JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Contents and Antioxidative Properties of Traditional Rye Breads

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The purpose of this research was to find out the effect of flour extraction rate on the antioxidative properties of traditional rye bread and then to compare the bioactive compounds content and antioxidant properties of rye breads with commercial wheat roll. Four types of rye flour with different extraction rates of 100 (whole meal dark flour), 95 (brown flour), 90 (brown flour), and 70% (light flour) originated from Warko rye cultivar were used for traditional bread baking with sourdough fermentation. Four types of the respective rye breads were analyzed for their potentially beneficial components, including tocopherols and tocotrienols, total phenolics and flavonoids, reduced glutathione, and inositol hexaphosphates. Moreover, the phenolic acids profile was provided. The Trolox equivalent antioxidant capacity (TEAC) of the breads was evaluated using free radical scavenging activities of 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-picrylhydrazyl radical ($DPPH^{\bullet}$). The superoxide dismutase-like activity (SOD-like activity) was evaluated as free radical scavenging activities of PBS extracts against superoxide anion radicals ($O_2^{\bullet-}$). The results were compared to whole meal rye bread as well as to wheat roll taken as representative example of wheat based bakery product. The studies showed that flour extraction rates strongly affected the content of bioactive compounds and antioxidative properties of traditionally baked rye breads. The incorporation of the rye flours with extraction rates from 100 down to 70% in the formulation caused decrease in tocopherol (T), tocotrienol (T3), inositol hexaphosphate (IP6), and phenolic compound (TPC) contents in rye breads. No changes in reduced glutathione (GSH) contents were noted between each type of rye bread. A significant decrease in Trolox equivalent antioxidant capacity and radical DDPH scavenging activity was also found in bread formulated on flour with an extraction rate of 70% in comparison to the breads formulated on flour with extraction rates from 100 to 90%. The highest SOD-like activity was noted for rye bread formulated on flour with an extraction rate of 70%. The four types of rye breads showed better antioxidative properties and higher antioxidant contents when compared to wheat roll with one exception made to tocopherols and tocotrienols.

KEYWORDS: Rye flour extraction rates; rye breads; wheat roll; bioactive compounds; antioxidant capacity; SOD-like activity

INTRODUCTION

Rye (*Secale cereale* L.) is considered a primitive crop with low yield and long and weak straw. Although the total production of rye has diminished, its use as a food for humans has increased slightly over the 1990s. In 1995, the worldwide food consumption of rye accounted for about 8 million metric tons, which is about 35% of total production. The rest was used as feed (*1*).

The rye whole grain contains a large variety of substances, especially those that are biologically active and demonstrate antioxidative properties, which include free radical scavengers, reducing agents, potential complexers of prooxidant metals, and quenchers of the singlet oxygen formation (*2*). Among cereals, rye is the only one with a whole grain culture, and the consumption of rye should be increased in light of this benefit. Nutritionists worldwide recommend an increased consumption

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10.1021/jf062425w CCC: $37.00
Published on Web 01/17/2007

of whole grain products and dietary fiber (*3*). Now, consumers are increasingly interested in health and their knowledge on the relationship between diet and well-being has been raised. In this respect, rye is likely to gain interest and popularity (*4*). Rye is second to wheat, the most commonly used grain in the production of bread (*1*).

The principal nutritional benefit of processing is to increase the bioavailability of the nutrients present in the grain. Essentially, this is brought about by making the cereal grain a better substrate for digestive enzymes. This is achieved at both a physical and/or a chemical level. In the milling process, the grains may be fractionated into different types of flour. The proportion of the original rye that is ultimately converted to flour is referred to as the extraction rate. Typical values for light flours are between 72 and 80%, between 85 and 98% for brown flours, and 100% for wholemeal dark flour. Wholemeal flour is the most popular rye flour for baking; however, rye flour with an extraction rate of about 80% is also widely used. In addition to the traditional use of different types of rye flour, various types of rye flakes and breakfast cereals with rye contents up to 55% are also available (*5*).

Baking is the most common technique used in flour processing for human food. The baking technology of rye is different from that of wheat. The majority of the rye bread is prepared with sourdough fermentation method. Sourdough is classically made by mixing rye flour with water and allowing it to ferment. Bakeries usually have their own sourdough, which is maintained by a back-slopping procedure. The microorganisms (lactic acid bacteria) originate mainly from the flour. During the fermentation period, because of the enzymatic activity of the microflora, flavor compounds are formed. The main components formed are lactic acid and acetic acid. After fermentation, more flour, water, and other ingredients are mixed to the sourdough to make the dough. The dough is left to rise for a short period, after which the breads are shaped, left to rise again, and baked (*6*).

Rye bread is recommended as an integral part of the diet because it is a good source of biologically active substances, especially those demonstrating antioxidative properties. Moreover, the fermentation makes some additional benefits for consumers because this process increases the solubility of pentosans in bread, which is optimal at lower pH, reduces enzymatic activity, which improves dough processability, and enables the use of rye with low falling numbers (*7*). Moreover, nutritional value, despite the better bioaccesibility of minerals due to destruction of phytic acid, is probably enhanced by nonenzymatic browning reactions occurring during baking due to Maillard reactions. This wide range of products is responsible for the attractive brown color and flavor of bread. Recently, it has been shown that Maillard reaction products, depending on the reaction stage, have the antioxidant activity (*8*−*10*).

Today, after several decades of diminishing rye consumption, the increased knowledge on the relationship between diet and well-being has raised the consumers' interest toward rye-based products, including rye bread. However, little is known about the effect of flour extraction rate on the antioxidative properties of traditional rye bread. Therefore, the aim of the present research was to investigate the effect of flour extraction rate on the antioxidative properties of traditional rye bread as affected by the incorporation of flour with different extraction rates in the formulation. The second aim was to compare the bioactive compounds content and antioxidant properties of rye breads with commercial wheat roll.

Table 1. Rye Bread and Wheat Roll Dough Formulation and Baking Conditions

ingredient and conditions	type of rye bread				wheat roll
	I/100%	II/95%	III/90%	IV/70%	
sour (g)	800	800	800	800	
rye flour (g) (extraction: 100, 95, 90, and 70%)	600	600	600	600	
wheat flour type 650 (g)					980
water (g)	300	300	300	300	560
salt (g)	20	20	20	20	13
yeast (g)					15
fermentation: temperature (°C)	28	28	28	28	28
time (min)	30	30	30	30	120
pieces of dough (g)	350	350	350	350	50
proofing (75% RH) temperature (°C)	28	28	28	28	28
time (min)	90	81	78	30	30
baking: temperature (°C)	230	230	230	230	230
time (min)	35	35	35	35	18

MATERIALS AND METHODS

Reagents. Glutathione (γ-glutamyl-cysteinyl-glycine; GSH), inositol hexaphosphoric acid (dodecasodium salt) from corn, oxidized glutathione (GSSG), (±)-catechin, ferulic, caffeic, *p*-coumaric, sinapic, and vanillic acids, bovine serum albumin (fraction V; BSA), 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 (St. Louis, MO). Standards of tocopherol (α-T, β-T, γ-T, and δ-T) and tocotrienol (α-T3, β-T3, and γ-T3) were obtained from Merck (Darmstadt, Germany). The superoxide dismutase kit (Ransod, Catalog no. SD 125) originated from Randox Laboratories Ltd. (Crumlin, United Kingdom). Dowex AG 1-X8 resin was from Bio-Rad Laboratories, Inc. (Hercules, CA). Folin−Ciocalteu's reagent and other reagents of reagent-grade quality were from POCh (Gliwice, Poland).

Characterization of Rye Flours and Breads. Rye grains of the cultivar Warko were selected from breeding materials grown in central Poland (Danko, Plant Breeding Co., Laski) in 2004, tempered to 14.0% moisture, and milled on a Quadrumat Senior laboratory mill (Brabender) to obtain a straight grade flour with extraction rates of 100, 95, 90, and 70%. Samples from three replications were stored at 4 °C until analysis. Flours and breads were characterized with the following analyses: moisture, ash, protein, and starch contents. The protein content was measured following AACC method 46-11B using Foss Tecator apparatus whereas starch content was determined by the polarymetric method (*11*). Moisture and ash contents of flours were analyzed according to AOAC 15.950.01 and 15.955.03, respectively (*12*). All analyses were made in triplicate.

Rye Breads and Wheat Rolls Baking Methods. Rye breads were baked using traditional fermentation generated by lactic acid bacteria without baker's yeast addition. The three-stage method was used to make dough. Sourdough starters, as the first stage, were prepared by mixing 36% of each type of flour and 64% of water. These mixtures were left to ferment for 48 h at 28 °C. The second-stage sourdough was prepared by mixing 300 g of the respective sourdough starter, 300 g of the same flour as used for starter, and 300 mL of water. The mixture was left to ferment for 3 h at 28 °C. The dough was prepared as shown in **Table 1**. At least four loaves of each type of bread were baked in duplicate. For comparison, commercial wheat roll based on the wheat flour with an extraction rate of approximately 70% was provided by a local bakery in Olsztyn (Poland). The wheat rolls were baked using a single-phase method as shown in **Table 1**. The breads and wheat roll were sliced (1 cm thick) and dried in an electric convection oven (40 °C) for 24 h. The dried material was ground and sieved though a 60-mesh screen to obtain powdered bread and powdered wheat roll.

Preparation of Bread Crude Extracts. Powdered bread and wheat roll were extracted in triplicate with phosphate-buffered saline (PBS),

pH 7.4 (1 g per 15 mL), or with 80% aqueous methanol (1 g per 10 mL) for 2 h of shaking at 37 °C. Samples were then centrifuged at 12000*g* for 15 min in a Beckman GS-15 R centrifuge (Beckman Instruments, Fullerton, CA). The fresh PBS extracts were used to determine their ability to scavenge superoxide anion radicals, whereas 80% methanolic extracts were used to determine their ability to scavenge $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals and total phenolic compounds (TPCs) and total flavonoids (TFs).

Determination of Total Phenolic Content and Phenolics Profile. The content of TPCs was determined in 80% methanolic extracts according to Shahidi and Naczk (*13*). Exactly 0.25 mL of the respective extract was mixed with 0.25 mL of Folin−Ciocalteu's reagent (previously diluted with water 1:1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution, and 4 mL of water. The mixture was allowed to stand at room temperature for 25 min and centrifuged at 2000*g* for 10 min. The supernatant absorbance was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Kyoto, Japan). The data were calculated on ferulic acid equivalents. For phenolics profile, extraction was performed for 2 h at 37 °C using 80% aqueous methanol (1/10; w/v), and then, the samples were centrifuged at 2600*g* at 4 °C for 15 min. Following the evaporation of methanol in a rotary evaporator at 45 °C, the remaining water solutions were lyophilized. Separation of phenolic acids was carried out according to Amarowicz and Weidner (*14*). An aqueous suspension of extract (5 mL) was mixed well with 5 mL of 4 M NaOH and hydrolyzed for 4 h at room temperature under a nitrogen atmosphere. After acidification to pH 2 with 6 M HCl, free phenolic acids and those liberated from esters were extracted five times into 15 mL of diethyl ether using a separatory funnel. Then, ether was evaporated; the dry residue was dissolved in 2 mL of methanol and filtered trough a 0.45 μm filter. The samples obtained in this way were injected onto a high-performance liquid chromatography (HPLC) column. Phenolic acids were analyzed using a Shimadzu HPLC system (Shimadzu Corp.) consisting of an LC-10AD pump, SCTL 10A system controller, and SPD-M 10A photodiode array detector. Phenolic acid separation was carried out by using a prepacked LiChrospher 100 RP-18 column (4 mm × 250 mm, 5 μm; Merck). The mobile phase water−acetonitrile−acetic acid (88:10:2; v/v/v) was delivered at a rate of 1 mL/min (*14*). The detection was monitored at 260 and 320 nm.

Determination of Total Flavonoid Content. The TF content was determined with a colorimetric method (*15*). Briefly, 0.25 mL of 80% methanolic extract was diluted with 1.25 mL of distilled water. Then, 75 μL of a 5% $NaNO_2$ solution was added, and the mixture was allowed to stay at room temperature. After 6 min, 150 μL of a 10% $AlCl_3 \times 6H_2O$ solution was added, and the mixture was allowed to stand for another 5 min. After that, 0.5 mL of 1 M NaOH was added. The solution was well-mixed, and the absorbance was measured immediately against blank at 510 nm using a spectrophotometer (UV-160 1PC, Shimadzu) in comparison with the standards prepared similarly with known (±)-catechin concentrations. Then, the results were expressed as mg of catechin equivalents.

Determination of Tocopherols and Tocotrienols Content. Tocopherols (α-T, β-T, γ-T, and δ-T) and tocotrienols (α-T3, β-T3, and γ-T3) were extracted with methanol (0.5 g of sample/7 mL). After evaporation, extracts were redissolved in *n*-hexane and tocols were separated by HPLC on Lichrospher Si 60 5-μm particle size, 4 mm × 250 mm column, according to the method described by Paterson and Qureshi (*16*). The HPLC system consisted of a Shimadzu model LC pump series 10 AD and a Shimadzu RF-535 fluorescence spectrometer (Shimadzu). The mobile phase was 0.5% isopropanol in hexane pumped at a flow rate of 1 mL/min, and the compounds were detected at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. The contents of tocols were calculated from the peak areas using standard curves of tocopherols (α-T, β-T, γ-T, and δ-T) and tocotrienols (α-T3, β-T3, and γ-T3).

Determination of Inositol Hexaphosphate Content. Inositol hexaphosphate was determined as follows: Exactly 0.5 g of the respective types of bread powders was extracted with 20 mL of 0.5 M HCl for 5 h using a BM1 magnetic stirrer. The extract was centrifuged at 3500*g* for 40 min (Centrifuge MPW-360, Factory of Precise Mechanics, Warsaw, Poland), and the supernatants were decanted, frozen overnight (−18 °C), thawed at room temperature, and recentrifuged at 3500*g* for 40 min. The supernatants (15 mL) were evaporated under reduced pressure to dryness at 40 °C and dissolved in 15 mL of 0.025 M HCl. The samples were then transferred to the minicolumns filled with Dowex AG 1-X8 resin, from which the inositol phosphates were eluted using 2 M HCl (5 × 4 mL). After the solvent had been removed by evaporation with the stream of air, the dry residue was dissolved in a mobile phase. Then, the samples were analyzed with HPLC method (*17*, *18*) using a Shimadzu chromatograph (LC-10 AD pump, refractometric detector RID-6A, CTO 6A column oven) and a Nova-Pak C_{18} column (Waters Corp., Milford, MA). The mobile phase was a mixture of methanol and 0.05 M formic acid (51:49, v/v) and 1.5 mL/100 mL tetrabutylammonium hydroxide pumped at a flow rate of 0.4 mL/min. Inositol hexaphosphoric acid (dodecasodium salt) from corn was the external standard, and injections were made with a 20 μL loop.

Determination of Reduced Glutathione Content. Reduced glutathione was extracted from the samples according to Smith et al. (*19*) and determined according to the spectrofluorometric method (*20*). The detailed procedure was described previously (*21*). The assays were performed using a Perkin-Elmer LS 50 B Luminescence Spectrometer (Perkin-Elmer Corp., Norwalk, CT).

Total Antioxidant Capacity by Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The TEAC assay was based on the reduction of the $ABTS^{\bullet+}$ radical cation by antioxidants present in 80% methanolic extracts. The $ABTS^{\bullet+}$ radical cation was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture had to remain for 12−24 h until the reaction was complete and the absorbance was stable. TEAC was determined following the procedure described by Re et al. (*22*) with a minor modification. For measurements, the $ABTS^{\bullet+}$ solution was diluted with 80% methanol to the absorbance of 0.700 ± 0.020 at 734 nm. For the photometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 μL of the extracts or Trolox standards were mixed and measured immediately at 30 °C after 6 min at 734 nm using a spectrophotometer (UV-160 1PC, Shimadzu). Appropriate solvent blanks were run in each assay. The TEAC of 80% methanolic extracts was calculated on the basis of percentage inhibition of absorbance at 734 nm using a Trolox standard curve.

DPPH Radical Scavenging Assay. The radical DPPH scavenging activity (RSA) assay was based on a modified method of Brand-Williams et al. (*23*). In this assay, antioxidants present in the sample reduced the $DPPH^{\bullet}$ radicals, which had an absorption maximum at 515 nm. The $DPPH^{\bullet}$ radical solution was prepared by dissolving 10 mg of DPPH in 25 mL of 80% methanol. First, the extinction of the disposable cuvette with 250 μL of the methanolic $DPPH^{\bullet}$ solution and 2.1 mL 80% methanol was measured as blank. Then, the cereal 80% methanol extracts (100 μL) were added to 250 μL of the methanolic $DPPH^{\bullet}$ solution and 2 mL of 80% methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 20 min. The decrease in absorbance of the resulting solution was monitored at 517 nm for 20 min using a spectrophotometer (UV-160 1PC, Shimadzu). The Trolox standard solution (concentration, 0.1−2.0 mM) in 80% methanol was prepared and assayed at the same conditions. $DPPH^{\bullet}$ scavenging activity was expressed in terms of Trolox equivalents, on the basis of percentage inhibition of absorbance at 515 nm of standards and samples.

Superoxide Dismutase (SOD)-like Activity Assay. The SOD-like activity of the PBS extracts was measured according to SOD Ransod kit. The assays were performed using a thermostated recording spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu) adjusted to 37 °C inside the chamber. The test required 50 μL of sample, with a read time of 3 min. The results were finally calculated on miligrams of soluble protein assayed according to the bicinchonic acid (BCA) protein microassay (*24*). The SOD with the activity of 5.3 U/mL was used as a standard and was a part of the reagent kit. In general, one unit of the SOD activity was defined as the amount of enzyme required to inhibit the rate of reduced adenine nucleotides (NADH, NADPH) oxidation of the control by 50%. The percent of the reaction inhibition of the sample was plotted against log of different SOD activities (SOD/mL) giving a standard curve, and then, the SOD-like activity of the sample was calculated as SOD units/mL of the

Table 2. Proximate Chemical Composition of Flours with Different Extraction Rates Originated from Warko (W) Rye Variety[a]

rye variety/ flour extraction rate	%			
	dry matter	protein content	ash content	starch content
W/100%	88.5 ± 0.04 a	11.02 ± 0.02 a	1.8 ± 0.01 a	53.3 ± 0.28 a
W/95%	88.3 ± 0.02 a	10.55 ± 0.09 be	1.7 ± 0.01 b	54.0 ± 0.14 bc
W/90%	88.3 ± 0.02 a	10.44 ± 0.02 ce	1.6 ± 0.06 c	54.2 ± 0.28 ce
W/70%	88.0 ± 0.01 b	7.93 ± 0.12 d	0.7 ± 0.02 d	56.6 ± 0.14 d

[a] Data are expressed as means ± standard deviation ($n = 3$). Within each column for each type of rye flour, means with the same letter are not significantly different ($P \leq 0.05$).

Table 3. Proximate Chemical Composition of Rye Breads Baked with Flours of Different Extraction Rates[a]

rye bread/ flour extraction rate	%			
	moisture content	protein content	ash content	carbohydrates content
type I/100%	27.5 ± 0.04 a	10.32 ± 0.01 a	2.22 ± 0.04 a	45.54 ± 0.18 a
type II/95%	26.7 ± 0.02 b	10.47 ± 0.02 a	2.31 ± 0.08 a	49.35 ± 0.13 b
type III/90%	25.2 ± 0.02 c	10.20 ± 0.01 a	2.15 ± 0.14 a	50.86 ± 0.28 b
type IV/70%	30.4 ± 0.01 d	8.50 ± 0.01 b	1.33 ± 0.01 b	50.14 ± 0.04 b

[a] Data are expressed as means ± standard deviation ($n = 3$). Within each column for the each type of rye bread, means with the same letter are not significantly different ($P \leq 0.05$).

investigated extract. The results were finally calculated on 100 g of dry matter basis of the respective breads and wheat roll.

Statistical Analysis Method. Data were subjected to one-way analysis of variance) using the Fischer LSD test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, MD) for Windows using a Pentium PC.

RESULTS AND DISCUSSION

Characterization of Rye Flours and Breads. The proximate chemical composition of rye flour with extraction rates of 100, 95, 90, and 70% are compiled in **Table 2**. The effect of flour extraction rate was found with respect to the protein, starch, and ash contents. The light flour with an extraction rate of 70% contained the highest amount of starch when compared to the wholemeal flour and two kinds of brown flours. In contrast, with the decreasing percent of extraction rate from 100 down to 70%, a decrease in protein and ash content was noted. The proximate chemical composition of rye breads baked with flours of different extraction rates is shown in **Table 3**. The effect of flour extraction rate was found with respect to the protein and ash contents. The breads formulated on dark and brown flours contained more protein and ash when compared to bread type IV formulated on light flour with an extraction rate of 70%.

Total Phenolics, TF Content, and Phenolics Profile. The content of TPCs in rye breads, depending on the flour extraction rates, ranged from 1.72 mg/g d.m. for bread type I to 1.29 mg/g d.m. for bread type IV. The pool of TFs was only 11.2 to 7.6% of TPC present in breads (**Table 4**). The breads formulated on rye flours with extraction rates from 100 to 90% showed the highest content of TPC when compared to the bread formulated on flour at an extraction rate of 70%. Moreover, the content of TPC as well as TF was about 2−3-fold higher when compared to wheat roll. Breads baked with flour extraction rates from 100 to 90% were a better source of phenolic compounds as well as flavonoids than the ones baked with light flour. Because phenolic compounds have drawn attention because of their antioxidative, antiinflammatory, anticarcinogenic, and antimutagenic properties, more and more products have been analyzed. Cereals contain a wide range of phenolic compounds, and what is more important, their high consumption may play a beneficial role in human health (*24*).

Table 4. Content of Total Phenolic Compounds (TPC) and Total Flavonoids (TF) in Rye Breads and Wheat Roll[a]

bread	TPC[b] (mg/g d.m.)	TF[c] (μg/g d.m.)
type I/100%	1.72 ± 0.03 a	171.40 ± 3.29 a
type II/95%	1.68 ± 0.04 a	196.98 ± 3.29 b
type III/90%	1.76 ± 0.04 a	177.21 ± 4.93 a
type IV/70%	1.29 ± 0.01 b	98.14 ± 1.64 c
wheat roll	0.86 ± 0.01 c	44.65 ± 1.64 d

[a] Data are expressed as means ± standard deviation ($n = 3$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$). [b] The data were calculated as mg of ferulic acid equivalents. [c] The data were calculated as μg of (±)-catechin equivalents.

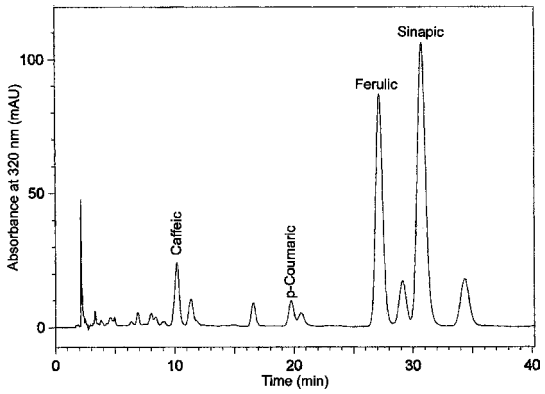

Figure 1. Typical HPLC chromatogram of phenolic acids recorded at 320 nm. The extract was prepared from the bread type III based on brown rye flour with an extraction rate of 90%.

Table 5. Phenolic Acid Contents (as Free and Those Liberated from Esters) in Rye and Wheat Bakery Products (μg/g d.m.)[a]

phenolic acid	type I/ 100%	type II/ 95%	type III/ 90%	type IV/ 70%	wheat roll
caffeic	1.6 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	0.8 ± 0.1
p-coumaric	1.8 ± 0.2	2.3 ± 0.3	1.8 ± 0.2	1.9 ± 0.2	0.8 ± 0.1
ferulic	18.5 ± 1.5	24.8 ± 2.0	20.0 ± 1.6	10.3 ± 0.8	4.9 ± 0.4
sinapic	33.3 ± 3.3	31.3 ± 3.0	29.1 ± 2.9	18.9 ± 1.9	5.1 ± 0.5
vanillic	ND	ND	ND	ND	3.3 ± 0.3
total	55.2	60.2	52.8	32.8	14.9

[a] Data are expressed as means ± standard deviation ($n = 3$); ND, not detected.

Phenolics Profile of Rye Breads and Wheat Roll. A typical HPLC chromatogram of phenolic acids recorded at 320 nm is shown in **Figure 1**; however, vanillic acid was analyzed at a wavelength of 260 nm. In rye breads and wheat roll, almost the same profile of phenolic acids was provided. According to the retention times, caffeic, ferulic, *p*-coumaric, and sinapic acids were found in respective rye breads whereas additionally vanillic acid was found in wheat roll. Ferulic and sinapic acids were predominant among analyzed acids in this material. The rye flour extraction rate and technological process as baking affected the content of phenolic acids in products (**Table 5**). The highest content and the most beneficial profile of phenolics acids were noted in rye breads formulated on flour with an extraction rate of 95%. Rye bread based on light flour, similarly to wheat roll, showed the lowest contents of phenolics acids. In general, rye

Table 6. Content of Tocopherols and Tocotrienols in Rye and Wheat Bakery Products (μg/g d.m.)[a]

	tocopherols (T)					tocotrienol (T3)			
bread	α	β	γ	δ	total	α	β	γ	total
type I/100%	2.51 ± 0.08	0.61 ± 0.05	0.30 ± 0.01		3.42 ± 0.06 a	1.85 ± 0.08	1.53 ± 0.14		3.38 ± 0.18 a
type II/95%	0.61 ± 0.03	0.17 ± 0.01	0.23 ± 0.03		1.01 ± 0.06 b	0.41 ± 0.04	0.65 ± 0.04		1.06 ± 0.08 b
type III/90%	0.76 ± 0.03	0.14 ± 0.01	0.29 ± 0.02		1.19 ± 0.04 b	0.35 ± 0.02	0.52 ± 0.02		0.87 ± 0.03 b
type IV/70%	0.76 ± 0.06	0.14 ± 0.03	0.37 ± 0.09		1.27 ± 0.08 b	0.14 ± 0.01	0.24 ± 0.02		0.38 ± 0.02 c
wheat roll	1.74 ± 0.07	0.28 ± 0.01	0.96 ± 0.03	0.04 ± 0.01	3.02 ± 0.09 c	0.53 ± 0.03	2.72 ± 1.02		3.25 ± 2.02 a

[a] Data are expressed as means ± standard deviation ($n = 3$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$).

breads were a better source of phenolics when compared to wheat bakery products such as wheat roll. It can be concluded that the profile of phenolics can be modulated by the type and quality of flours as well as by dough preparation and parameters of baking process.

Phenolic acids are mainly localized in the outer part of cereal grains (*4*). For example, ferulic acid in rye grain is the most abundant phenolic compound present in bran. For this reason, consumption of whole meal rye products increases the intake of beneficial ferulic acid. Boscov-Hansen et al. (*26*) showed no significant changes in the content of phenolic compounds during rye bread baking. In contrast, in some cases such as sourdough preparation, the level of total phenolic compounds may increase. It resulted from the increased acidity; however, it was strongly dependent on the type of the sourdough process (*27*).

Tocopherols and Tocotrienols Content. The content of tocopherols and tocotrienols is compiled in **Table 6**. In this study, the content of tocopherols (α-T, β-T, γ-T, and δ-T) in rye breads, depending on the flour extraction rate, ranged from 3.42 to 1.19 μg/g d.m. The main tocopherol in rye breads and wheat roll was α-T; however, the latest contained a small quantity of δ-T. The tocotrienols content noted in rye bread ranged from 3.38 to 0.38 μg/g d.m. The main rye bread tocotrienol's fraction was formed by α-T3 and β-T3. The highest level of tocopherols and tocotrienols was found in bread with a flour extraction rate of 100%. This level was comparable with the data obtained for wheat roll in which the content of tocopherols and tocotrienols was 3.02 μg/g d.m., and 3.25 μg/100 g d.m., respectively. The bread typed II, III, and IV showed lower contents of these compounds. The data showed that the content of tocopherols and tocotrienols strongly depended on the refining process, in which the outer layer of grain is usually removed (*28*). That is why the food industry is obliged to add vitamin E to bakery products since bakeries prefer rather light flours for bread making. Moreover, during the baking process, the loss of these bioactive compounds was noted as their content in flours with respective extraction rate was approximately 3-fold higher (data not shown). This similar loss of tocols was observed by Liukkonen et al. (*29*). This effect could result from the reduction of tocopherols and tocotrienols during sourdough fermentation with air contact (*27*). Food rich in tocopherols and tocotrienols may have positive effects on human health and well-being due to the fact that these bioactive compounds can protect polyunsaturated fatty acids against oxidative damage in cell membranes. This food also may have anticancerogenic properties because of tocol's ability to destroy nitrite—a component in the food chain associated with the production of some type of cancers (*4*). Tocopherols and tocotrienols are probably involved in antitumor processes in tissue (*30*). Moreover, the content of α-T in the diet decreases the risk of cardiovascular diseases (*31*). For these reasons, consumption instead of supplementation of products rich in tocopherols and tocotrienols should be recommended.

Table 7. Content of Inositol Hexaphosphate (IP6) and Reduced Glutathione (GSH) in Rye Breads and Wheat Roll[a]

bread	IP6 (μmol/g d.m.)	GSH (nmol/g d.m.)
type I/100%	1.26 ± 0.01 a	174.41 ± 10.58 a
type II/95%	0.40 ± 0.01 b	179.02 ± 12.36 a
type III/90%	0.44 ± 0.02 b	173.55 ± 8.18 a
type IV/70%	0.57 ± 0.01 c	178.85 ± 16.82 a
wheat roll	0.39 ± 0.02 b	109.08 ± 6.30 b

[a] Data are expressed as means ± standard deviation ($n = 3$). Within each column, means with the same letter are not significantly different ($P \leq 0.05$).

Inositol Hexaphosphate and Reduced Glutathione Content. Wholemeal cereal products are an important source of minerals such as K, P, Mg, and Zn, but unfortunately, their bioavailability is limited by phytic acid. On the other hand, phytic acid can play a significant role in prevention from carcinogenesis and cardiovascular diseases. Rye and wheat contain phytic acid, which is mostly located in the outer layer of the kernel as the magnesium−potassium salt (*27*). This corresponds with our data, which showed that the highest level of inositol hexaphosphate (IP6) (1.26 μmol/g d.m.) in bread was formulated on flour with an extraction rate of 100% (**Table 7**). The bread types II, III, and IV formulated on flours with extraction rates from 95 down to 70% had lower IP6 contents, which were comparable to the IP6 level in wheat roll (0.39 μmol/g d.m.). The similar relation between type of flour and IP6 content in bread was observed by Garcia-Estepa et al. (*32*). The presented results were confirmed by firm evidence showing the losses of phytic acid during bread preparation (*27*). It was also indicated that this reduction was connected with the action of phytase, which resulted in a decrease of IP6 from approximately 13 to 100% (*27*). It was also found that the highest amount of IP6 in breads baked also without yeast, and it was suggested that the loss of phytic acid during bread making is strongly dependent on both phytase presence in flour and baking temperature. According to Plaami (*33*), the activity of phytase and its heat resistance in rye products is much more superior when compared to wheat products, as was shown in our experiment.

The content of reduced glutathione (GSH) in rye bread type I based on wholemeal flour was 174 nmol/g d.m. while the amount of GSH found in wheat roll was only 109 nmol/g d.m. (**Table 7**). It was noted that the flour extraction rate has no impact on the content of this compound in the four types of bread analyzed in this study. Even though the level of glutathione in flours is low, it still plays a significant role in redox reactions occurring in flour as well as in bread making (*34*). It was also suggested that the content of glutathione in flour was related to rheological properties of dough. Nevertheless, no clear relation between content of reduced glutathione and baking performance was observed (*35*). The results obtained in this study indicate a higher resistance of rye bread against oxidative damages when compared to wheat roll. Protective effects of

Table 8. Values of TEAC, RSA, and SOD-like Activity of the Four Types of Rye Bread Baked with Rye Flours of Different Extractions Rates in Comparison to Wheat Roll[a]

	TEAC (mmol Trolox/100 g d.m.)	RSA (mmol Trolox/100 g d.m.)	SOD (U/100 g)
bread type I	0.881 ± 0.009 a	0.485 ± 0.008 a	306 ± 6.3 a
bread type II	0.851 ± 0.017 b	0.488 ± 0.004 a	340 ± 10.7 b
bread type III	0.809 ± 0.033 c	0.486 ± 0.001 a	304 ± 3.8 a
bread type IV	0.507 ± 0.016 d	0.371 ± 0.009 b	389 ± 13.9 c
wheat roll	0.277 ± 0.004 e	0.212 ± 0.001 c	191 ± 18.5 d

[a] Data are expressed as means ± standard deviation ($n = 3$). Within each column for the each bakery product, means with the same letter are not significantly different ($P \leq 0.05$).

GSH have been widely known, and they include protection against oxidative destruction in systems in which scavenging of free radicals, elimination of lipids peroxidation products, preservation of thiol-disulfide status of proteins, and repair processes occur (*36*). Moreover, the consumption of foods high in glutathione may be associated with about a 50% reduction in the risk of oral and pharyngeal cancer (*37*).

Total Antioxidant Capacity. The 80% methanol extracts of the four types of rye bread and wheat roll were examined for their free radical scavenging activity against $ABTS^{\bullet+}$ cation radical. The results were compared to the free radical scavenging activity of Trolox. The obtained data expressed as TEAC are presented in **Table 8**. Rye breads formulated on flours with extraction rates from 100 to 90% had the highest scavenging activity. In this case, the calculated TEAC values were at similar levels (0.88 to 0.81 mmol Trolox/100 g d.m.). These results were almost 3-fold higher when compared to TEAC of wheat roll. It was also found that TEAC of rye bread formulated on flour with an extraction rate of 70% was lower by approximately 40% when compared to TEAC of whole meal rye bread. Because of the fact that milling processes caused a decrease in the amount of antioxidants and bioactive compounds in flours (*38*), the TEAC values of rye breads partly depend on the flour extraction rate.

DPPH Scavenging Activity. The 80% methanol extracts of the four types of rye breads and wheat roll were also used for the determination of their DPPH RSA. The obtained results compared to the RSA of Trolox are presented in **Table 8**. The RSA of the four types of rye breads ranged from 0.49 to 0.37 mmol Trolox/100 g d.m. Breads formulated on flours with the extraction rates ranging from 100 to 90% showed about 25% higher radical scavenging activity when compared to the bread formulated on flour with the extraction rate of 70%. The lowest radical DPPH scavenging activity was noted for wheat roll, and it was decreased approximately 60% when compared to whole meal rye bread. According to evidence recently reported with respect to the wheat grain, the antioxidants that are able to scavenge directly the DPPH radicals are mostly present in bran fractions of the grains (*25*). Moreover, it was noted that during rye bread preparation when sourdough is formed, the RSA increased in methanol extracts due to the fact that the level of extractable phenolic compounds increased (*28*).

SOD-like Activity. The SOD-like activities of the four types of rye breads and wheat roll are presented in **Table 8**. The SOD-like activity of rye breads ranged from 389 to 304 U/100 g d.m. and was the highest for rye bread formulated on flour with an extraction rate of 70%. In contrast to the scavenging activities against DPPH and ABTS radicals, the rye bread type I showed approximately 30% lower SOD-like activity when compared to rye bread type IV. Moreover, the 2-fold lower SOD-like activity was noted for wheat roll when compared to wholemeal bread. The SOD-like activity expresses the cumulative action of antioxidants to scavenge superoxide anion radicals by nonenzymatic and by SOD action. The latest catalyze the dismutation of two superoxide radical anions into hydrogen peroxide and oxygen, and its essential role is connected with removing damaging reactive oxygen species (ROS) from the cellular environment (*39*). The ability of cereal-based products to scavenge superoxide anion radical, especially by low molecular weight antioxidants, can be important in human nutrition. For example, in humans, the loss or dysfunction of SOD can trigger the ROS-mediated pathologies like coronary heart disease, atherosclerosis, and diabetes (*40*). However, there is still little known about the SOD-like activity in food products, which could prevent the formation of superoxide radical in the gastric tract. Thus, data provided in this study expand our knowledge.

In summary, it can be stated that flour of different extraction rates used for bread production strongly affected the content of bioactive compounds and antioxidative properties of traditionally baked rye breads. The differences in the contents of bioactive compounds and antioxidative properties found between the whole meal rye bread and the wheat roll indicate that rye bread is a better source of antioxidative compounds and also resulted in higher TEAC, RSA, and SOD-like activities. Therefore, the results provided in this study suggest that rye bread based on flour with extraction rates from 100 down to 90% and formulated using a traditional sourdough method should be recommended for human nutrition.

ACKNOWLEDGMENT

This article is a part of the Ph.D. thesis of A.M.

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Received for review August 23, 2006. Revised manuscript received December 6, 2006. Accepted December 12, 2006. These studies were supported by the Polish State Committee for Scientific Research (Project PBZ-KBN-094/P06/2003/13).

JF062425W