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ANTIOXIDANT PROPERTIES OF SPELT BREAD

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In this study the antioxidant properties of laboratory baked spelt breads and their separated crumb and crust were compared to those obtained for wheat and rye bread. Phosphate buffered saline (PBS) and 80% aqueous methanol extracts were prepared to this end. Antioxidant properties were presented as TEAC (Trolox Equivalent Antioxidant Capacity), RSA DPPH (Radical Scavenging Activity of DPPH radicals), FCR (Reducing capacity by Folin-Ciocalteu's reagent application) and SOD-like activity (Superoxide dismuatse – like activity). Spectrophotometric methods based on free radical scavenging activities (TEAC, RSA DPPH, SOD-like activity) and reducing power (FCR) were found applicable for the evaluation of the antioxidant capacity of spelt breads. The studies showed that spelt breads and their crumbs formulated on white flours possessed similar antioxidant capacity comparable to that of wheat bread, however the spelt bread crusts showed higher antioxidant capacity than the wheat bread crust. Moreover, the reducing capacity of spelt breads and separated crumbs and crusts was significantly higher in comparison with that of wheat samples but lower than that of rye samples. The highest antioxidant capacity and reducing capacity were provided by rye bread and its separated crumb and crust.

INTRODUCTION

Nowadays people are interested in the consumption of healthy cereal based products, especially when grains originate from ecological farms. Growing public awareness and an increasing knowledge about positive health aspects of such cereal-based products initiate changes in customer nutritional habits [Andlauer & Furst, 1998]. Therefore, people search for new sources of healthy diet in order to improve their well-being. Currently, the worldwide cereal-based food production is based mainly on wheat grain processing and, to a smaller extent, on rye-based products. An alternative to those commonly cultivated and consumed wheat and rye-based food could be processed spelt grain (*Triticum aestivum* subsp. *spelta*). Spelt grain was the predominant bread cereal from the 5th century until the beginning of the 20th century [Ruibal-Mendieta *et al.,* 2002]. The cultivation of spelt has declined during the last decades, however recent interest in the use of spelt grain for bread-making offers a new resurgence in its cultivation. In fact, spelt cultivations has been a tradition in Europe, particularly in Germany, where it is widely used for preparing grain-based foods [Ranhotra *et al.,* 1995]. This ancient crop is still cultivated in many European countries (*e.g.* Belgium, Germany Austria, Slovenia, and northern parts of Italy) and has been introduced in the United States in the 1980s [Oplinger *et al.,* 1990]. In Poland, cultivation of this cereal in not so popular and should be increased since spelt flour and spelt bread and pasta are now promoted and marketed [Ceglińska, 2003; Tyburski & Żuk-Gołaszewska, 2005]. Spelt demonstrates a higher resistance to environmental factors and can be cultivated in harsh ecological conditions, without the use of pesticides when compared to common wheat [Bonafaccia *et al.,* 2000]. Moreover, spelt is more tolerant of poorly-drained and low-fertility soils than other commonly cultivated cereals. The spelt-based products are easily digestible and have a slightly higher protein content than wheat products. The main difference in the nutritional value of spelt and wheat grains is the variation in the amount and type of prolamines. This is probably why some people suffering from food allergy tolerate foodstuffs originating from spelt-based products [Bonafaccia *et al.,* 2000]. Spelt has also been recommended for treatment of colitis ulcerosa, neurodermitis and other allergies as well as high blood cholesterol [Hertzka & Strehlow, 1988; Strechlow *et al.,* 1991].

Scarce literature is available on the nutritional quality of spelt grains and spelt-based product [Grela *et al.,* 1993, 1996; Ranhotra *et al.,* 1996; Moudry & Dvoracek, 1999; Bonafaccia *et al.,* 2000], but there are no principal studies related to the antioxidant properties of spelt bread. Recently our group provided the milling and baking characteristics of different strains of spelt wheat originated from Polish breeding and showed a profile of antioxidants in spelt breads formulated on white spelt flours [Zieliński *et al.,* 2008]. Therefore, the present study focused on the characterization of the antioxidant properties of previously investigated spelt breads and their parts (crumbs and crusts), in comparison with commonly consumed wheat and rye breads formulated also on the respective white flours.

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Antioxidant properties of breads were evaluated with spectrophotometric methods based on free radical scavenging activities of bread extracts against $ABTS^{\bullet+}$ radical cations (TEAC assay), 2,2-diphenyl-1-picryhydrazyl radicals (RSA DPPH assay) and superoxide anion radicals (SOD-like activity). The reducing capacity of breads was provided by means of Folin-Ciocalteu's phenol reagent (FCR) application.

MATERIALS AND METHODS

Reagents

Ferulicacid, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). A superoxide dismutase kit (RANSOD, Cat No SD 125) originated from Randox Laboratories Ltd, (Crumlin, UK). Folin-Ciocalteu's phenol reagent and other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

Materials

Spelt grains originated from Polish breeding STH 915, STH 975 and STH 974 spelt strains whilst rye grain of the cultivar Dańkowskie Złote was selected from breeding materials grown in central Poland (DANKO, Plant Breeding Co., Laski). White spelt and rye flours with extraction rate of 65-70% were obtained after milling in a Quadrumat Senior mill (Germany). A white wheat flour with extraction rate of 70% was provided by a local shop in Olsztyn (Poland).

Flours characterization

Flours were characterised for the content of protein, ash and starch. Protein content was measured following AACC method [AACC, 2000] using 46-11B Using Foss Tecator apparatus whereas starch content was determined with the polarimetric method [AACC, 2000]. Ash content of flours was analysed according to AOAC method 15.955.03 [AOAC, 1990]. All analyses were made in triplicate.

Bread making procedures

Spelt breads were baked using the single-stage method (Figure 1). Baking trials were performed in triplicate as described by Zieliński *et al.* [2008]. Rye and wheat breads were produced according to the method described in details by Michalska *et al.* [2007]. The crumb and crust were separated manually. Samples were freeze-dried, ground and sieved through a 60-mesh screen. Powdered samples were stored at –20°C until analysis.

Preparation of PBS and 80% methanol extracts

Powdered whole bread, crumb and crust were extracted in triplicate with PBS pH 7.4 (1 g per 15 mL) or with 80% aqueous methanol (1 g/10 mL) for 2 h of shaking at 37°C. Samples were centrifuged at 12,000 × g for 15 min in a Beckman GS-15 R centrifuge (Beckman Instruments, Fullerton CA, USA) and then the fresh supernatants were used for the determination of antioxidant properties of breads and their separated parts.

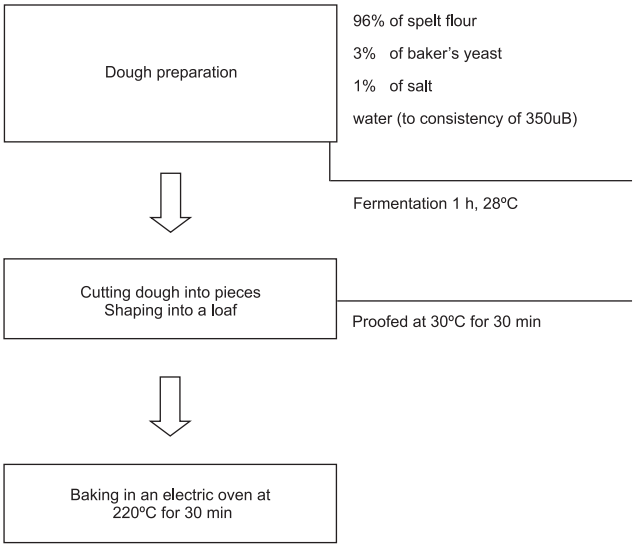

FIGURE 1. Simplified flow diagram of spelt bread making process.

Antioxidant capacity assays

The Trolox Equivalent Antioxidant Capacity (TEAC) of the breads and their crumb and crust were evaluated using free radical scavenging activities of PBS and 80% methanol extracts against $ABTS^{\bullet+}$ radical cation (ABTS radical cation decolorization method), whereas radical scavenging activity (RSA) was determined against 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$) in 80% methanol extracts. The superoxide dismutase-like activity (SOD-like activity) was evaluated as free radical scavenging activities of PBS extracts against superoxide anion radicals ($O_2^{-\bullet}$). FCR reducing capacity of breads was provided by means of Folin-Ciocalteu reagent (FCR) application. The analytical details were as previously reported by Michalska *et al.* [2007].

Statistical analysis

Data were subjected to a multifactor analysis of variance (ANOVA) using the least-squared difference test with Statgraphic 5.0 Program (Statistical Graphic, Rockville, Md., USA) and multiple correlation using Statistica 5.1 Program (Statsoft, Tulsa, Okla, USA) for Windows using a PC-Pentium.

RESULTS AND DISCUSSION

Characterization of white spelt, wheat and rye flours

The proximate chemical composition of three types of white spelt flours originated from STH 915, STH 975 and STH 974 spelt strains, and white wheat and rye flours, is compiled in Table 1. The effect of flour origin was found for spelt flours in respect to protein and starch content. Spelt flour obtained from STH 975 strain showed a higher content of starch and a lower content of protein than the two flours originated from the remaining spelt strains. A similar relationship was found for white wheat flour which was characterised by the highest content of protein and an average content of starch among all the tested flours. In contrast, white rye flours had the protein level similar to those found for spelt flours, the highest ash content and the lowest starch content.

TABLE 1. Proximate chemical composition of white spelt, wheat and rye flours with extraction rate of 65–70%.

Flour origin	Protein (%)	Ash (%)	Starch (%)
Spelt flour - STH 915 strain	8.9	0.51	78.2
Spelt flour - STH 975 strain	7.5	0.52	82.3
Spelt flour - STH 974 strain	8.8	0.55	78.6
Rye flour - cv. Dańkowskie	7.9	0.73	56.7
Wheat flour - local shop	9.2	0.50	74.9

Data expressed as mean ± standard deviation (n = 3). The content of protein, ash and starch is reported on fresh flour basis.

Trolox Equivalent Antioxidant Capacity (TEAC) and DPPH Radical Scavenging Activity (RSA DPPH)

The PBS and 80% methanol extracts of spelt, wheat and rye breads and their separated parts were examined for their free radical scavenging activity against $ABTS^{\bullet+}$ cation radicals. The PBS extracted almost twice (spelt and wheat bread) and three times (rye bread) more antioxidant compounds from the whole breads and their separated parts than 80% methanol did. The data are compiled in Table 2 and Table 3, respectively. The spelt bread formulated on flour originated from STH 915 strain possessed the highest ability to scavenge $ABTS^{\bullet+}$ cation radicals in both PBS and 80% methanol extracts when compared to the remaining spelt breads, and the results were statistically different ($p<0.05$). In contrast, spelt bread formulated on flour originated from STH 974 strain showed the lowest TEAC in comparison with STH 915, STH 975, wheat and rye breads. Moreover, the average TEAC calculated for three spelt breads derived from PBS (5.76 μmol Trolox/g d.m.) and 80% methanol (1.96 μmol Trolox/g d.m.) measurements showed the similar antioxidant capacity as wheat bread. In contrast, this average TEAC was twice lower when compared to TEAC values provided for rye breads. These findings indicate the importance of the origin of flour taken for the bread-making since the flours tested had the same extraction rate.

TABLE 2. Trolox Equivalent Antioxidant Capacity (TEAC) of spelt, wheat and rye breads and their separated parts (μmol Trolox/g d.m.). The results were based on PBS extracts.

Type of bread	Whole bread	Crust	Crumb
Spelt bread STH 915	8.00 ± 0.23^{a}	9.95 ± 0.51^{a}	5.13 ± 0.04^{a}
Spelt bread STH 975	5.21 ± 1.08^{b}	9.61 ± 1.27^{a}	4.63 ± 0.36^{a}
Spelt bread STH 974	4.07 ± 0.20^{b}	6.44 ± 0.50^{b}	4.84 ± 0.28^{a}
Wheat bread	5.90 ± 0.09^{c}	7.34 ± 1.27^{ab}	6.06 ± 0.53^{b}
Rye bread	11.13 ± 0.88^{d}	15.39 ± 1.55^{c}	8.71 ± 0.22^{c}

Data expressed as mean ± standard deviation (n = 3). Within each column related to whole bread, crust and crumb data, means with the same letter are not significantly different ($p \leq 0.05$).

TABLE 3. Trolox Equivalent Antioxidant Capacity (TEAC) of spelt, wheat and rye breads and their separated parts (μmol Trolox/g d.m.). The results were based on 80% methanol extracts.

Type of bread	Whole bread	Crust	Crumb
Spelt bread STH 915	2.02 ± 0.01^{a}	4.11 ± 0.29^{a}	1.32 ± 0.13^{ab}
Spelt bread STH 975	2.18 ± 0.08^{b}	5.94 ± 0.64^{ab}	2.21 ± 1.57^{ab}
Spelt bread STH 974	1.67 ± 0.07^{c}	5.87 ± 0.73^{b}	1.08 ± 0.13^{a}
Wheat bread	2.77 ± 0.04^{d}	3.30 ± 0.15^{a}	2.42 ± 0.26^{ab}
Rye bread	3.98 ± 0.01^{e}	8.43 ± 0.23^{b}	2.99 ± 0.04^{b}

Data expressed as mean ± standard deviation (n = 3). Within each column related to whole bread, crust and crumb data, means with the same letter are not significantly different ($p \leq 0.05$).

It is also well-known that baking conditions are responsible for the formation of Maillard reaction products (MRPs) in breads, which is essential to give positive notes to the food products such as improving their flavor, colour and texture [Rada-Mendoza *et al.,* 2004]. Therefore, the parts separated from bread, namely crumb and crust, were further investigated for their ability to scavenge $ABTS^{\bullet+}$ radicals. The TEAC values, based on PBS and 80% methanol extracts, provided for crumbs separated from all tested breads were slightly lower or had similar values (STH 975 and wheat bread) when compared with those provided for the respective whole breads. This finding indicates thermal resistance up to 100°C of PBS and 80% methanol extracted antioxidants formulating the antioxidant capacity of crumb since the temperature of the crumb never exceeds the boiling point of water, and the center of loaf does not reach the maximum temperature until the end of the baking process [Chang, 2006]. The TEAC values, based on PBS extracts, provided for crusts separated from the three types of spelt, wheat and rye breads were higher within the range from 24%-84%, 24% and 38% when compared with those provided for the respective whole breads. Moreover, antioxidants extracted with 80% methanol formulated almost twice and three fold-higher TEAC of crust in comparison to those being responsible for TEAC of whole breads. The order of increasing TEAC of the crusts was as follows: wheat bread < spelt breads < rye bread. This phenomena cannot be explained by the thermal resistance of antioxidant compounds but rather by the effect of baking conditions which favoured the formation of Maillard reaction products (MRPs) in the bread crust. The temperature of the crust can reach about 205°C when oven temperature remains at constant zone of 220-240°C [Chang, 2006]. Formulation of these compounds could be related to Maillard reaction that occurred during thermal treatment [Del Castillo *et al.,* 2002]. The antioxidants responsible for the formulation of TEAC of crust extractable with PBS and 80% methanol were highly correlated ($r=0.94$), but lower correlation coefficients were calculated in respect to the whole breads ($r=0.83$) and separated crumbs ($r=0.77$).

It has been widely known that the radical system used for determination of antioxidant capacity may influence the results obtained. Therefore, at least two or more radical systems are required to investigate the radical scavenging capacities of food extracts [Yu *et al.,* 2002]. In this study, DPPH radical scavenging activity test was applied using 80% methanol extracts. Within this assay, the coloured stable DPPH radicals are reduced in the presence of an antioxidants or hydrogen donors to non-radical DPPH-H molecules. The scavenging activity of DPPH radicals enables the evaluation of the hydrogen-donating potency of phenolic compounds as well as of the newly formed ones due to the Maillard reaction progress [Liyana-Pathirana *et al.,* 2006]. The DPPH radical scavenging activity (DPPH RSA) provided for the whole breads and

TABLE 4. DPPH Radical Scavenging Activity (DPPH RSA) of spelt, wheat and rye breads and their separated parts (μmol Trolox/g d.m.). The results were based on 80% methanol extracts.

Type of bread	Whole bread	Crust	Crumb
Spelt bread STH 915	2.49 ± 0.05^{a}	2.43 ± 0.02^{a}	2.50 ± 0.06^{a}
Spelt bread STH 975	2.68 ± 0.01^{b}	3.12 ± 0.16^{b}	2.43 ± 0.01^{a}
Spelt bread STH 974	2.31 ± 0.08^{c}	2.30 ± 0.07^{a}	2.26 ± 0.03^{b}
Wheat bread	2.12 ± 0.01^{d}	2.41 ± 0.05^{a}	1.69 ± 0.03^{c}
Rye bread	3.06 ± 0.05^{e}	4.20 ± 0.05^{c}	3.12 ± 0.02^{d}

Data expressed as mean ± standard deviation (n = 3). Within each column related to whole bread, crust and crumb data, means with the same letter are not significantly different (p≤0.05).

their separated parts is compiled in Table 4. The DPPH RSA of the analysed whole breads ranges from 2.12 to 3.06 μmol Trolox/g d.m. The DPPH RSA of spelt breads formulated on white flour originated from STH 915, STH 975 and STH 974 strains showed higher values by 14.8%, 20.9% and 0.82% when compared to the wheat bread. The significantly highest ability to scavenge DPPH radicals was found in relation to rye bread whilst the lowest to wheat bread. The order of increasing RSA DPPH of the whole breads and their crumb and crust was as follows: wheat bread < spelt breads < rye bread. In this study, the ability of 80% methanol extracts to scavenge DPPH and $ABTS^{\bullet+}$ radicals was comparable. It was important to find out the same level of DPPH RSA values in whole breads and separated crumbs. Moreover, DPPH RSA of whole breads and crusts was at a comparable level, with one exception made to STH 975, wheat and rye breads, where an increase by 16%, 4% and 37% was found, respectively. This finding indicates that, due to the baking process, the newly formed compound had no ability to scavenge DPPH radicals. The TEAC and RSA DPPH values based on the examined 80% methanol extracts from whole breads and their separated crumbs were poorly correlated (r=0.63 and r=0.30), but a higher correlation was noted in relation to the separated breads (r=0.88).

SOD-LIKE ACTIVITY

The free radical scavenging activity against superoxide anion radicals ($O_2^{-\bullet}$) was measured in PBS extracts of spelt, wheat and breads and their separated crumbs and crusts (Table 5). The highest SOD-like activity was noted for whole spelt bread STH 915 and its crumb and crust when compared to all analysed respective bread samples. The whole bread STH 915, its crumb and crust showed almost twice higher SOD-like activity in comparison with wheat bread and its separated parts, and higher by 69%, 31% and 0.8% when compared to the respective rye samples. The SOD-like activity of bread crusts was slightly higher than the values noted for whole breads, whereas those related to crumbs were lower with one exception made to rye crumb, for which no difference was found. This finding indicates that the antioxidant properties of spelt bread as well as other breads and their parts strongly depend on the type of radical being scavenged within the assay used. The results suggest that new compounds with ability to scavenge the superoxide anion radicals were formed in bread crust during baking. Moreover, these PBS-soluble compounds were able to scavenge $ABTS^{\bullet+}$ radicals since a positive correlation was found between TEAC and SOD-like activity values (r=0.73). In contrast, no correlation was noted between the whole bread and crumb TEAC and SOD-like activity (r=-0.04 and r=-0.21, respectively).

TABLE 5. Superoxide dismutase-like activity (SOD-like activity) of spelt, wheat and rye breads and their separated parts (U/g dm). The results were based on PBS extracts.

Type of bread	Whole bread	Crust	Crumb
Spelt bread STH 915	3.52 ± 0.63^{a}	3.77 ± 0.16^{a}	2.75 ± 0.05^{a}
Spelt bread STH 975	2.57 ± 0.13^{b}	3.36 ± 0.08^{ab}	2.09 ± 0.04^{b}
Spelt bread STH 974	2.54 ± 0.39^{b}	2.78 ± 0.47^{cb}	2.16 ± 0.14^{b}
Wheat bread	1.92 ± 0.19^{b}	2.14 ± 0.40^{c}	1.40 ± 0.12^{c}
Rye bread	2.08 ± 0.05^{b}	3.74 ± 0.11^{a}	2.10 ± 0.25^{b}

Data expressed as mean ± standard deviation (n = 3). Within each column related to whole bread, crust and crumb data, means with the same letter are not significantly different (p≤0.05).

FCR reducing capacity

From evaluation of data presented at the First International Congress on Antioxidants Methods in 2004 as well as from consideration of potential end uses of antioxidants, it was proposed that, among procedures and applications for different assays, the Folin-Ciocalteu method and possibly the Trolox equivalent antioxidant capacity (TEAC) be considered for standardization [Prior *et al.,* 2005]. In this study, the FCR reducing capacity by means of the Folin-Ciocalteu reagent application was taken for the evaluation of antioxidant properties of spelt, wheat and rye breads and their separated crumbs and crusts [Huang *et al.,* 2005]. The reducing capacity of whole breads and their separated crumb and crust based on PBS and 80% methanol extracts is shown in Figures 2 and 3, respectively. The reducing capacity of whole spelt bread PBS extracts ranged from 0.47±0.01 to 0.87±0.02 (mg FA equiv./g d.m.). The lowest value was noted for spelt bread STH 974, whereas no statistical difference was found in relation to spelt breads STH 915 and STH 975. The latter showed reducing capacity being comparable to that of wheat bread but significantly lower in comparison with rye breads. The FCR reducing capacity of crumbs, evaluated on PBS extracts, had lower reducing capacity whilst the crusts showed significantly higher capacity when compared to that of the whole bread PBS extracts. A similar trend in the case of reducing capacity and its distribution in relation to crumbs and crusts was found in 80% methanol extracts (Figure 3). The provided results indicate that phosphate buffered saline (PBS) and a mixture of methanol/water (4:1, v/v). were both applicable for extraction of bread sample due to determination of the FCR reducing power. Moreover, the white flours from spelt and rye used for bread-making yielded a better profile of antioxidants and baking process was more prone to the formation of Maillard reaction products, which could influence the enhancement of FRC values as it was shown in Figures 2 and 3, respectively. It was supported by the high FCR reducing capacity values noted for crust originated from all tested breads, irrespective of the extraction system used.

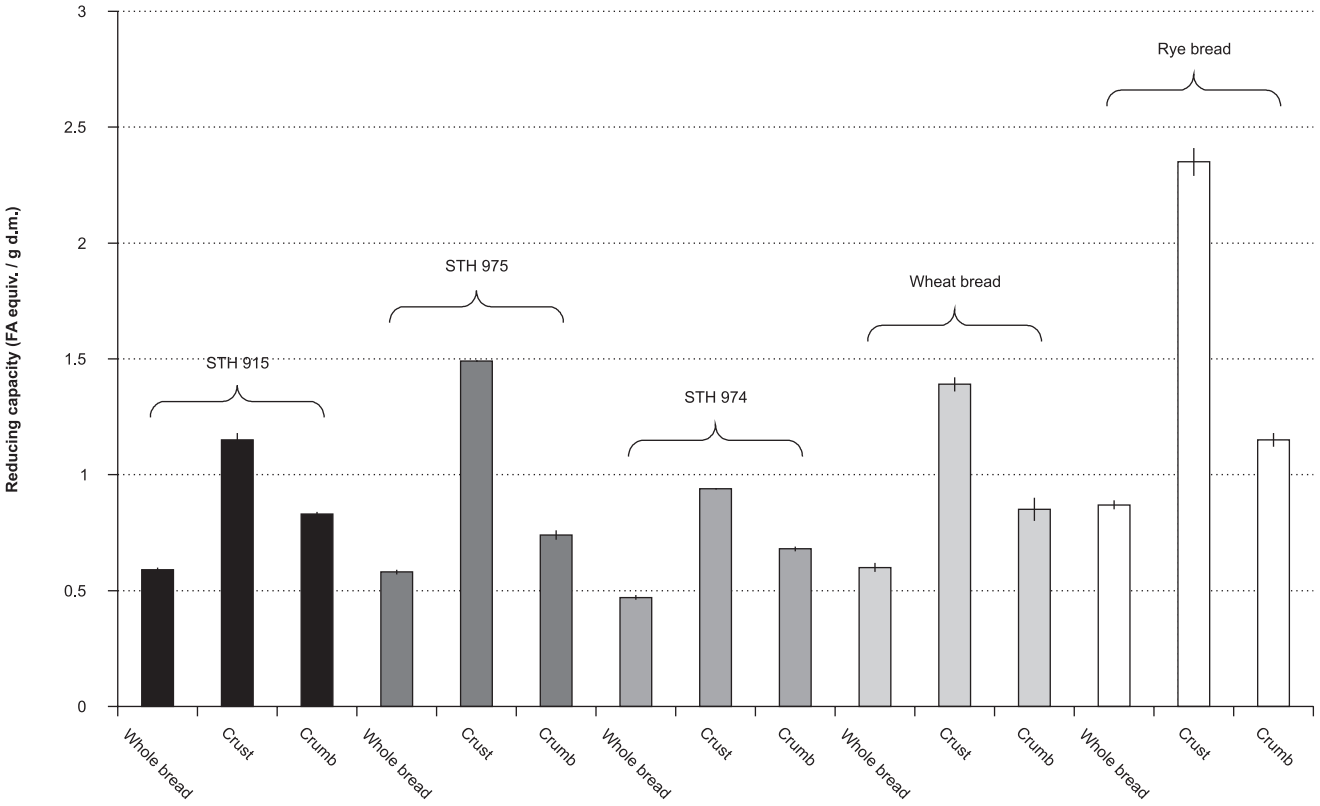

FIGURE 2. FCR reducing capacity of spelt, wheat and rye breads and their separated parts (crumb and crust). Sample extraction was carried out with PBS.

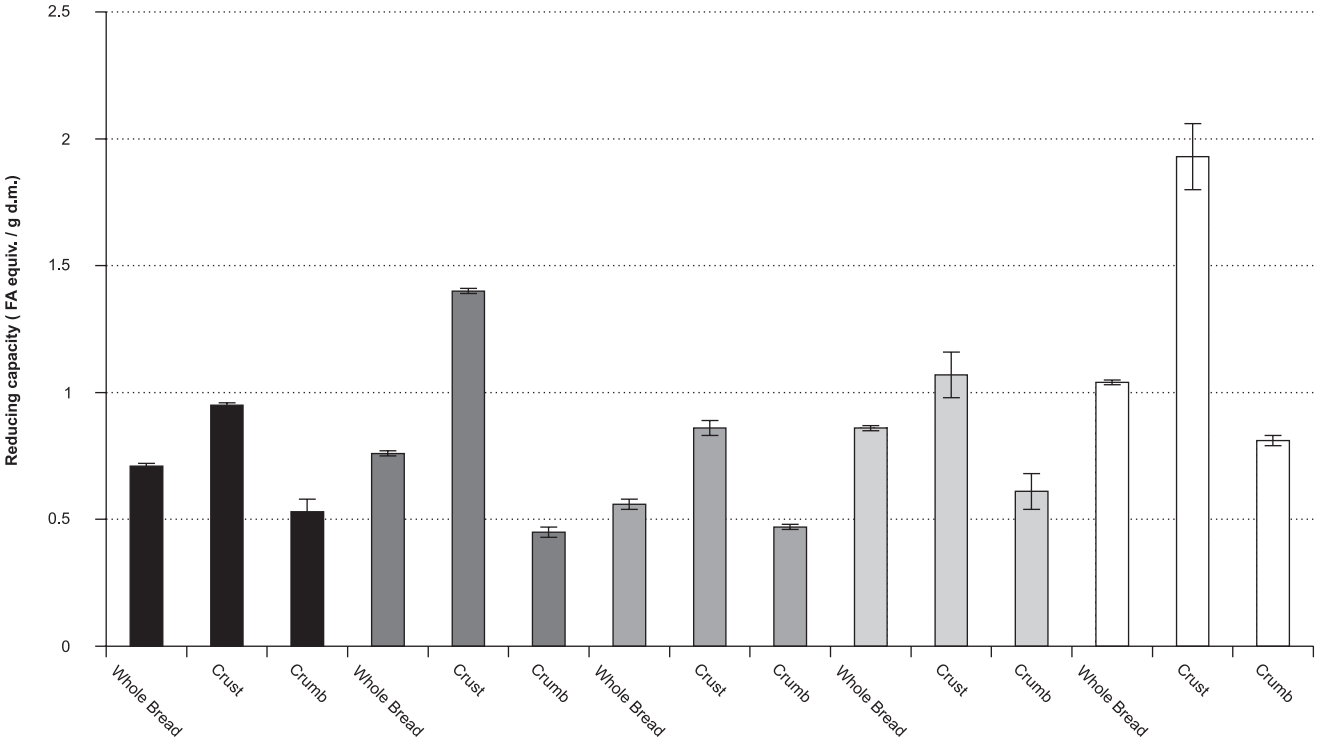

FIGURE 3. FCR reducing capacity of spelt, wheat and rye breads and their separated parts (crumb and crust). Sample extraction was carried out with 80% methanol.

CONCLUSIONS

The study is the first to provide the antioxidant capacity of spelt bread as well as its crumb and crust and compare it to those obtained for wheat and rye respective samples. Spectrophotometric methods based on free radical scavenging activities (TEAC, RSA DPPH, SOD-like activity) and reducing power (FCR) turned out to be applicable for the evaluation of the antioxidant capacity of spelt breads. The studies showed that spelt breads formulated on white flours displayed similar antioxidant activity comparable to that of wheat bread however the spelt bread crusts were better source of antioxidant compounds then wheat bread crust. The highest antioxidant capacity and reducing power were provided by rye bread and its separated crumb and crust.

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