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The Bioaccessible Reducing Capacity of Buckwheat-Enhanced Wheat Breads Estimated by Electrochemical Method

Danuta Zielińska

Abstract

The application of cyclic voltammetry (CV) technique for the determination of bioaccessible reducing capacity of buckwheat-enhanced white wheat breads (BEWWBs) and buckwheat-enhanced dark wheat breads (BEDWBs) was addressed. Buckwheat flour (BF) or flour from roasted buckwheat groats (BFR) were used to substitute white (WWF) or dark wheat flour (DWF) at 10, 20, 30, and 50% w/w on total flour basis in bread formula. The study showed that substitution of 10, 20, 30, and 50% of WWF or DWF by BF or BFR in bread formula resulted in almost linear increase of the reducing capacity of BEWWBs and BEDWBs. After digestion of BEWWBs, the bioaccessible reducing capacity was up to fivefold higher than the reducing capacity of the corresponding undigested breads, and in all cases was also higher than that noted for a soluble fraction of the digestible portion of white wheat bread (WWB). In contrast, the bioaccessible reducing capacity of BEDWBs was only up to twofold higher but in all cases did not exceed the value noted for digested dark wheat bread (DWB). Our results indicate that CV methodology is suitable for obtaining rapid electrochemical profile of a bread sample after digestion useful for evaluation of their selected functional properties.

Keywords: wheat bread, buckwheat, digestion, cyclic voltammetry, bioaccessible reducing capacity

1. Introduction

In recent years, common buckwheat is gaining interest in the development of new food products due to health-promoting, biofunctional properties, gluten freeness, and its high nutritional value [1]. The high nutritional value of buckwheat attributed to a balanced amino acid composition and high contents of vitamin B_1 and B_2, lysine, flavonoids, phytosterols, soluble carbohydrates, D-*chiro*-inositol, fagopyritols, and thiamin-binding proteins has been described [2]. Buckwheat is also rich in antioxidant compounds such as flavonoids, phenolic acids, tocopherols, reduced glutathione, inositol phosphates, and melatonin [3, 4]. Therefore, based on the above evidences, ingredients derived from buckwheat could be attractive for the bakery industry [5].

Wheat flour is usually used in bread making, but more often it is demonstrated that the usage of buckwheat flour as an ingredient in bakery goods can provide

beneficial health effects [6–11]. The buckwheat-enhanced wheat bread is an attractive model of polyphenol-rich bread for an in vitro investigation of the impact of digestion on the bioaccessible reducing/antioxidative capacity as well as on the potential bioaccessibility of wide spectrum of bioactive compounds. The selection on buckwheat flour for formula of model polyphenol-enriched breads was due to the several publications indicating the potential use of buckwheat flour as a functional ingredient in bakery product formulations [12].

Several methods to measure antioxidant properties have been proposed and were recently reviewed [13–17]. Among others, scavenging of stable radicals such as DPPH and ABTS, oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), ferric-reducing antioxidant power (FRAP), and cupric ion (Cu^{2+})-reducing power (CUPRAC) were employed in foods [13]. Electrochemical methods, used for the determination of reducing activity, have been still developing. Among different electrochemical techniques, the most widely used for this purpose is cyclic voltammetry (CV). The main advantage of CV is its capability to rapidly observe the total redox behavior over a wide potential range without the necessity of measuring the specific reducing capacity of each component alone. In contrast to the abovementioned methods, electrochemical assays are low-cost and usually do not require time-consuming sample preparation. CV is based on the analysis of the anodic current (AC) waveform, which is a function of the reactive potential of a given compound in the sample or a mixture of compounds. The CV tracing indicates the ability of a compound to donate electrons at the potential of the anodic wave [18]. A CV also provides information describing the integrated reducing capacity without the specific determination of the contribution of each individual component. Therefore, in the past couple of years, CV has been suggested as an instrumental methodology for the evaluation of the reducing capacity of various food products [16, 19–21]. From the current point of view, the electrochemical methods should be used to assess the reducing capacity of food in vitro to cover all aspects of antioxidant efficacy in vivo [22, 23]. Recently it was demonstrated that that in vitro digestion of buckwheat-enhanced wheat breads was the crucial step in the formation of the antioxidant capacity due to the release of the high amount of phenolic compounds [24].

Since the use of electrochemical methods ensures the measurement of the bioaccessible reducing capacity of food as it could occur in vivo, the objective of this work was to show an application of a cyclic voltammetry (CV) technique for determination of the bioaccessible reducing capacity of a soluble fraction from a digestible portion of buckwheat-enhanced wheat breads.

2. Materials and methods

2.1 Chemicals and reagents

α-Amylase (A1031-5KU), pepsin (P7000), pancreatin (P7545), bile salts extract (B8631), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were from POCH, (Gliwice, Poland). Water was purified with a Milli-Q-system (Millipore, Bedford, USA).

2.2 Buckwheat-enhanced wheat bread preparation

White wheat flour (WWF), dark wheat flour (DWF), and buckwheat flour (BF) from common buckwheat var. Kora were purchased from a healthy food store

in Olsztyn, Poland. The flour from roasted buckwheat groats (BFR) originated from a local company in Poland. BF or BFR were used to replace WWF or DWF or at level of 10, 20, 30, and 50% (w/w). Buckwheat-enhanced white wheat breads (BEWWBs), buckwheat-enhanced dark wheat breads (BEDWBs), and reference white (WWB) and dark wheat bread (DWB) were baked in a laboratory bakery. **Table 1** shows the buckwheat-enhanced wheat bread formulation and baking conditions. Three pieces of each type of bread were baked. Samples were freeze-dried, milled and sieved through a mesh of 0.6 mm, and then were stored at −20°C before using for analysis.

2.3 Preparation of buckwheat-enhanced wheat bread crude extracts for measurement of reducing capacity by cyclic voltammetry (CV)

The lyophilized and milled 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 minutes [24]. Next, samples were centrifuged for 5 minutes (16,100 × *g*, 4°C) (5415 R centrifuge, Eppendorf, Germany). After that, the 67% methanol extracts were directly used to determine the reducing capacity.

Ingredient and conditions	Substitution level (%)				
	0	10	20	30	50
BEWWBs					
WWF (g)	350	315	280	245	175
BF (g)	–	35	70	105	175
BFR (g)	–	35	70	105	175
BEDWBs					
DWF (g)	350	315	280	245	175
BF (g)	–	35	70	105	175
BFR (g)	–	35	70	105	175
Water (mL)	228	228	228	228	228
	250	250	250	250	250
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)	37	37	37	37	37
Time (min)	90	90	90	90	90
Pieces of dough (g)	250	250	250	250	250
Proofing (75% rh)					
Temperature (°C)	37	37	37	37	37
Time (min)	25	25	25	25	25
Baking					
Temperature (°C)	250	250	250	250	250
Time (min)	30	30	30	30	30

BEWWBs, buckwheat-enhanced white wheat breads; BEDWBs, buckwheat-enhanced dark wheat breads; WWF, white wheat flour; DWF, dark wheat flour; BF, buckwheat flour; BFR, buckwheat flour from roasted groats.

Table 1.
Buckwheat-enhanced wheat bread formulation and baking conditions.

2.4 In vitro digestion of buckwheat-enhanced wheat breads

The buckwheat-enhanced wheat breads were in vitro digested as described in details [25] with some modifications. The protocol included three steps: saliva (pH 7.0), gastric (pH 2.0), and intestinal digestion (pH 7.5). Briefly, 10 g of lyophilized and milled buckwheat-enhanced wheat breads and reference wheat breads were suspended in 80 mL of deionized water. An α-amylase solution (77 U/mg solid) was added to the samples at a proportion of 3.25 mg/10 g of sample dry matter (d.m.) in 1 mM $CaCl_2$, pH 7.0. Then, samples were shaken in a water bath at 37°C for 30 minutes. For the gastric digestion, the pH was reduced to 2.0 with 6 N HCl, and pepsin solution (738 U/mg) was added in the amount of 0.5 g/10 g of sample d.m. in 0.1 N HCl. The incubation was continued under the same conditions for 120 minutes. In the next step, the pH was adjusted to 6.0 with 6 M NaOH, and a mixture of pancreatin (activity 8×USP) and bile salts extract was added. Subsequently, the pH was increased to 7.5 with 6 M NaOH, and water buffered to a pH of 7.5 was introduced to obtain a final volume of 150 mL. Then, the samples were incubated at 37°C for 120 minutes. After incubation, the digestive enzymes were inactivated by heating at 100°C for 4 minutes and cooled for centrifugation at 5000 rpm for 60 minutes at 4°C in an MPV-350R centrifuge (MPW Med. Instruments, Warsaw, Poland). The supernatants obtained were freshly used for the evaluation of the bioaccessible reducing capacity of buckwheat-enhanced wheat breads.

2.5 Measurement of reducing capacity of buckwheat-enhanced wheat breads by cyclic voltammetry (CV)

The cyclic voltammogram (CV tracing) provides information describing the integrated reducing capacity without the specific determination of the contribution of each electroactive component. It is based on the analysis of the anodic current (AC) waveform, which is a function of the reductive potential of a given compounds in the extract. The total reducing 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 (Ia), reflecting the concentration of the components. However, more often the area under the AC wave (S; related to the total charge) is calculated since it is a better parameter reflecting the reducing capacity of the sample [18].

In this study, the cyclic voltammetry experiments were performed in 67% methanol bread crude extracts and soluble fraction obtained after digestion mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v) according to Zielińska et al. [26]. The sodium acetate-acetic buffer acted also as a supporting electrolyte for cyclic voltammetry measurements. A micro-electrochemical cell (with 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 ultrapure 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 area under the anodic current (AC) waveform of the voltammogram (S) related to the total charge was calculated as it was shown for dark wheat bread extract (**Figure 1**). The cyclic voltammograms of Trolox solutions over the concentration range of 0.05–2.5 mM were determined. The reducing capacity of buckwheat-rich wheat breads was expressed in terms of μmol Trolox equivalent (TE)/g of dry matter (DM).
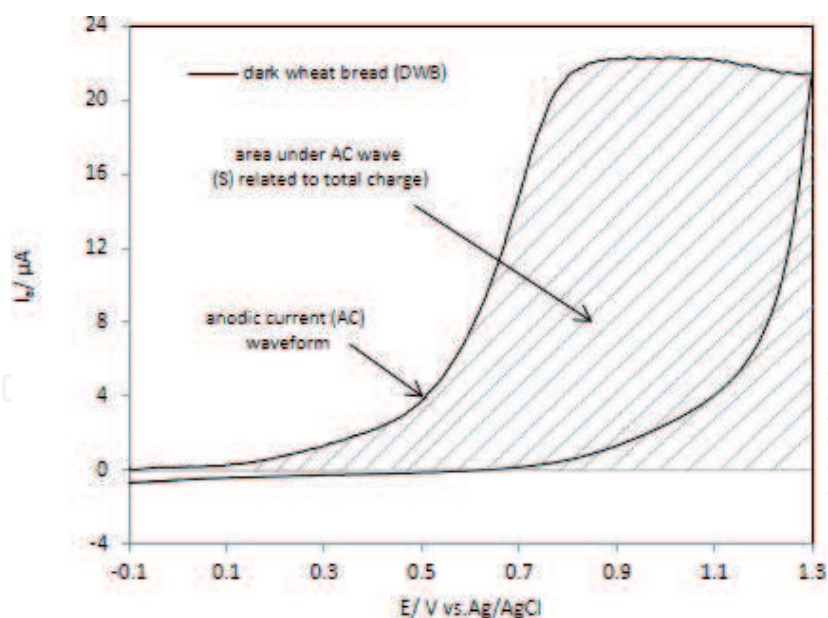
Figure 1.
Cyclic voltammogram of undigested dark wheat bread extract (DWB). The area under the anodic current (AC) waveform related to the total charge is indicated, and the mode of its calculation is presented. Measurements were performed with 67% methanol extract of DWB (100 mg/mL) mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v) and scan rate 100 mV s⁻¹.

2.6 Statistical analysis

Results of the chemical analyses are illustrated as mean values and the standard deviation of three independent measurements. 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 and discussion

3.1 Reducing capacity of buckwheat-enhanced wheat breads

This reducing capacity of BEDWBs and BEWWBs was based on the electrochemical behavior and chemical properties of the electroactive compounds present in bread [27].

The cyclic voltammograms of 67% MeOH extracts from BEWWBs and BEDWBs breads before digestion were recorded as shown in **Figure 2**. The shape of the anodic waves was typically due to the response of several antioxidants with different oxidation potentials [11, 28]. The study showed that the substitution of WWF or DWF at levels of 10, 20, 30, and 50% w/w on total flour basis caused almost linear increase of the reducing capacity of undigested BEWWBs and BEDWBs breads (**Figure 3**). For example, the highest level of WWF substitution (50%) by BF resulted in almost fivefold increase in the reducing capacity, while the effect of substitution with BFR at 50% level was even higher, being above eightfold as compared to the reference WWB. Similarly, the reducing capacity of BEDWBs was higher 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 total flour basis caused a lower increase of the reducing capacity of BEDWBs as compared to BEWWBs. The highest level of DWF substitution (50%) by BF of BFR resulted in 2.2 and 2.6-fold increase of the reducing capacity of BEDWBs as compared to the reference DWB. These findings are in agreement with those provided by Lin et al. [6] which showed a fivefold
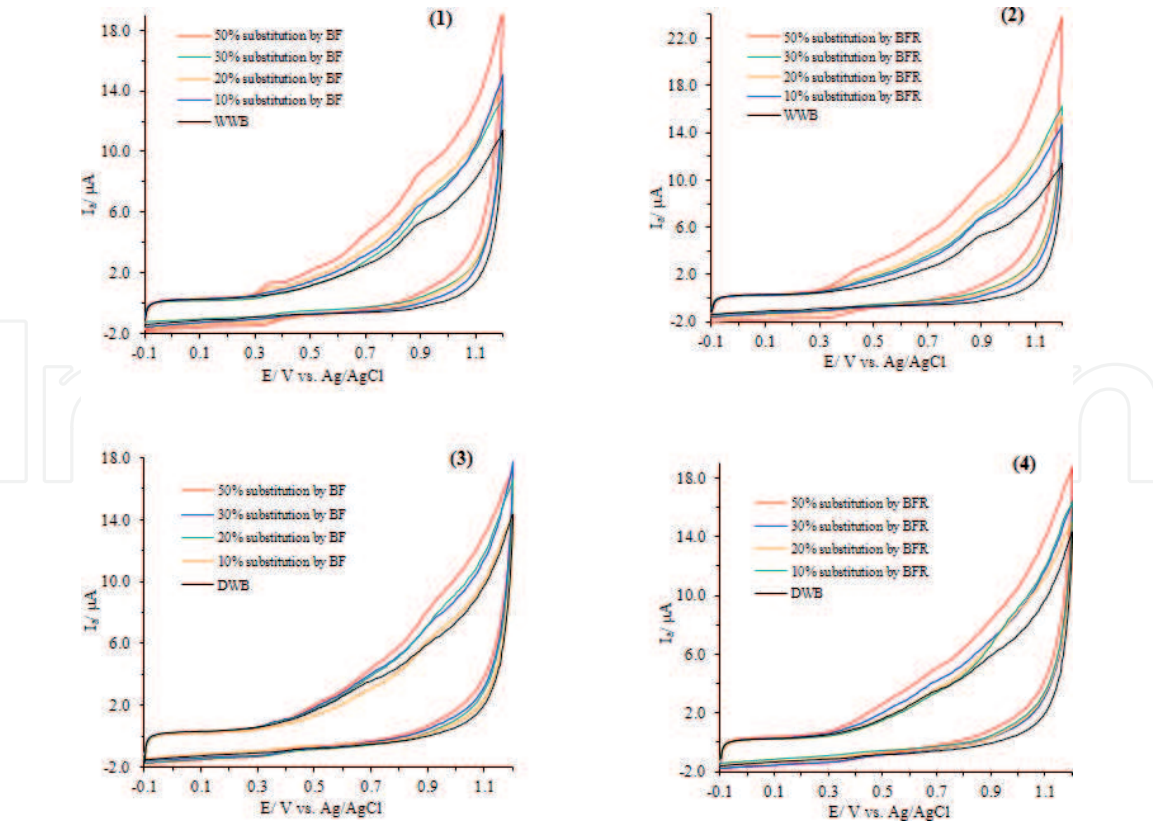
Figure 2.
Cyclic voltammograms of undigested buckwheat-enhanced white wheat breads (BEWWBs) and undigested buckwheat-enhanced dark wheat breads (BEDWBs). Upper figure, BEWWBs with (1) BF and (2) BFR substitution; lower figure, BEDWBs with (3) BF and (4) BFR substitution. Measurements were performed with 67% methanol extracts (100 mg/mL) mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v) and scan rate 100 mV s^{-1}. The higher total charge under anodic current wave indicates a higher reducing capacity of the investigated bread extracts.
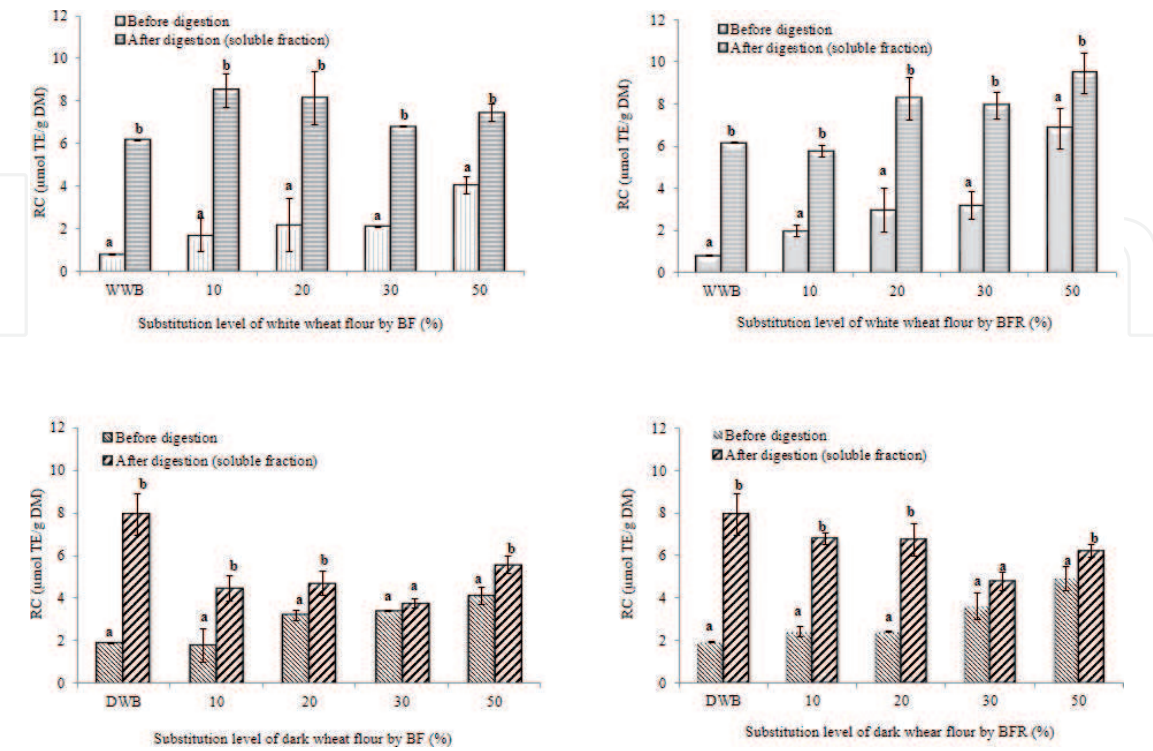

Figure 3.
Reducing capacity of buckwheat-enhanced white wheat breads (BEWWBs) and buckwheat-enhanced dark wheat breads (BEDWBs) before and after digestion in vitro. Upper figures, BEWWBs with BF and BFR substitution; lower figures, BEDWBs with BF and BFR substitution.
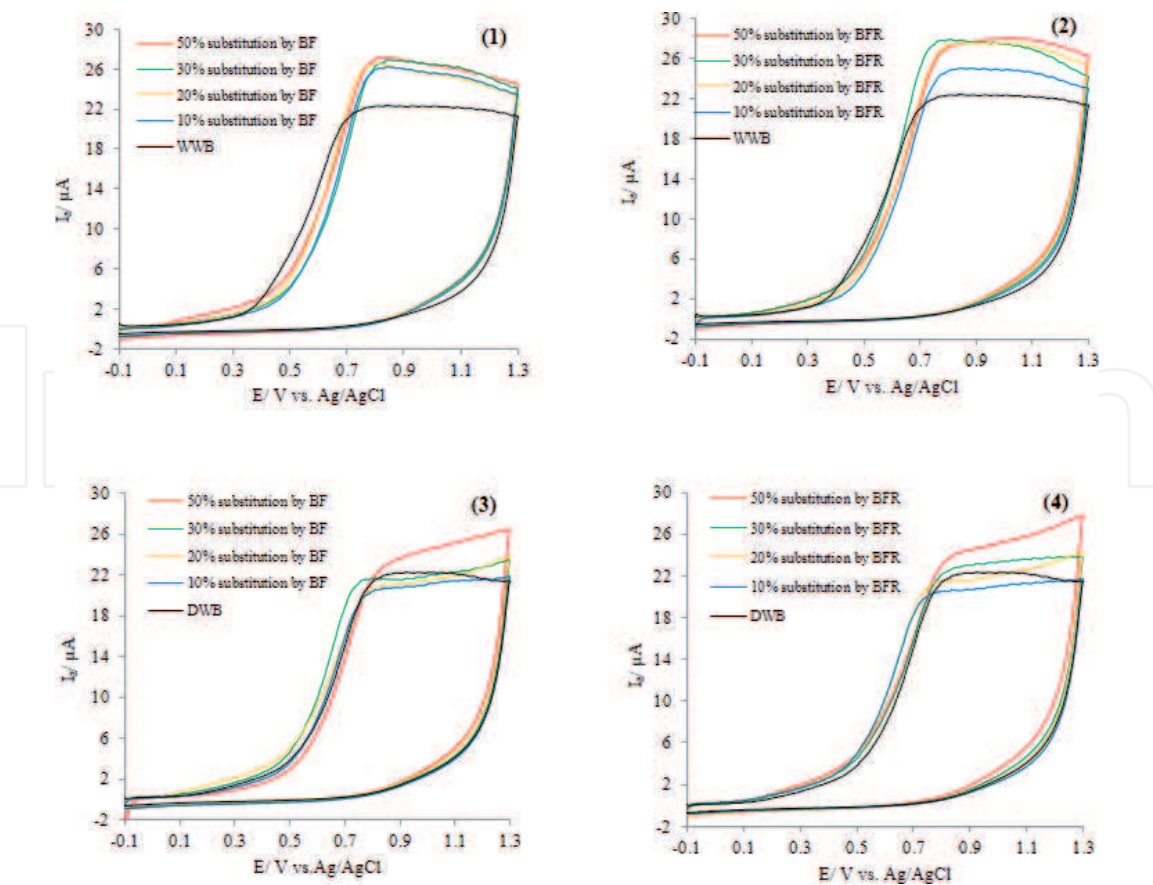
Figure 4.
Cyclic voltammograms of soluble fraction obtained after digestion of buckwheat-enhanced white wheat breads (BEWWBs) and buckwheat-enhanced dark wheat breads (BEDWBs). Upper figure, BEWWBs with (1) BF and (2) BFR substitution; lower figure, BEDWBs with (3) BF and (4) BFR substitution. Measurements were performed with soluble fraction obtained after digestion mixed with 0.1 M sodium acetate-acetic buffer (pH 4.5) at ratio 1:1 (v/v) and scan rate 100 mV s^{-1}.

increase in reducing power of buckwheat-enriched wheat bread at 15% substitution level using flour from unhusked buckwheat.

The cyclic voltammograms of soluble fraction obtained after digestion of BEWWBs and BEDWBs breads were recorded as shown in **Figure 4**. Comparison of the cyclic voltammograms recorded for undigested bread (**Figure 2**) with those recorded for soluble fraction obtained after digestion (**Figure 4**) showed broadened anodic waves due to the response of several reducing compounds with different oxidation potentials, including mainly released from the bread matrix phenolics compounds as described by Szawara-Nowak et al. [24]. After digestion of BEWWBs, the reducing capacity was higher by 21% (at substitution level of 50% by BF) and by 53% (at substitution level of 50% by BFR) than the reducing capacity of the corresponding digested reference WWB (**Figure 3**). In contrast, after digestion of BEDWBs, the reducing capacity was lower by 20% (substitution level of 50% by BF) and by 22% (substitution level of 50% by BFR) than the reducing capacity of the corresponding digested reference DWB. In contrast to digested BEWWBs, the reducing capacity of digested BEDWBs in all cases of substitution did not exceed the value noted for digested DWB. Available evidences based on the recent studies clearly indicate that the observed increase of reducing capacity of digested buckwheat-enhanced white and dark wheat breads was due to the release of phenolics from their conjugation forms as well as cell wall matrices. Szawara-Nowak et al. [18] showed significantly higher content of total phenolic compounds after in vitro gastrointestinal digestion of buckwheat-enhanced wheat breads as compared to the undigested ones. Moreover, the other scientists also demonstrated that simulated

gastrointestinal conditions significantly increased the total phenolic compounds of extracts obtained from wheat whole grains and their flour, germ, and bran fractions [7, 29, 30]. It should be also pointed out that as the higher reducing capacity was observed in breads formulated with participation of BFR than BF, then a contribution of MRPs originating from this ingredient can be suggested [31].

It should be mentioned that practical limitation of applied CV methodology was that the working electrode. It 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.

3.2 Bioaccessible reducing capacity of buckwheat-enhanced white and dark wheat breads

From a nutrition perspective, the definition of bioaccessibility is the fraction of a compound that is released from the food matrix in the gastrointestinal lumen and used for intestinal absorption [32]. This definition can be extended for the functional properties of food since it is closely related to the bioaccessibility of compounds responsible for the formation of this functional property. The in vitro digestion model has been widely used to study the complex multistage process of human digestion [33]. Bioaccessibility is a major factor that should be taken into account when assessing the potential health benefits of functional foods. Different contributors affect bioaccessibility. It can be affected by the composition of the digested food matrix, the synergisms and antagonisms of the different components, and the pH, temperature, and texture of the matrix [34]. Bioactive compounds are susceptible to multiple effects during digestion due to the effects of pH and enzymes, and in the present study, the bioaccessible reducing capacity of buckwheat-enhanced wheat breads was determined for the first time after an in vitro digestion by cyclic voltammetry method.

The reducing capacity of the digested BEWWBs and BEDWBs was significantly higher than those noted for the undigested corresponding breads (**Figure 3**). Therefore, for better evaluation of the bioaccessible reducing capacity in vitro, we introduced the reducing capacity bioaccessibility index (PRC):

$$PRC = RC_{GD}/C_{bread}.$$

where RC_{GD} is the reducing capacity after simulated gastrointestinal digestion (GD) and RC_{bread} is the reducing capacity of BEWWBs, BEDWBs, WWB, and DWB, respectively.

PRC value ˃ 1 indicates high bioaccessibility; PAC value < 1 indicates low bioaccessibility. The similar factor was introduced by Gawlik-Dziki et al. [35] as a useful parameter to study the bioaccessibility of phenolics from coffee and coconut. The PRC values of BEWWBs and BEDWBs are shown in **Figure 5**. PRC ranged from 5.0 to 1.8 for digested BEWWBs with participation of BF and from 2.9 to 1.4 for digested BEWWBs with participation of BFR. The PRC index ranged from 2.5 to 1.4 for digested BEDWBs with participation of BF and from 2.8 to 1.3 for digested BEDWBs with participation of BFR. These values indicate high bioaccessible reducing capacity of buckwheat-enhanced wheat breads. It was also found that the reducing capacity bioaccessibility index (PRC) for BEWWBs and BEDWBs depends on the level of substitution WWF or DWF by BF or BFR. As the level of substitution was higher, then the PRC was lower despite of the high reducing capacity of bread samples. This finding indicates that not only real value of reducing capacity but also its bioaccessibility should be taken into account when functional properties of buckwheat-enhanced wheat breads are proposed for consumers. Therefore, the substitution level of wheat flours by BF or BFR at level up to 20–30% seems to be the best well-balanced.
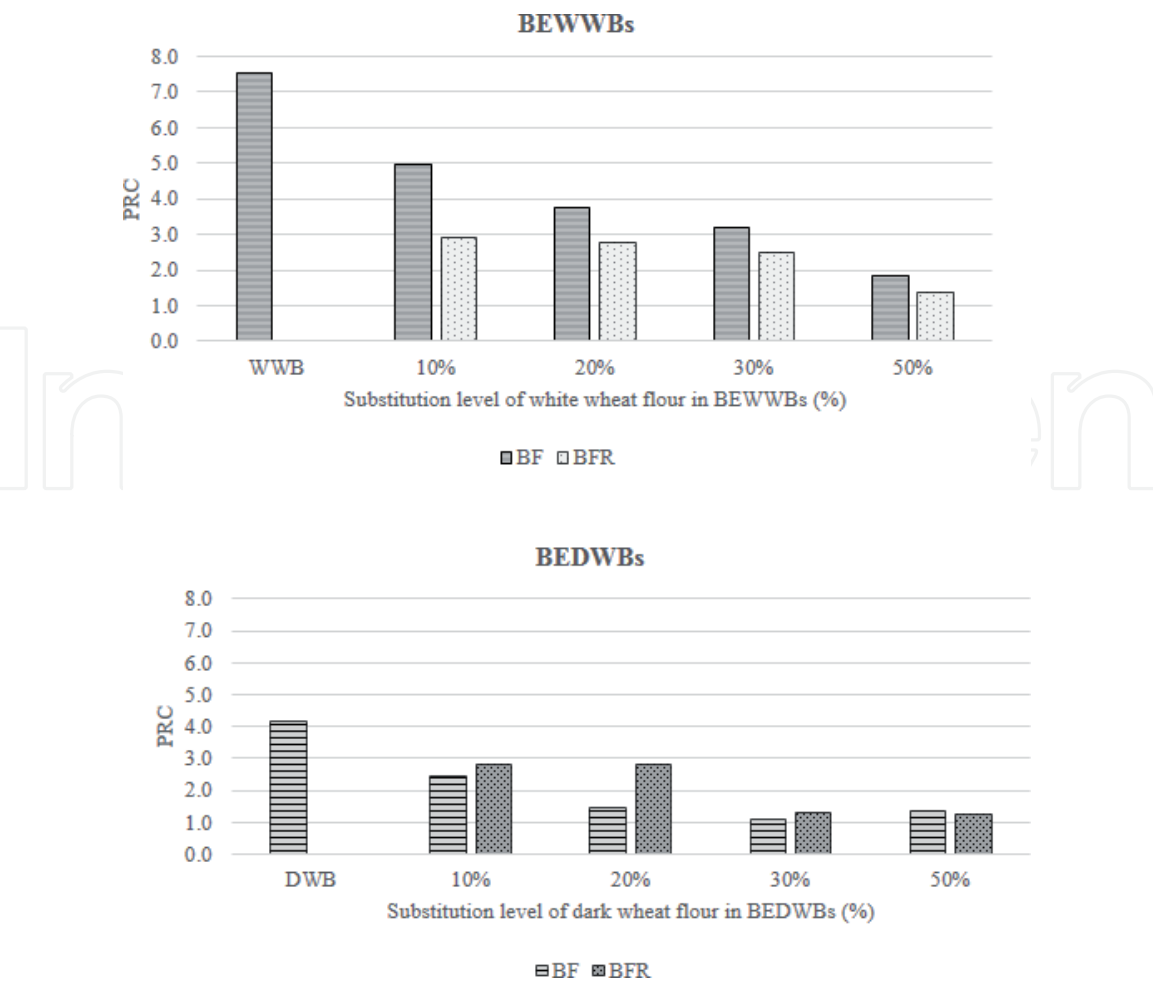
Figure 5.
The reducing capacity bioaccessibility index (PRC) of buckwheat-enhanced white wheat breads (BEWWBs) and buckwheat-enhanced dark wheat breads (BEDWBs).

4. Conclusions

The use of cyclic voltammetry allowed to show higher reducing capacity of digested in vitro buckwheat-enhanced wheat breads as compared to the undigested ones. Therefore, the bioaccessible reducing capacity of buckwheat-enhanced wheat breads seems to be an important factor characterizing the functional properties of bread among others. The reducing capacity bioaccessibility index (PRC) of buckwheat-enhanced wheat breads allowed to indicate the beneficial level of wheat flour substitution by buckwheat flours. The CV methodology is suitable for screening studies and allows obtaining a rapid electrochemical profile of a bread sample after digestion useful for evaluating their selected functional properties such as bioaccessible reducing capacity as proposed in this study.

Acknowledgements

Conflict of interests

The author declares that there are no conflict of interests regarding the publication of this paper.

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