided by CV assay was comparable to their reducing power determined by the potassium ferricyanide method (**Table 3**), and antioxidant capacity provided by photochemiluminescence (**Table 2**). In contrast, reducing capacity of BEDWBs was about threefold lower than antioxidant capacity determined against ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radicals.
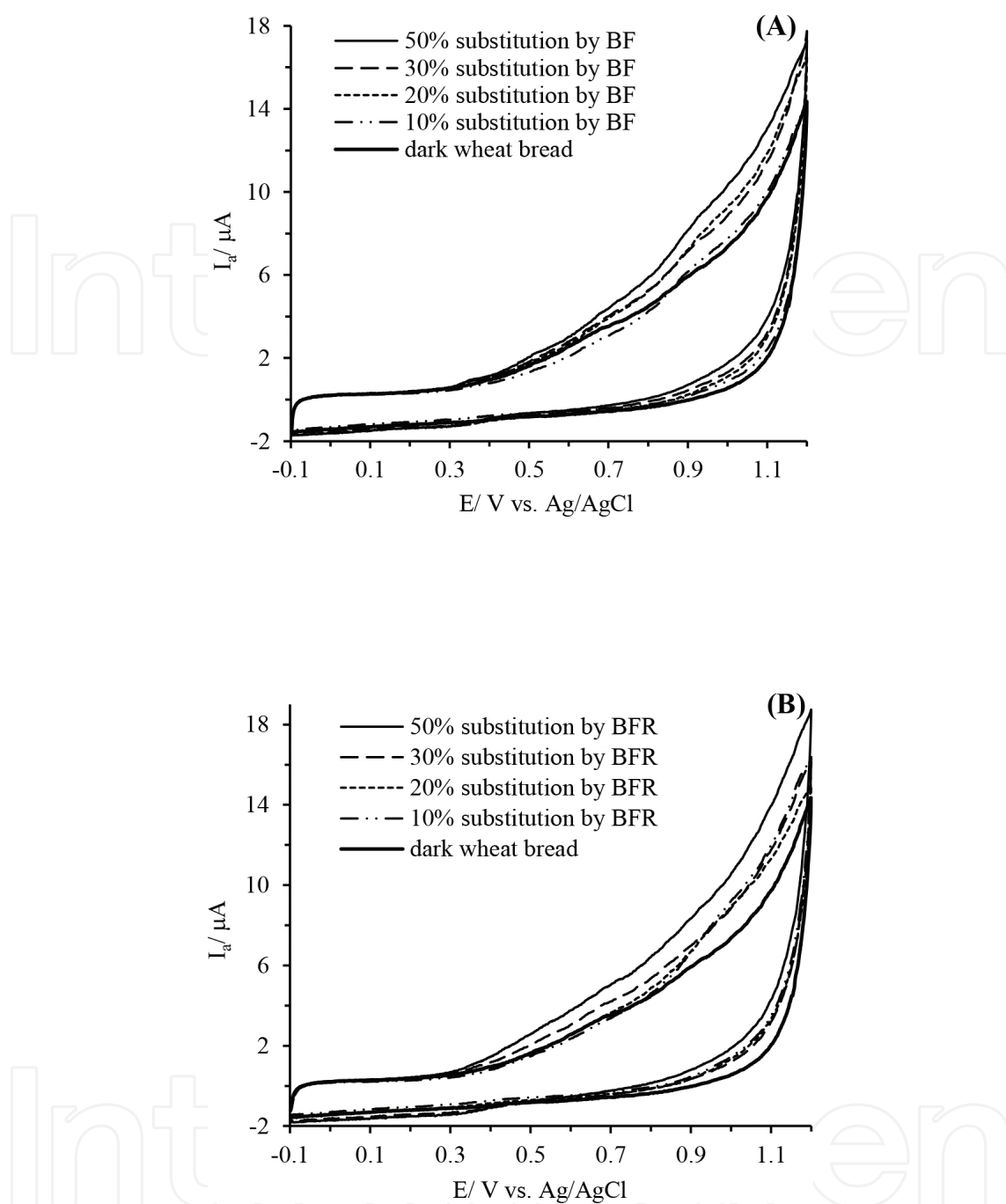
Figure 3. Cyclic voltammograms of buckwheat-enriched dark wheat breads. (A) DWF was substituted by BF. (B) DWF was substituted by BFR. Measurements were performed with 67% methanol extracts (100 mg/mL) mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v); scan rate 100 mV s^{-1}. The higher total charge under anodic current wave indicates a higher reducing capacity of the investigated bread extracts.

3.3. Fe(II) chelating capacity (ChC) of buckwheat-enhanced dark wheat breads

The results of Fe(II) chelating capacity of BEDWBs are summarized in **Table 3**. It was found that DWB as well as all types of BEDWBs contained compounds with Fe(II) chelating capacity. Both buckwheat types of flour were a good source of these compounds since substitution of

DWF by BF or BFR at levels of 10, 20, 30 and 50% w/w on the total flour basis resulted in increased chelating capacity of breads. The highest Fe(II) chelating capacity was noted for BEDWBs with 50% substitution of DWF by BF or by BFR. This level of DWF substitution resulted in 40% increase in the chelating capacity of bread as compared to the reference DWB (9.89 ± 0.01 µmol EDTA/g d.m.).

4. Discussion

The obtained results show that substitution of DWF by two types of buckwheat flour, especially by BF, enhanced the antioxidant properties of BEDWBs. This clear beneficial effect may be due to the enrichment of DWB in bioactive compounds, including rutin with well-recognized antioxidant properties. These results are consistent with the results obtained by Zielińska et al. [14] and Zieliński et al. [35]. Similarly, Lin et al. [21] showed that supplementation of wholegrain buckwheat flour in wheat bread resulted in increase of the antioxidant properties more than the application of light buckwheat flour. Whereas Yoo et al. [15] and Błaszczak et al. [16] found that rutin content in buckwheat groats is greatly reduced by thermal processing (by approximately 60%). This finding may explain the lower antioxidant capacity of buckwheat-enhanced dark wheat bread based on flour from roasted groats as compared to bread formulated with buckwheat flour as it was shown in this study. Many researchers also argue that phenolic compounds as well as compounds formed in Maillard reaction (e.g. HMF, furfural and acrylamide) play a significant role in scavenging of free radicals [36, 37]. However, the formation of Maillard reaction compounds can disguise actual reduction of phenolic contents and antioxidant capacity as well as loss of antioxidant activity in bread samples throughout the heat treatment [38].

Furthermore, the present study showed that BEDWBs were more effective scavengers of radical cation (ABTS$^{\bullet+}$) than DPPH$^{\bullet}$ radicals and superoxide anion radical (O$_2^{-\bullet}$). These differences were statistically significant ($p < 0.05$) (**Table 2**). An inverse relationship was found in the reference DWB. This trend in the rank of the radical scavenging activity was demonstrated by Floegel et al. [39] and Xu et al. [40]. Similarly, Sakač et al. [38] observed clear differences in antioxidant capacity between the light buckwheat enriched bread and wholegrain buckwheat enriched bread.

One of the significant mechanisms to defend against oxidative damage and lipid peroxidation is to chelate metal ions. In this study, we observed significant differences in metal chelating activity and reducing power between the reference DWB and BEDWBs (**Table 3**). Especially, supplementation of BFR has contributed to an increased metal chelating activity of BEDWBs. Whereas, Sakač et al. [38] found significant differences in antioxidant capacity measured by metal chelating activity and reducing power between the light and wholegrain buckwheat enriched breads. Enhancement of the antioxidant activity of bread after application of buckwheat flour from milled roasted groats can be related to the modification and/or degradation of phenolic compounds and formation of Maillard reaction products such as melanoidins, which may also act as antioxidants [36, 37]. It is possible that the enhancement of bread

with BF or BFR contributed to metal chelating activity due to the rutin content, since rutin is well-known as a potent metal chelator. Symonowicz and Kolanek [27] reported that the interactions of phenolic compounds with metal ions caused the formation of chelates. Metal chelation may be important to limit the formation of free radicals, thus reduce oxidative stress. Several studies have confirmed that flavonoids possess antioxidant properties due to their ability to chelate metal ions [26, 27]. Filipčev et al. [18] observed clear differences in metal chelating activity between the buckwheat and rye cookies. They also noticed that buckwheat enriched cookies (in amount 30, 40 and 50%) show a higher antioxidative properties than cookies enriched in rye. In this study a special focus was put on the cyclic voltammetry methodology, which allowed rapid screening of the electrochemical profile of buckwheat-enhanced dark wheat bread samples. This reducing capacity of BEDWBs was based on the electrochemical behaviour and chemical properties of the electroactive compounds being in bread [41]. In this study was found that substitution of DWF by BF or BFR at levels of 10, 20, 30 and 50% w/w on total flour basis caused almost a linear increase of the reducing capacity of BEWBs (**Table 3**). It should be mentioned that practical limitation of CV methodology was that the working electrode had to be frequently cleaned to remove residues of sample from its surface and to maintain its sensitivity. However, the advantage of CV was related not to do requiring the use of reactive chemicals.

5. Conclusions

This paper shows the beneficial role of the addition of buckwheat in bakery products. It highlights aspects of buckwheat as a food ingredient and possible use as flour in bakery products. The obtained results indicate that the improved antioxidant properties of buckwheat-enhanced dark wheat bread might be enhanced due to the incorporation of phenolic compounds, mainly rutin and quercetin, which had been shown to possess antioxidant activity. Overall, buckwheat breads could be developed as a source of antioxidant activity for humans.

6. Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

Author details

Dorota Szawara-Nowak[1], Danuta Zielińska[2], Henryk Zieliński[1] and
Małgorzata Wronkowska[1*]

*Address all correspondence to: m.wronkowska@pan.olsztyn.pl

1 Division of Food Sciences, Institute of Animal Reproduction and Food Research of the Polish
Academy of Sciences, Olsztyn, Poland

2 Department of Chemistry, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

References

[1] Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprocess Technol. 2011;89:217–233. DOI: 10.1016/j.fbp. 2010.04.008

[2] Mohamed DA, Al-Okbi AY, El-Hariri DM, Mousa I. Potential health benefits of bread supplemented with defatted flaxseeds under dietary regimen in normal and type 2 diabetic subjects. Pol. J. Food Nutr. Sci. 2012;62:103–108. DOI: 10.2578/ v10222-011-0049-x

[3] Kasprzak M, Rzedzicki Z. Application of grasspea wholemeal in the technology of white bread production. Pol. J. Food Nutr. Sci. 2012;62:207–213. DOI: 10.2478/ v10222-012-0056-6

[4] Jiang P, Burczynski F, Campbell C, Pierce G, Austria JA, Briggs CJ. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum, F. tataricum*, and *F. homo-tropicum* and their protective effects against lipid peroxidation. Food Res. Int. 2007;40:356–364. DOI: 10.1016/j.foodres.2006.10.009

[5] Campbell C. Buckwheat crop improvement. Fagopyrum 2003;20:1–6.

[6] Zielińska D, Zieliński H. Low molecular weight antioxidants and other biologically active components of buckwheat seeds. In: Dobránszki J, editor. Buckwheat 1. The European Journal of Plant Science in Biotechnology. 2009. Vol. 3 (Special Issue 1), pp. 29–38.

[7] Zieliński H, Michalska A, Piskuła MK, Kozłowska H. Antioxidants in thermally treated buckwheat groats. Mol. Nutr. Food Res. 2006;50:824–832. DOI: 10.1002/mnfr.200500258

[8] Pashikanti S, de Alba DR, Boissonneault GA, Cervantes-Laurean D. Rutin metabolites: Novel inhibitors of nonoxidative advanced glycation end products. Free Radic. Biol. Med. 2010;48:656–663. DOI: 10.1016/j.freeradbiomed.2009.11.019

[9] Zielińska D, Szawara-Nowak D, Zieliński H. Antioxidative and anti-glycation activity of buckwheat hull tea infusion. Int. J. Food Proper. 2013;16:228–239. DOI: 10.1080/10942912.2010.551308

[10] Wijngaard HH, Arendt EK. Buckwheat. Cereal Chem. 2006;83:391–401. DOI: 10.1094/CC-83-0391

[11] Serra A, Macia A, Romero M-P, Reguant J, Ortega N, Motilva M-J. Metabolic pathway of the colon metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. Food Chem. 2012;130:383–393. DOI: 10.1016/j.foodchem.2011.07.055

[12] Alam M, Bristi N, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm. J. 2013;21:143–152. DOI: 10.1016/j.sps.2012.05.002

[13] Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol. 2013;51:15–25. DOI: 10.1016/j.fct.2012.09.021

[14] Zielińska D, Szawara-Nowak D, Michalska A. Antioxidant capacity of thermally-treated buckwheat. Pol. J. Food Nutr. Sci. 2007;57:465–470.

[15] Yoo J, Kim Y, Yoo S-H, Inglett GE, Lee S. Reduction of rutin loss in buckwheat noodles and their physicochemical characterisation. Food Chem. 2012;132:2107–2111. DOI: 10.1016/j.foodchem.2011.12.065

[16] Błaszczak W, Zielińska D, Zieliński H, Szawara-Nowak D, Fornal J. Antioxidant properties and rutin content of high pressure-treated raw and roasted buckwheat groats. Food Bioproc. Technol. 2013;6:92–100. DOI: 10.1007/s11947-011-0669-5

[17] Bojňanská T, Frančaková H, Chlebo P, Vollmannová A. Rutin content in buckwheat enriched bread and influence of its consumption on plasma total antioxidant status. Czech J. Food Sci. 2009;27:236–240.

[18] Filipcev B, Simurina O, Sakac M, Sedej I, Jovanov P, Pestoric M, Bodroza-Solarov M. Feasibility of use of buckwheat flours as an ingredient in ginger nut biscuit formulation. Food Chem. 2011;125:164–170. DOI: 10.1016/j.foodchem.2010.08.055

[19] Torbica A, Hadnađev M, Dapcevic-Hadnađev T. Rice and buckwheat flour characterisation and its relation to cookie quality. Food Res. Int. 2012;48:277–283. DOI: 10.1016/j.foodres.2012.05.001

[20] Szawara-Nowak D, Koutsidis G, Wiczkowski W, Zieliński H. Evaluation of the in vitro inhibitory effects of buckwheat enhanced wheat bread extracts on the formation of advanced glycation end-products (AGEs). LWT-Food Sci. Technol. 2014;58:327–334. DOI: 10.1016/j.lwt.2013.03.005

[21] Lin L-Y, Liu H-M, Lin S-D, Mau J-L. Quality and antioxidant property of buckwheat enhanced wheat bread. Food Chem. 2009;112:987–991. DOI: 10.1016/j.foodchem.2008.07.022

[22] Sedej I, Sakač M, Mandic A, Misan A, Pestoric M, Simurina O, Canadanovic-Brunet J. Quality assessment of gluten-free crackers based on buckwheat flour. LWT-Food Sci. Technol. 2011;44:694–699. DOI: 10.1016/j.lwt.2010.11.010

[23] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 2005;53:4290–4302. DOI: 10.1021/jf0502698

[24] Magalhaes LM, Segundo MA, Reis S, Lima JLFC. Methodological aspects about *in vitro* evaluation of antioxidant properties. Anal. Chim. Acta. 2008;613:1–19. DOI: 10.1016/j.aca.2008.02.047

[25] López-Alarcóna C, Denicolab A. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Anal. Chim. Acta. 2013;763:1–10. DOI: 10.1016/j.aca.2012.11.051

[26] Vlachodimitropoulou E, Sharp PA, Naftalin RJ. Quercetin-iron chelates are transported via glucose transporters. Free Radic. Biol. Med. 2011;50:934–944. DOI: 10.1016/jfreeradbiomed.2011.01.005

[27] Symonowicz M, Kolanek M. Flavonoids and their properties to from chelate complexes. Biotechnol. Food Sci. 2012;76:35–41.

[28] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CA. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999;26:1291–1237. DOI: 10.1016/S0891-5849(98)00342-6

[29] Zielińska D, Szawara-Nowak D, Ornatowska A, Wiczkowski W. Use of cyclic voltammetry, photochemiluminescence and spectrophotometric methods for the measurement of the antioxidant capacity of buckwheat sprouts. J. Agric. Food Chem. 2007;55:9891–9898. DOI: 10.1021/jf07175z

[30] Popov I, Lewin G. Antioxidative homeostasis: Characterisation by means of chemiluminescent technique in methods in enzymology. In: Packer L, editor. Oxidants and Antioxidants, part B. Academic Press; Cambridge; Massachusetts; US. 1999, pp. 96–100.

[31] Wang J, Sun B, Cao Y, Tian Y. Protein glycation inhibitory activity of wheat bran feruloyl oligosaccharides. Food Chem. 2009;112:350–353. DOI: 10.1016/j.foodchem.2008.05.072

[32] Oyaizu M. Studies on products of browning reaction – Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 1986;44:307–315.

[33] Liyana-Pathirana CM, Shahidi F. Antioxidant and free radical scavenging activities of whole wheat and milling fractions. Food Chem. 2007;101:1151–1157. DOI: 10.1016/j.foodchem.2006.03.016

[34] Szawara-Nowak D, Zielinski H, Piskuła MK. Content of antioxidants and antioxidative properties of novel buckwheat flours. 5th Central European Congress on Food. 19th–

22nd May 2010, Bratislava, Slovak Republic, Book of Full Papers, pp. 235–241. ISBN: 778-80-89088-89

[35] Zieliński H, Michalska A, Amigo-Benavent M, del Castillo MD, Piskuła MK. Changes in protein quality and antioxidant properties of buckwheat seeds and groats induced by roasting. J. Agric. Food Chem. 2009;57:4771–4776. DOI: 10.1021/jf900313e

[36] Del Castillo MD, Gordon MH, Ames JM. Peroxyl radical-scavenging activity of coffee brews. Eur. Food Res. Technol. 2005;221:471–477. DOI: 10.1007/s00217-005-1209-1

[37] Horszwald A, Morales FJ, Del Castillo MD, Zieliński H. Evaluation of antioxidant capacity and formation of processing contaminants during rye bread making. J. Food Nutr. Res. 2010;49:149–159.

[38] Sakač M, Torbica A, Sedej I, Hadnađev M. Influence of bread-making on antioxidant capacity of gluten free breads based on rice and buckwheat flours. Food Res. Int. 2011;44:2806–2813. DOI: 10.1016/j.foodres.2011.06.026

[39] Floegel A, Kim D-O, Chung S-J, Sung I-K, Chun O-K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Compos. Anal. 2011;24:1043–1048. DOI: 10.1016/j.jfca.2011.01.008

[40] Xu F-Y, Gao Q-H, Ma Y-J, Guo X-D, Wang M. Comparison of tartary buckwheat flour and sprouts steamed bread in quality and antioxidant property. J. Food Qual. 2014;37:318–328. DOI: 10.1111/jfq.12101

[41] Zielińska D, Szawara-Nowak D, Zielinski H. Determination of the antioxidant activity of rutin and its contribution to the antioxidant capacity of diversified buckwheat origin material by updated analytical strategies. Pol. J. Food Nutr. Sci. 2010;60:315–321.