Quality and antioxidant properties of breads enriched with dry onion (Allium cepa L.) skin

Urszula Gawlik-Dziki a,*, Michał Świeca a, Dariusz Dzikib, Barbara Baraniak a, Justyna Tomiło b, Jarosław Czyż c

a Department of Biochemistry and Food Chemistry, Faculty of Food Science and Biotechnology, University of Life Sciences, Skroma Str. 8, 20-704 Lublin, Poland
b Department of Thermal Technology, Faculty of Production Engineering, University of Life Sciences, Doświadczalna Str. 44, 20-280 Lublin, Poland
c Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa Str. 7, 30-387 Cracow, Poland

Keywords: Breads Onion skin Functional food Antioxidants Bioaccessibility

ARTICLE INFO

The aim of the study was to investigate the effect on the antioxidant properties and sensory value of bread of adding ground onion skin (OS). For a determination of bioaccessibility and bioavailability in vitro the human gastrointestinal tract model was used. OS contained mastication-extractable quercetin (4.6 mg/g). Quercetin from OS was highly bioaccessible during in vitro conditions, but only approximately 4% of quercetin released during simulated digestion was bioavailable in vitro. The antioxidant potential of bread with OS was significantly higher than the activity noted in the control. In particular, OS addition significantly fortified bread with bioaccessible lipid oxidation preventers and compounds with reducing and chelating abilities. The 2–3% OS addition caused significant improvement of antioxidant abilities (further increases in the OS supplement did not increase the activity of bread). Sensory evaluation showed that replacement of wheat flour in bread with up to 3% OS powder gave satisfactory consumer acceptability.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Epidemiological studies strongly suggest that diet plays a significant role in the prevention of many chronic diseases. Thus, nowadays consumers prefer to eat healthier foods in order to prevent non-communicable diseases. For this reason industry and researchers are involved in optimising bread making technology to improve the quality, taste, functionality and bioavailability of food products such as bakery goods. Among the ingredients that could be included in bread formulation are herbs and spices, which may significantly improve its nutraceutical potential (Balestra, Cocci, Pinnavaia, & Romani, 2011).

Cereals are considered to be the best vehicle for fortification in developing countries, because ~95% of the population consumes cereals as a dietary staple. Wheat flour is a staple food of the population and it provides more than 50% of the total energy intake. Cereals are relatively inexpensive, and are grown and consumed worldwide by all economic classes. Approximately 600 million metric tons of wheat and maize flour are commercially milled annually and consumed as various flour products in nearly every nation of the world (Saeed, Anjum, & Akbar Anjum, 2011). The pro-health effect of whole grain products is connected with the high content of polyphenol antioxidants that are important in the control of degenerative diseases in which oxidative damage has been implicated (Gawlik-Dziki, Świeca, & Dzik, 2012; Lim, Park, Ghafoor, Hwang, & Park, 2011).

Unfortunately, many bioactive compounds found in the grain, especially antioxidants (phenolic compounds), are particularly concentrated in the bran and aleurone layer (Mateo Anson, Havenaar, Bast, & Haenen, 2010).

Nowadays, the food and agricultural product processing industries generate substantial quantities of phenolic-rich by-products, which could be valuable natural sources of antioxidants to be employed as ingredients. Phytochemicals, including phenolic compounds, are suggested to be the major bioactive compounds contributing to the health benefits of fruits, vegetables and grains (Mateo Anson et al., 2010; Roldan, Sanchez-Moreno, De Ancos, & Cano, 2008). Flavonoids are a group of phenolic compounds with antioxidant activity that have been connected to reducing the risk of major chronic diseases. Onion has been shown to be one of the major sources of dietary flavonoids in many countries. Specifically, onion has been characterised for its flavonol quercetin and querce- tin derivates. Quercetin is a strong in vitro antioxidant with the ability to scavenge free radicals and chelate metals, which in turn inhibits lipid peroxidation. Additionally, it possesses anti-inflammatory properties (Boots, Drent, de Boer, Bast, & Haenen, 2011; Gawlik-Dziki, Świeca, Sugier, & Cichocka, 2011).

* Corresponding author. Tel.: +48 81 4623327; fax: +48 81 4623324. E-mail address: urszula.gawlik@up.lublin.pl (U. Gawlik-Dziki).
2. Materials and methods

2.1. Dry onion skin-based supplement (OS) preparation

The food supplement was prepared as follows: dry onion (Allium cepa, var. Wolska) skin was washed twice with deionised water and dried in an oven at 50 °C. Once dried, the material was powdered using a laboratory mill and then sieved (60 mesh).

2.2. Chemicals

Ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenyl-sulphonic acid)-1,2,4-triazine), ABTS (2,2-diphenyl-1-picrylhydrazyl) α-amyrase, pancreatic, pepsin, bile extract, linoleic acid, ammonium thiocyanate and haemoglobin were purchased from Sigma–Aldrich company (Poznan, Poland). All others chemicals were of analytical grade.

2.3. Bread making

The flour used in the formula of control bread (C) was wheat bread flour (600 g), type 750 (average 0.75% ash content, humidity 14%). The flour was replaced with OS at 1%, 2%, 3%, 4%, 5% levels (O1, O2, O3, O4 and O5, respectively). The percentage of OS addition was chosen on the basis of a previous test on antioxidant activity (data not published). Besides this 6 g of instant yeast and 12 g of salt were used for dough preparation. The general quantity of water necessary for the preparation of the dough was established through the marking of water absorption properties in flour of a consistency of 350 Brabender units. The batches of dough were mixed in a spiral mixer for 6 min. After fermentation, the pieces of dough (300 g) were put into an oven heated up to a temperature of 230 °C. The baking time was 30 min. After baking, the bread was left to stand for 24 h at room temperature.

2.4. Sensory evaluation

Sensory evaluation was carried out on bread samples with the different percentages of onion skin. Subsequently, the samples were sliced (slices about 1.5 cm thick), coded with a number and served to untrained consumers. The panel consisted of 16 consumers (24–45 years old), who evaluated the bread’s overall acceptability. This hedonic test was used to determine the degree of overall liking for the different types of bread based on degree of liking or disliking according to a nine-point hedonic scale (1: dislike extremely, 5: neither like nor dislike, 9: like extremely). Plain water was used for mouth rinsing before and after each sample testing (Lim et al., 2011).

2.5. Extracts preparation

After baking, bread samples were allowed to cool down to room temperature for 24 h. Subsequently, the samples were sliced (slices about 1.5 cm thick). The crust was removed aseptically and kept frozen (at −20 °C) until analysis. After thawing, the slices were dried and then manually crumbled, ground in a mill and screened through a 0.5 mm sieve to obtain bread powder.

2.5.1. Buffer extracts (BEs)

Powdered samples of bread or dry onion skin (1 g DW) were extracted for 1 h with 20 mL of PBS buffer (phosphate buffered saline, pH 7.4). The extracts were separated by decantation and the residues were extracted again with 20 mL of PBS buffer. Extracts were combined and stored in darkness at −20 °C.

2.5.2. Gastrointestinally digested (GE) and absorbed (AE) in vitro extracts

In vitro digestion. Simulated saliva solution was prepared by dissolving 2.38 g Na2HPO4, 0.19 g KH2PO4, and 8 g NaCl, 100 mg of mucin in 11 l of distilled water. The solution was adjusted to pH = 6.75 and α-amylase (E.C. 3.2.1.1.) was added to obtain 200 U per mL of enzyme activity. For gastric digestion 300 U/mL of pepsin (from porcine stomach mucosa, pepsin A, EC 3.4.23.1) was prepared in 0.03 mol/L NaCl, pH = 1.2. Further, simulated intestinal juice was prepared by dissolving 0.05 g of pancreatin (activity equivalent 4 × USP) and 0.3 g of bile extract in 35 mL 0.1 mol/L NaHCO3 (Elles, Blaylock, Huang, & Gussman, 2000; Tan, Khiang Pen, & Al-Manbali, 2000).

The onion skin and bread samples were subjected to simulated gastrointestinal digestion as follows: 1 g of powdered sample was homogenised in a Stomacher laboratory blender for 1 min to simulate mastication in the presence of 15 mL of simulated salivary fluid; and subsequently, the samples were shaken for 10 min at 37 °C. The samples were adjusted to pH = 1.2 using 5 mol/L HCl; and subsequently, 15 mL of simulated gastric fluid was added. The samples were shaken for 60 min at 37 °C. After digestion with the gastric fluid, the samples were adjusted to pH = 6 with 0.1 mol/L of NaHCO3 and then 15 mL of a mixture of bile extract and pancreatin was added. The extracts were adjusted to pH = 7 with 1 mol/L NaOH and finally 5 mL of 120 mmol/L NaCl and 5 mL of mmol/L KCl were added to each sample. Once prepared, the samples were submitted for in vitro digestion for 120 min, at 37 °C and in darkness. Thereafter, samples were centrifuged and supernatants were used for further analysis.

In vitro absorption: Considering that antioxidant absorption takes place mainly at the intestinal digestion stage, the resulting mixture (fluids obtained after in vitro digestion) was transferred to dialysis sacks (D9777-100FT, Sigma–Aldrich), placed in an Erlenmeyer flask containing 50 mL of PBS buffer and incubated in a rotary shaker (2 times per 2 h, 37 °C). The PBS buffer, together with the compounds that passed through the membrane (dialysate), was treated as an equivalent of the raw material absorbed in the intestine after digestion.

2.5.3. Methanolic extracts

Powdered samples of dry onion skin (1 g) were extracted for 1 h with 25 mL of 80% methanol (v/v). The extracts were separated by decantation and the residues were extracted again with 25 mL of 80% methanol (v/v). Extracts were combined and stored in darkness at −20 °C.
2.6. HPLC determination of quercetin

Quercetin was analysed according to the method described by Świeca, Gawlik-Dziki, Kowalczyk, and Złotek (2012). Samples were analysed with a Varian ProStar HPLC System separation module (Varian, Palo Alto, CA) equipped with a Varian ChromSpher C18 reverse phase column (25 mm × 4.6 mm) column and a ProStar DAD detector. The column thermostat was set at 40 °C. The mobile phase consisted of 4.5% acetic acid (solvent A) and 50% acetonitrile (solvent B) and a flow rate of 0.8 mL/min. At the end of the gradient, the column was washed with 50% acetonitrile and equilibrated to the initial condition for 10 min. Gradient elution was used as follows: 0 min 92% A, 30 min 70% A, 45 min 60%, 80 min 61% A, 82 min 0% A, 85 min 0% A, 86 min 92% A, 90 min 92% A. Detection was carried out at 370 nm. Quantitative determinations were carried out with the external standard calculation, using calibration curves of the standard.

2.7. Free radical scavenging assay

The experiments were performed using an improved ABTS decolorization assay (Re et al., 1999). ABTS⁺ was generated by the oxidation of ABTS with potassium persulfate. The ABTS radical cation (ABTS⁺) was produced by reacting 7 mmol/L stock solution of ABTS with 2.45 mmol/L potassium persulphate (final concentration). The ABTS⁺ solution was diluted (with distilled water) to an absorbance of 0.7 ± 0.05 at 734 nm. Then, 40 μL of samples were added to 1.8 mL of ABTS⁺ solution and the absorbance was measured at the end time of 5 min. The ability of the extracts to quench the ABTS free radical was determined using the following equation:

\[
\text{scavenging } \% = \left( \frac{A_C - A_S}{A_C} \right) \times 100
\]

where \( A_C \) is the absorbance of control, \( A_S \) the absorbance of sample.

Antioxidant activities were determined as EC₅₀ - extract concentration provided 50% of activity based on dose-dependent mode of action.

2.8. Metal chelating activity

Chelating power was determined by the method of Guo, Lee, Chiang, Lin, and Chang (2001). The extract samples (5 mL) were added to a 0.1 mL of 2 mM FeCl₂ solution and 0.2 mL 5 mM ferrous solution and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine- Fe²⁺ complex formation was given below formula:

\[
\% \text{ inhibition} = \left[ 1 - \frac{(A_C - A_S)}{A_C} \right] \times 100
\]

where \( A_C \) is the absorbance of the control, \( A_S \) the absorbance in the presence of the sample present of the sample.

Antioxidant activities were determined as EC₅₀ - extract concentration provided 50% of activity based on dose-dependent mode of action.

2.9. Ferric reducing power (FRAP)

Reducing power was determined using the method described by Oyaizu (1986). Extracts (2.5 mL) were mixed with phosphate buffer (2.5 mL, 200 mmol/L, pH 6.6) and 2.5 mL of a 1 g/100 mL aqueous solution of potassium ferricyanide K₃[Fe(CN)₆]. The mixture was incubated at 50 °C for 20 min. A portion (0.5 mL) of 10 g/100 mL trichloroacetic acid was added to the mixture, which was then centrifuged at 25 × g for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of 0.1 g/100 mL FeCl₃, and the absorbance was measured at 700 nm. EC₅₀ value (ng/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis.

2.10. Inhibition of linoleic acid peroxidation

Antioxidant activity was determined as the degree of inhibition of the peroxidation of linoleic acid according to Kuo, Yeh, and Pan (1999) with modification. Ten microliters of sample was mixed with 0.37 mL 5 mmol/L phosphate buffer (pH 7) containing 0.05% Tween 20 and 4 mmol/L linoleic acid and then equilibrated at 37 °C for 3 min. The peroxidation of linoleic acid in the above reaction mixture was initiated by adding 20 μL 10 mmol/L FeCl₂ in water, followed by incubation in a shaking bath at 37 °C for 10 min. The reaction was stopped by adding 5 mL 0.6% HCl in ethanol to the reaction mixture. The hydroperoxide formed was assayed according to a ferric thiocyanate method with mixing in, sequentially, of 0.02 mol/L FeCl₂ (0.1 mL) and 30% ammonium thiocyanate (0.1 mL). The absorbance of the sample (Aₚ) was measured at 480 nm with a spectrophotometer (Lambda 40, Perkin-Elmer) for 5 min. The absorbance of the base control (A₀) was obtained without adding haemoglobin to the above reaction mixture; the absorbance of the maximal control (A₉₀₀) was obtained with no sample addition to the above mixture. Thus, the antioxidative activity of the sample was calculated as:

\[
AA\% = \left( 1 - \frac{(A₀ - Aₚ)}{(A₉₀₀ - A₀)} \right) \times 100
\]

Antioxidant activities were determined as EC₅₀ - extract concentration provided 50% of activity was based on dose-dependent mode of action.

2.11. Theoretical approach

The following factors were determined to better understand the relationships between biologically active compounds in the light of their bioaccessibility, bioavailability, and bioefficiency:

- quercetin bioaccessibility factor (ACP), which is an indication of the bioaccessibility of quercetin,

\[
ACP = C_{GE} / C_{BE}
\]

- relative quercetin bioavailability index (rAVP),

\[
rAVP\% = C_{AE} / C_{BE} \times 100\%
\]

where \( C_{GE} \) is the concentration of quercetin after simulated gastrointestinal digestion (GE), and \( C_{BE} \) is the concentration of quercetin after PBS extraction (BE); \( C_{AE} \) is the concentration of quercetin after simulated intestinal absorption (AE),

- the antioxidant bioaccessibility index (BAC), which is an indication of the bioaccessibility of antioxidative compounds,

\[
BAC = A_{BE} / A_{GE}
\]

- the antioxidant bioavailability index (BAV) which is an indication of the bioavailability of antioxidative compounds,

\[
BAV = A_{AE} / A_{BE}
\]

- the antioxidant bioefficiency index (BEE), which is an indication of the bioactivity of bioavailable antioxidant compounds,

\[
BEE = A_{BE} / A_{AE}
\]

where \( A_{BE} \) is EC₅₀ of the extract after simulated intestinal absorption (AE), \( A_{BE} \) is EC₅₀ of the raw extract (BE), \( A_{GE} \) is EC₅₀ of the extract after simulated gastrointestinal digestion (GE).
2.12. Statistical analysis

All experimental results were mean ± S.D. of three parallel measurements, and, unless stated otherwise, data were evaluated by two-way analysis of variance (Tukey test) using Statistica 6.0 software (StatSoft, Inc., Tulsa, USA). The statistical tests were carried out at a significance level of α = 0.05.

3. Results

3.1. Quercetin concentration and bioaccessibility

As shown in Fig. 1, dry onion skin (OS) contains about 24.5 mg/g DW of total quercetin. Buffer-extractable quercetin concentration averages about 4.6 mg/g DW. Most importantly, quercetin from OS was highly bioaccessible during in vitro conditions (about 8.2 mg/g DW, \( \text{ACP} = 1.76 \)). In terms of buffer-extractable quercetin, its bioavailability in vitro averaged about 7%, but just around 4% of quercetin released during simulated digestion was determined in dialysate.

As expected, quercetin content in bread was significantly positively correlated with OS addition (Table 1). Correlation coefficients average 0.99, 0.99 and 0.94 in the case of \( \text{BE, GE and AE} \), respectively. The highest quercetin content was found for \( \text{GE} \), whereas the lowest in the extracts after dialysis. Surprisingly, the highest bioaccessibility in vitro was determined for quercetin from the control bread. Based on \( \text{ACP} \) factor values it can be concluded that digestion in vitro released quercetin from the control bread (probably occurring in conjugated form), whereas values obtained for enriched breads indicate that in dry onion skin quercetin occurred mainly as an aglycone. Results concerning the availability of quercetin showed that the highest \( r_{\text{AVP}} \) values were obtained for low-supplemented bread (from 1% to 3% of functional supplement). However, despite the relatively low accessibility and availability in vitro, all bread containing OS showed significantly higher quercetin levels when compared with the control.

3.2. Determination of quality of enriched breads

The results of hedonic tests on different types of bread are given in Table 2. The colour of both crust and crumb of the enriched bread was much darker (brown) than that of the control bread. However, it had no negative influence on bread acceptability. The taste, aroma and overall acceptability of bread at substitution levels of 1–3% had the highest linking score. Higher levels of onion skin addition caused a sharp aroma and taste. For texture characteristics, no statistically significant difference was observed for all samples. The sensory characteristics linking results indicated that a partial replacement of wheat flour in bread with up to 4% onion skin powder gives satisfactory overall consumer acceptability. However, bread which contained 4% and 5% skin was rated comparatively lower, which might be due to excessive amounts of volatile compounds which negatively affected the aroma and taste of products.

3.3. Influence of onion skin (OS) addition on antioxidant potential of bread

Most of the antioxidant potential in plant foods is due to the properties of phenolic compounds, especially quercetin, which can act as reducing agents, free radical scavengers and hydrogen donors (Boots et al., 2011; Gawlik-Dziki et al., 2011; Wiczkowski, Nemeth, Buciński, & Piskula, 2003). Thus, antiradical activity, the ability to inhibit lipid peroxidation, and the reducing and chelating power of OS and bread were assayed in this work. Values are given by \( \text{EC}_{50} \), i.e. the concentration needed to cause 50% of the antioxidant effect, meaning that a lower \( \text{EC}_{50} \) relates to higher antioxidant activity.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Quercetin concentration [µg/100 g DW]</th>
<th>Factors</th>
<th>( r_{\text{AVP}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7.60 ± 2.83(^{a,b}) 24.49 ± 0.15(^{d}) 0.56 ± 0.30(^{a})</td>
<td>3.22</td>
<td>7.37</td>
</tr>
<tr>
<td>O1</td>
<td>44.00 ± 2.83(^{a}) 69.01 ± 5.25(^{d}) 4.45 ± 0.54(^{a})</td>
<td>1.57</td>
<td>10.10</td>
</tr>
<tr>
<td>O2</td>
<td>98.50 ± 23.62(^{a}) 139.50 ± 25.78(^{a}) 5.32 ± 0.10(^{a})</td>
<td>1.42</td>
<td>5.40</td>
</tr>
<tr>
<td>O3</td>
<td>127.00 ± 22.34(^{a}) 211.58 ± 32.98(^{a}) 6.44 ± 0.20(^{a})</td>
<td>1.67</td>
<td>5.07</td>
</tr>
<tr>
<td>O4</td>
<td>178.80 ± 6.79(^{a}) 337.59 ± 8.52(^{a}) 7.46 ± 0.25(^{a})</td>
<td>1.89</td>
<td>4.17</td>
</tr>
<tr>
<td>O5</td>
<td>231.00 ± 5.37(^{a}) 437.04 ± 53.52(^{a}) 8.19 ± 0.10(^{a})</td>
<td>1.89</td>
<td>3.55</td>
</tr>
</tbody>
</table>

Means ± SD followed by different letters are significantly different at \( α = 0.05 \).

### Table 2

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Substitution level (%)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crumb colour</td>
<td>7.9(^{a}) 8.5(^{b}) 8.3(^{ab}) 8.2(^{ab}) 8.1(^{ab})</td>
<td>8.3(^{b})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aroma</td>
<td>7.6(^{a}) 8.1(^{b}) 8.3(^{b}) 7.5(^{b}) 6.2(^{a})</td>
<td>4.3(^{d})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>7.5(^{a}) 7.4(^{b}) 7.2(^{a}) 7.3(^{b}) 7.2(^{a}) 7.4(^{b})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste</td>
<td>7.2(^{a}) 8.4(^{b}) 8.5(^{b}) 8.2(^{b}) 4.1(^{d}) 3.2(^{d})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>7.5(^{a}) 7.9(^{b}) 7.8(^{b}) 7.7(^{ab}) 5.2(^{d}) 3.1(^{d})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Nine-point hedonic scale with 1, 5 and 9 representing extremely dislike, neither like nor dislike, and extremely like, respectively. Means with different letter superscript within a same row are significantly different (\( α < 0.05 \)).
(EC50 = 40.62 mg DW/mL), whereas the activity of lipid oxidation preventers increased significantly (EC50 = 15.32 mg DW/mL). High activity was also observed for potentially bioaccessible (GE extracts) antiradical and reductive compounds (EC50 = 6.44 and 7.46 mg DW/mL, respectively). After simulated absorption (AE extracts) a higher activity was observed for radical scavengers, lipid oxidation preventers and chelating compounds, whereas activity reductive compounds decreased significantly when compared with initial activity (BE).

### 3.3.2. Antioxidant activity of breads

Onion skin addition strongly influenced the antiradical activity of bread. The antiradical activity of bread BE was relatively low. Digestion in a simulated gastrointestinal system released active compounds from all samples. It is interesting that a 2–3% OS addition caused significant improvement in scavenging abilities, while a further increase in OS supplement did not increase the activity of the studied bread. The activity of AES was comparable with the activity of GEs (Table 3).

Fortification with dry onion skin increased the chelating power of bread. Digestion in vitro released compounds with strong chelating power from OS-enriched bread, whereas the activity of the control sample was decreased significantly. Most importantly, the activity of samples with a 2–5% OS addition did not differ significantly. Very interesting results were obtained for samples after simulated absorption. Potentially bioavailable metal ion chelators from the control bread (AE) were less active than those obtained from OS-enriched bread. Generally, there was no significant difference in terms or activity among the group of enriched bread samples (Table 3).

All the bread studied possessed the ability to inhibit lipid peroxidation. The activity of fortified bread samples was significantly higher than the activity of the control; however, no linear relation was found between the level of dry onion skin (OS) and activity. With regards to BE, the highest activity was determined for bread with a 3% OS addition (O3). Simulated gastrointestinal digestion significantly decreased activity of control and O1 bread, while for other samples activity was unchanged or slightly increased (Table 3).

The reducing power of bread was strongly dependent on its composition. The activity of samples with an OS addition was significantly higher than the activity of the control. Digestion in vitro released compounds with strong reducing power. It should be noted that the activities of bread enriched with 2–5% OS did not differ significantly. Also, taking into account samples obtained after simulated absorption no linear correlation between activity and OS addition was found (Table 3).

### 3.3.3. Bioaccessibility, bioavailability and bioefficiency in vitro

An important factor determining biological activity is the bioavailability of active compounds. For this reason, in this study bioavailability in vitro was measured.

---

**Fig. 2.** Comparison of antioxidant potential (A) and bioaccessibility, bioavailability and bioefficiency in vitro (B) of onion skin phytochemicals. Bars represent means ± SD. Means, within separate antioxidant properties, followed by different letters are significantly different at \( p < 0.05 \) using one-way Anova (Tukey test). BE – buffer extract, GE – extract after digestion in vitro, AE – extract after absorption in vitro AA – antiradical activity, CHEL – chelating power, LPO – ability to inhibit lipid peroxidation, RED – reducing power, BAC – antioxidant bioaccessibility index, BAV – antioxidant bioavailability index, BEF – antioxidant bioefficiency index.
pH conditions and/or digestive enzyme action. The interaction between the conditions occurring in living organisms, e.g. changeable activity of plant-derived compounds is the interaction between these conditions. Significantly lower (the BAV with chelating and reducing power were most active during digestion against oxidative damage. Bioactive compounds released from endogenous bioaccessible lipid prevention compounds able to protect the upper part of the gastrointestinal tract from oxidative damage. Bioactive compounds released from enriched bread were poorly bioavailable during in vitro conditions (the BAV was equal to or significantly lower than that determined for control). The BEF values indicated that bioavailable compounds with chelating and reducing power were most active during in vitro conditions. Significantly lower BEF values were obtained for lipid oxidation preventers and radical scavengers.

These data indicate that an important factor modifying the bioactivity of plant-derived compounds is the interaction between them and/or food matrix. A crucial role in the bioactivity is also played by conditions occurring in living organisms, e.g. changeable pH conditions and/or digestive enzyme action.

Table 3
Antioxidant activity of breads extracts.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Sample</th>
<th>Buffer extract EC50 (mg DW/mL)</th>
<th>Digestion extract EC50 (mg DW/mL)</th>
<th>Absorption extract EC50 (mg DW/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>C</td>
<td>248.18 ± 6.89</td>
<td>44.91 ± 2.89</td>
<td>45.36 ± 3.58</td>
</tr>
<tr>
<td></td>
<td>O1</td>
<td>164.52 ± 5.65</td>
<td>37.33 ± 1.89</td>
<td>31.23 ± 2.89</td>
</tr>
<tr>
<td></td>
<td>O2</td>
<td>101.37 ± 4.59</td>
<td>31.62 ± 2.58</td>
<td>34.10 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>O3</td>
<td>65.07 ± 2.87</td>
<td>33.16 ± 1.06</td>
<td>29.17 ± 2.36</td>
</tr>
<tr>
<td></td>
<td>O4</td>
<td>67.71 ± 2.11</td>
<td>31.86 ± 2.74</td>
<td>34.94 ± 1.58</td>
</tr>
<tr>
<td></td>
<td>O5</td>
<td>57.71 ± 1.98</td>
<td>29.69 ± 1.69</td>
<td>22.93 ± 5.58</td>
</tr>
<tr>
<td>CHL</td>
<td>C</td>
<td>29.05 ± 1.69</td>
<td>45.03 ± 3.54</td>
<td>17.56 ± 1.29</td>
</tr>
<tr>
<td></td>
<td>O1</td>
<td>24.63 ± 1.58</td>
<td>16.92 ± 2.99</td>
<td>9.06 ± 5.84</td>
</tr>
<tr>
<td></td>
<td>O2</td>
<td>24.95 ± 0.56</td>
<td>10.61 ± 2.58</td>
<td>7.10 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>O3</td>
<td>18.05 ± 1.89</td>
<td>10.76 ± 0.87</td>
<td>7.39 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>O4</td>
<td>20.03 ± 1.98</td>
<td>8.88 ± 0.18</td>
<td>8.76 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>O5</td>
<td>16.29 ± 0.33</td>
<td>8.34 ± 1.02</td>
<td>6.61 ± 0.98</td>
</tr>
<tr>
<td>LPO</td>
<td>C</td>
<td>18.75 ± 0.56</td>
<td>28.92 ± 1.89</td>
<td>11.24 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>O1</td>
<td>14.67 ± 0.87</td>
<td>24.07 ± 2.58</td>
<td>8.76 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>O2</td>
<td>13.97 ± 1.02</td>
<td>10.46 ± 0.33</td>
<td>8.65 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>O3</td>
<td>10.95 ± 0.58</td>
<td>10.51 ± 0.12</td>
<td>7.31 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>O4</td>
<td>11.96 ± 0.77</td>
<td>10.60 ± 1.21</td>
<td>6.77 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>O5</td>
<td>13.47 ± 1.45</td>
<td>8.78 ± 0.47</td>
<td>6.19 ± 0.25</td>
</tr>
<tr>
<td>RED</td>
<td>C</td>
<td>100.85 ± 1.87</td>
<td>46.77 ± 5.79</td>
<td>27.96 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>O1</td>
<td>59.29 ± 1.88</td>
<td>9.35 ± 0.68</td>
<td>6.46 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>O2</td>
<td>30.34 ± 2.11</td>
<td>6.94 ± 1.87</td>
<td>7.45 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>O3</td>
<td>23.56 ± 2.39</td>
<td>6.79 ± 0.69</td>
<td>6.34 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>O4</td>
<td>19.98 ± 0.87</td>
<td>6.19 ± 0.11</td>
<td>6.17 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>O5</td>
<td>17.03 ± 2.01</td>
<td>5.39 ± 0.28</td>
<td>3.83 ± 0.89</td>
</tr>
</tbody>
</table>

Means ± SD within separate antioxidant properties followed by different letters are significantly different at p < 0.05 using two-way ANOVA (Tukey's test).

**4. Discussion**

In recent years, many studies have been carried out to find potential sources of bioactive compounds for wheat breads. Based on the fact that onion skin contains significantly higher levels of flavonoids than the edible portion of the vegetable (about 2–10 g/kg) (Leighton et al., 1992) and is waste material from the food industry, we decided to evaluate the dry onion skin as a functional supplement for bread making. The primary flavonoid in onion skin is quercetin aglycone (about 84% of total quercetin), small amounts of quercetin diglucoside, quercetin 4-glucoside and in some cases, isorhamnetin monoglucoside or kaempferol monoglucoside are also present (Wiczkowski et al., 2008). The food supplement used in these studies contained about 24.56 mg/g DW of quercetin aglycone (Fig. 1); however, the level of buffer-extractable quercetin aglycone concentration was significantly lower – 4.6 mg/g DW. Results concerning total quercetin content obtained in these studies are generally in agreement with those presented by Wiczkowski et al., 2003. Additionally, it must be emphasised that in most studies concerning flavonoid content in onion these compounds were isolated with organic solvents. Kim, Jo, Jang, Lee, and Kwon (2010) proved that extraction of quercetin with 90% ethanol enriched the final extracts about 15-fold in comparison to buffer extraction. Cited authors proposed the use of 95% EtOH extract from dry onion skin as a component for production of functional foods. In our opinion, the onion skin-based supplement proposed in this study is cheaper, easier to produce and safer for consumers. It should be noted that, in spite of the highly enhanced efficiency of extraction using ethanol, this technology requires the removal of the extraction medium (additional costs). Bread making technology does not allow for the use of ethanol extracts due to their negative influence on yeast action.

High biologically active compound content can be used as the first criterion during selection of food supplements. However, a compound needs to be able to reach its primary site of action in order to be biologically active; in other words, it needs to be bioavailable. In dry onion skin, unlike in the case of onion flesh, quercetin aglycone does not appear in crystalline form but it is dispersed in the onion matrix, which makes it very easily accessible (Wiczkowski et al., 2008). Additionally, according to cited investigators quercetin may form some complexes with cell wall components. An increase in free quercetin content may also be linked with disruption of quercetin conjugates by changeable pH conditions and digestive enzyme action (Fig. 1). Data concerning the bioavailability of quercetin from OS were difficult to interpret. Most importantly, bioavailability assays in vitro were quite difficult and many models were used. Wiczkowski et al. (2003) suggested that more hydrophobic quercetin may pass through biological membranes to reach intestinal enterocytes via passive transport, while for more polar quercetin glucosides two mechanisms were proposed: either prior to absorption they may be hydrolysed in the intestinal lumen by β-glucosidases and free quercetin thus released is absorbed as above, or they may enter enterocytes via an Na+/glucose transporter. Results obtained in our work suggested that antioxidants and/or quercetin from dry onion skin may be highly bioavailable during in vivo conditions. These results were in accordance with those obtained by Wiczkowski et al. (2008); however, further study is needed.

As expected, quercetin content in enriched bread was significantly, positively correlated with onion skin addition irrespective of the kind of extract. Data confirming the fact that quercetin from onion skin occurs mainly in free form were obtained when the ACP values were compared. Digestion in vitro caused release of quercetin conjugates from wheat flour, whereas in the case of enriched bread the ACP values were significantly lower despite of the high
content of quercetin (Table 1). This tendency was not visible when the availability factor values (AVP) were compared. This parameter clearly shows that quercetin from enriched bread was more bioavailable than from the control. Most importantly, the ACP values obtained for bread with higher OS additions suggested the appearance of slightly bioavailable in vitro quercetin-protein complexes. These results were in agreement with those obtained in our previous experiments (data not shown). As summarised in Fig. 1, compounds contained in onion skins may use different mechanisms to act as antioxidants. These results confirmed those of previous studies (Kim et al., 2010; Suh, Lee, Cho, Kim, & Chung, 1999). However, in the recent literature there is a lack of papers with a comprehensive study of antioxidant potential (determined using various methods) in the light of bioaccessibility, bioavailability and the bioefficiency of dry onion skin compounds. Thus, in our study we attempt to discuss this problem.

Buffer-extractable phytochemicals from OS were the most effective radical scavengers, and chelating and reducing factors; however, their ability to prevent lipid oxidation was relatively low. Taking into account bioaccessibility, bioavailability and bioefficiency in vitro it may be concluded that the most bioaccessible were compounds able to chelate metal ions; slightly lower BAC values were obtained for antiradical and reductive phytochemicals, whereas the bioaccessibility of lipid oxidation preventers was very low. Surprisingly, metal chelators were strongly bioavailable in vitro, in contrast to reductive and lipid oxidation preventers. The relatively high values of the bioefficiency factors of onion skin antioxidants should also be emphasised. Thus, our data confirm the high nutraceutical potential of onion skin phytochemicals.

Despite of thermal processing during bread making, onion skin addition significantly increased the antioxidant potential of supplemented bread. This is especially important in the light of the fact that phenolics are quite heat unstable and the baking process might have resulted in some heat damage to phenolic compounds (Cheynier, 2005). In our study, in all cases the antioxidative potential of the control sample was significantly lower in comparison with supplemented bread. Taking into account BEs containing potentially mastication-extractable phytochemicals it is observed that OS addition was a crucial factor affecting the elevation of antiradical potential. Significant enhancement of the antioxidant potential of enriched bread has been observed in many studies (Chlopicka, Pasko, Gorinstein, Jedryas, & Zagrodzki, 2012; Fan, Zhang, Yu, & Ma, 2006; Gawlik-Dziki, Dziki, Baraniak, & Lin, 2009; Glei, Kirmse, Habermann, Persin, & Pool-Zobel, 2006; Lim et al., 2011). Nevertheless, in most of the cited studies organic solvents (MeOH, EtOH) were used for antioxidant potential estimation; only some studies were performed using in vitro conditions. Moreover, comparison of these results was difficult due to different supplements and models of the gastrointestinal tract. The findings and implications resulting from the in vitro experiments described must now be validated in human studies.

The results obtained in this study clearly showed that during preparation new functional food changes occurring during digestion must be taken into consideration. The amount of bioaccessible
food polyphenols and thus antioxidants may differ quantitatively and qualitatively from polyphenols included in food databases. Compilation of the results obtained for AEs and BEs showed significant elevation of activity in almost all samples. This tendency was strongly visible in the case of antiradical and reducing compounds. Most importantly, activities of extracts obtained after absorption in vitro were significantly higher than those observed for BEs. This fact may indicate the high bioefficiency of phytochemicals from bread enriched with dry onion skin. However, particularly noteworthy is the fact that antioxidant values obtained after digestion of bread are better than those of the buffer extract and they remain quite constant after dialysis despite the much lower quercetin content in the dialysates (Table 1). It should be emphasised that the control bread has a rather high antioxidant potential; however, the antioxidant potential of dialysates was significantly, positively correlated with the OS addition level ($r = 0.76, 0.72, 0.62$ and 0.73 for AA, CHEL, LPO and RED, respectively). These results may be explained by the fact that bread contains bioaccessible phenolic antioxidants, especially ferulic acid and alkylresorcinols (Mateo Anson, Hemery, Bast, & Haenen, 2012; Mateo Anson, Van Den Berg, Havenaar, Bast, & Haenen, 2009); these are likely to be bioavailable in vitro and may interact synergistically with quercetin. These results confirmed previously observed synergistic interactions between plant antioxidants (Gawlik-Dziki, 2012). Additionally, thermally processed foods may contain various levels of Maillard reaction products (MRPs) that have been reported to have antioxidant activity through scavenging oxygen peroxyl, hydroxyl and DPPH radicals, copper and Fe$^{2+}$ chelators (Gawlik-Dziki et al., 2009).

Onion skin constitutes a valuable supplement for developing bread with enhanced functional properties. In the light of the present data it can be concluded that a 2–3% supplementation with OS was optimal for improving the antioxidant potential of bread without compromising its sensory quality.

References


