Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 980–988
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant contents and properties as quality indices of rye cultivars

Henryk Zieliński [a,*], Alicja Ceglińska [b], Anna Michalska [a]

[a] *Division of Food Science, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Tuwima 10, P.O. Box 55, 10-718 Olsztyn 5, Poland*
[b] *Warsaw Agricultural University, 02-787 Warsaw, Poland*

Received 2 June 2006; received in revised form 29 November 2006; accepted 8 January 2007

Abstract

Contents and properties of antioxidants in the whole grain, a fraction of pericarb with testa and endosperm with embryo of rye (*Secale cereale* L.) cv. Amilo, cv. Warko and cv. Dańkowskie Złote, grown in 2004, in Poland, were studied as quality indices of rye cultivars. The characteristics of rye cultivars were based on the trolox equivalent antioxidant capacity (TEAC) of the phosphate-buffered saline (PBS) and 80% methanolic extracts determined by the $ABTS^{\cdot+}$ radical-scavenging method, and on superoxide dismutase-like activity (SOD) of PBS extracts. The results obtained were supported by the determination of the total phenolic compounds (TPC), total flavonoids (TF), tocopherols (T) and tocotrienols (T3), inositol phosphates (IP), reduced (GSH) and oxidized glutathione (GSSG) contents, and glutathione peroxidase activity (GPx) of the respective extracts originating from the whole grain and its morphological fractions. The reduced/oxidized glutathione status of three rye cultivars was examined as a potential index of balance between oxidative stress and antioxidant system. The results of this study suggest that the content and properties of antioxidants in pericarb with testa fraction rather than in the whole grain may serve as quality indices of rye cultivars. The determination of correlation coefficients between antioxidant properties and antioxidant content should be carried out in this respect.

Keywords: Antioxidant capacity; Superoxide dismutase-like activity; Bioactive compounds; Rye grain extracts

1. Introduction

Rye (*Secale cereale* L.) has been considered to be a primitive crop with low yield, long and weak straw, and problematic behaviour regarding sprouting in the year. Its positive features, in cultivation practices, include low requirements regarding soil and fertilization, as well as a relatively good overwinter ability. Therefore rye has gained popularity, especially in areas with relatively poor soils, such as wide sandy ridges in Poland and some areas in Germany. The greatest rye producer used to be the former Soviet Union. Poland, which produces about 6 million tonnes, and Germany with almost 5 million tonnes, are the largest producers nowadays (Salovaara & Autio, 2001). Although the total production of rye has diminished, its use as a food for humans increased slightly during the 1990s. In 1995, the worldwide food consumption of rye accounted for about 8 million metric tonnes, which is about 35% of the total production. The rest was used as feed (Bushuk, 2001).

Rye grain, like other cereal grains, contributes significant quantities of energy, protein, selected micronutrients and non-nutrients to a human diet (Edge, Jones, & Marquart, 2003). Rye is an excellent raw material for healthy and tasty foods and it has a high fibre content. The whole grain contains a large variety of substances, especially those that are biologically active and demonstrate antioxidant properties, which include free radical-scavengers, reducing agents, potential complexes of prooxidant metals and quenchers of the formation of singlet oxygen (Zieliński, 2002). Although antioxidants in grain and grain products are known, their potential contribution to health, through diet,

* Corresponding author. Tel.: +48 89 5234682; fax: +48 89 5240124.
E-mail address: haziel@pan.olsztyn.pl (H. Zieliński).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.002

has essentially been ignored (Miller, Rigelhof, Marguart, Prakash, & Kanter, 2000). Cereal and cereal-based foods, including rye grain, represent the bulk of all foods consumed. However, their contribution to human nutrition and health should be considered cumulative and collective, together with the consumption of fruits and vegetables (EU-Air Concerted Action, 1998). The latest clinical evidence shows that the consumption of whole foods, such as fruits, vegetables, and whole grain products, and not just their known purified antioxidants, is correlated with a reduced risk of chronic diseases (Willcox, Ash, & Catignani, 2004).

Nutritionists world-wide recommend an increased intake of whole grain products and dietary fibre (Adams & Engstrom, 2000; Lang & Jebb, 2003). In 43 out of 45 epidemiological studies, whole grains, in particular, have been shown to reduce the risk of cancer (Slavin, Marquart, & Jacobs, 2000). It is hypothesized that the biological activities of natural antioxidants and other phytochemicals, in addition to digestion-resistant polysaccharides in the whole grains, contribute to this reduction (Temple, 2000). Now that consumers are increasingly interested in health and their knowledge of the relationship between diet and well-being has increased, rye is likely to gain interest and popularity (Andlauer & Furst, 1999). In addition, the cereal industry has responded to the growing demand, and the variety of rye cereal products is increasing. Whole meal rye flour is the most popular for bread baking; however, rye flour with an extraction rate of about 80% is widely used as well. In addition to the traditional use of different types of rye flour, various types of rye flakes, and breakfast cereals with rye contents of up to 55% are also available (Steller, 1995).

At present, literature about the quality indices of rye cultivars is scare (Franz & Sampson, 2006). Therefore, the aim of this study was to show the content and properties of antioxidants as quality indices of rye cultivars.

2. Materials and methods

2.1. Reagents

Glutathione (γ-glutamyl-cysteinyl-glycine; GSH), sodium phytate, bovine serum albumin (fraction V; BSA), oxidized glutathione (GSSG), (±) catechin, ferulic acid, 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, USA). Standards of tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) were obtained from Merck and Sigma. The superoxide dismutase kit (RANSOD, Cat No SD 125) and glutathione peroxidase kit (RANSEL, Cat No RS504) originated from Randox Laboratories Ltd, UK. All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

2.2. Sample preparation

Rye grain samples, grown in 2004, were obtained from a local plant breeding station in Poland. The samples included rye cv. Amilo, cv. Warko and cv. Dańkowskie Złote. Samples from three replications were chosen for analysis. Whole-grain samples were dehulled using a laboratory dehuller and fractions of pericarb with testa and endosperm with embryo were separated manually by sieving through a set of sieves. All samples were ground in a WZ-1 laboratory mill (Factory of Machines and Mechanisms for the Food Industry, Żnin, Poland). Ground samples were stored at 4 °C prior to extraction.

2.3. Preparation of phosphate-buffered saline and 80% methanolic extracts

The whole grain and fraction of pericarb with testa and endosperm with embryo were ground and then extracted in triplicate with phosphate-buffered saline, pH 7.4 PBS (15 ml per 1 g of sample), or with 80% aqueous methanol (1/10; w/v) with 2 h of shaking at 37 °C. They were then centrifuged at 12000*g* for 15 min in a Beckman GS-15 R centrifuge (Beckman Instruments, Inc., Palo Alto, CL, USA). The fresh PBS extracts were used to determine their ability to scavenge superoxide anion radicals, $ABTS^{\cdot+}$ radicals, and the contents of soluble proteins, total phenolic compounds and total flavonoids, whereas 80% methanolic extracts were used to determine their ability to scavenge $ABTS^{\cdot+}$ radicals and the content of total phenolic compounds and total flavonoids.

2.4. Analytical methods

2.4.1. General

All samples were analysed in duplicate for dry matter, nitrogen and ash, using the AOAC methods (AOAC, 1990).

2.4.2. Determination of inositol phosphates (IP3–IP6) by HPLC

Ground raw whole grain 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) and the supernatant was decanted, frozen overnight (−18 °C), thawed at room temperature and recentrifuged at 3500*g* for 40 min. The supernatant (15 ml) was vacuum-evaporated to dryness at 40 °C and dissolved in 15 ml of 0.025 M HCl. The samples were then transferred to the mini-columns 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, the dry residue was dissolved in a mobile phase. Then the sample was analysed with HPLC according to the methods of Sandberg and Ahderinne (1986) and Sandberg et al. (1989) using a Shimadzu chromatograph (LC-10 AD pump, refractometric detector RID-6 A, CTO 6A column oven) and a Nova-Pak C_{18} column. The mobile phase was a mixture of

methanol and 0.05 M formic acid (51/49), (v/v) and 1.5 ml/100 ml of tetrabutylammonium hydroxide (TBA-OH). The flow rate was 0.4 ml/min. Sodium phytate was the external standard and injections were made with a 20 μl loop.

2.4.3. Determination of reduced (GSH) and oxidized glutathione (GSSG)

Extraction was conducted according to Smith, Vierheller, and Thorne (1988). Rye grains (3 g) were ground and then the flour was transferred to a centrifuge tube and mixed with phosphate buffer (15 ml; 0.2 M, with EDTA, 1 mM, pH 7.5) and potassium chloride (KCl, 0.33 g). The mixture was homogenised for 30 s using a Polytron homogeniser at full speed. Polyvinylpolypyrrolidone (PVPP; 0.25 g) was added and, after thorough mixing, the mixture was centrifuged (2000*g*, 10 min; 4 °C). After centrifugation, the supernatant was kept on ice and assayed for soluble protein (SP); the activity of glutathione reductase (GPx) and the content of reduced and oxidised glutathione. GSH and GSSG were determined according to the spectrofluorometric method of Hissin and Hilf (1976). The detailed procedure was described previously (Zieliński, Honke, Troszyńska, & Kozłowska, 1999). The assays were performed using a Perkin–Elmer LS 50 B Luminescence Spectrometer. The data were calculated on a dry matter basis of the whole grain and its morphological fractions.

2.4.4. Determination of glutathione peroxidase activity (GPx)

Glutathione peroxidase activity was assayed with the glutathione peroxidase kit (RANSEL, Randox Laboratories Ltd). The assay was based on the fact that glutathione reductase catalyzes the reduction of oxidised glutathione (GSSG) in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is oxidised to $NADP^+$. The amount of GSSG reduced to GSH was calculated from the change in absorbance at 340 nm. One unit (U) of glutathione reductase reduces 1 μmol GSSG/min at 30 °C and at pH 7.5. The detection limit was 0.05 U/g of dry matter of rye grains.

2.4.5. Determination of tocopherols and tocotrienols by HPLC

Tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) were extracted with methanol (0.5 g of sample/7 ml) and then the evaporated extracts were redissolved in *n*-hexane. The tocols were separated by HPLC on Lichrospher Si 60, 5 μm particle size, 4 × 250-mm column, according to the method described by Paterson and Qureshi (1993). Twenty microlitres of each sample were injected onto the column. The HPLC systems consisted of a Shimadzu model LC pump series 10 AD, and a Shimadzu RF-535 fluorescence spectrometer. The mobile phase was 0.5% isopropanol in hexane. Flow rate was 1 ml/min, and the peaks were detected using an excitation wavelength of 295 nm and 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, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) obtained from Merck and Sigma.

2.4.6. Determination of total phenolic compounds (TPC)

The content of total phenolic compounds (TPC) was determined in PBS and 80% methanolic extracts according to Shahidi and Naczk (1995). Exactly 0.25 ml (aliquot) of each extract was mixed with 0.25 ml of Folin-Ciocalteu reagent (previously diluted with water 1:1 v/v) and 0.5 ml of saturated sodium carbonate (Na_2CO_3) solution and 4 ml water. The mixture was allowed to stand at a room temperature for 25 min and was then centrifuged at 5000 rpm for 10 min. Supernatant absorbance was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The data were calculated as ferulic acid equivalents.

2.4.7. Determination of total flavonoid content (TF)

Total flavonoid content was determined by the colorimetric method described by Jia, Tang, and Wu (1998). Briefly, 0.25 ml of the PBS or 80% methanolic extract was diluted with 1.25 ml of distilled water. Then, 75 μl of a 5% $NaNO_2$ solution were added, and the mixture was allowed to stand at a room temperature. After 6 min, 150 μl of a 10% $AlCl_3 \times 6\ H_2O$ solution were added, and the mixture was allowed to stand for a further 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 the prepared blank at 510 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan) in comparison with the standards prepared similarly with known (±) catechin concentrations. Then the results were expressed as mg of catechin equivalents. Data are reported as means and standard deviation for at least three replications.

2.4.8. Determination of SOD-like activity

The superoxide dismutase-like activity of the PBS extracts was measured with a superoxide dismutase kit (RANSOD, Cat No SD 125). The assays were performed using a thermostatted recording spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu, Japan) adjusted to 37 °C inside the cuvettes. The test requires 50 μl of sample, with a read time of 3 min. The results were finally converted into milligrammes of soluble protein assayed according to the bicinchonic acid (BCA) protein microassay (Smith et al., 1985). Superoxide dismutase with an activity of 5.3 U/ml was used as a standard and was supplied as part of the reagent kit. In general, one unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of reduced adenine nucleotides (NADH, NADPH) oxidation of the control by 50%. Then, extrapolation of 50% inhibition values in the samples allows calculation of the enzyme activity. The percent of the reaction inhibition was plotted against $\log_{10}$ 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 investigated extract.

2.4.9. Determination of trolox equivalent antioxidant capacity (TEAC)

This test was based on the reduction of the $ABTS^{\cdot+}$ radical cation by antioxidants present in PBS and 80% methanolic extracts. The $ABTS^{\cdot+}$ 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. Trolox equivalent antioxidant capacity was determined, following a procedure described by Re et al. (1999) with a minor modification described below. For measurements, the $ABTS^{\cdot+}$ solution was diluted with PBS or 80% methanol, respectively, to the absorbance of 0.700 ± 0.020 at 734 nm. For the photometric assay, 1.48 ml of the $ABTS^{\cdot+}$ solution and 20 μl of the extracts or trolox standards were mixed and measured immediately after 6 min at 734 nm at 30 °C using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). Appropriate solvent blanks were run in each assay. The trolox equivalent antioxidant capacities of the PBS and 80% methanolic extracts were calculated, using the trolox standard curve, on the basis of percentage inhibition of absorbance at 734 nm.

2.5. Statistical analysis

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

3. Results and discussion

3.1. General

The aim of this study was to show the content and properties of antioxidants as quality indices of rye cultivars. Therefore, the contents of antioxidants and their properties in the whole grain and its different morphological fractions were analyzed, along with the proximate chemical composition of whole rye grains.

3.2. Chemical composition of whole rye grains

The means of dry matter, protein, starch and crude ash contents (%) in the whole grain of rye cultivars selected for study are shown in Table 1. Whole grain cv. Warko had the highest protein and crude ash contents; however, starch content noted in this variety was lower by 4% and 2% when compared to cv. Amilo and cv. Dańkowskie Złote, respectively. The statistical analysis of the data showed that protein and starch were still the main factors responsible for the discrimination of the quality of rye grain. Therefore, the rye grain cv. Warko, with a high content of protein and ash and low starch content, seems to be more required for the cereal industry for further processing.

3.3. Inositol phosphates (IP3–IP6) content of whole grain and its morphological fractions

Only inositol hexaphosphate (IP6) was present in the whole grain, and its content ranged from 7.76 to 10.9 μmol/g d.m., depending on the cultivar (Fig. 1). No trace amounts of the lower forms of inositol phosphates were detected. Rye grain cv. Amilo appeared to be the richest in IP6. These results confirm that rye whole grains are a rich source of inositol hexaphosphate which, in the diet, has the potential to reduce the risk of colon cancer (Lantzsch, 1990; Shamsuddin, 1995).

3.4. Reduced and oxidised glutathione contents, glutathione status and glutathione peroxidase activity of whole grain and its morphological fractions

The contents of reduced and oxidized glutathione, the reduced/oxidized glutathione status (GSH/GSSG ratio) and glutathione peroxidase activity (GPx) in whole grains

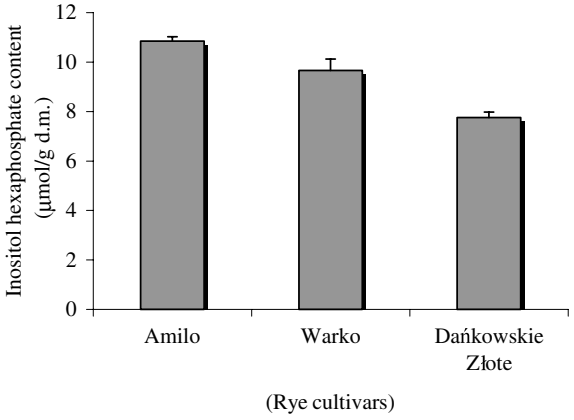

Fig. 1. Inositol hexaphosphate content in the whole grain of different rye cultivars.

Table 1
Means of dry matter, protein, starch and crude ash contents in the whole grain of rye cultivars[A]

Rye cultivar	Dry matter (%)	Protein content (%)	Starch content (%)	Crude ash (%)
Amilo	88.47 ± 0.04^{a}	8.65 ± 0.21^{a}	55.7 ± 0.14^{a}	1.59 ± 0.05^{a}
Warko	88.29 ± 0.01^{a}	11.6 ± 0.13^{b}	53.3 ± 0.28^{b}	1.86 ± 0.05^{b}
Dańkowskie Złote	88.50 ± 0.01^{a}	10.3 ± 0.15^{c}	54.0 ± 0.14^{c}	1.81 ± 0.16^{bc}

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

and their morphological fractions are shown in Table 2. The content of GSH in the whole grain ranged from 324 to 401 nmol/g d.m. (cv. Amilo). The fractions of endosperm with embryo of the three rye cultivars examined were richer in GSH than were the whole grains and pericarb with testa fractions. This points to rye grain cv.Warko as the best rye cultivar when assessed only in terms of GSH content. This GSH distribution within the endosperm with embryo fraction is convenient, with the well known role of glutathione in maintaining redox status of disulfide bonds during dough preparation. Nevertheless, Li, Bollecker, and Schofield (2004) did not observe any clear relationship between the content of reduced glutathione and baking performance. The high content of oxidized glutathione was noted in pericarb with a testa fraction when compared to the content noted for the whole grain and endosperm with an embryo fraction of the three rye cultivars tested. Since the outer layer of the grain is responsible for grain protection against oxidizing environmental factors, the reduced/oxidized glutathione status (GSH/GSSG ratio) was calculated in order to show the resistance of the investigated rye grains against oxidative stress. It was noted that Warko and Dańkowskie Z łote cultivars were more resistant to this stress (2.15 for cv. Wako and 2.37 for cv. Dańkowskie Złote) as compared to Amilo cv. (1.83). In contrast to our previous data indicating a potential hierarchy of resistance of wheat, rye, barley, oat and buckwheat against oxidative stress, the data provided herein for glutathione status of the whole grain were not statistically significantly different (Zieliński et al., 1999). Our findings indicate that the GSH/GSSG ratio of the whole grain is of limited usability for rye cultivar selection, in contrast to its applicability for other problems, such as dormancy, germination and grain aging (Pheifer & Briggs, 1995). The GSH/GSSG ratio of pericarp with testa fraction, however, can be recommended as one among other parameters describing the rye technological quality. This conclusion was strongly supported by the highest SOD-like activity found in the extracts of pericarb with testa fractions of the rye cultivars studied (Fig. 2c). The activity of GPx in the whole grain of the three rye cultivars ranged from 0.41 U/g d.m. (cv. Amilo) to 0.68 U/g d.m. (cv. Warko) and remained at a similar level for the endosperm with embryo and pericarb with testa fractions.

3.5. Tocopherol and tocotrienol contents of whole grain and its morphological fractions

Tocopherol and tocotrienol profiles of the whole grains and their morphological fractions are shown in Table 3. The highest level of tocopherols was found in the whole grain of rye cv. Warko and in its fraction of endosperm with embryo where the main tocopherols were α-T and β-T. In contrast, γ-T was only present in these three rye cultivars in small quantities. The main tocotrienols found in rye were only α-T3 and then β-T3. The richest fraction, in which they were present, was the fraction of pericarb and testa of rye cv. Dańkowskie Złote. Tocotrienol contents of the whole grains and the fraction of endosperm with embryo of the investigated cultivars were twice as low as tocopherol content of pericarb and testa fraction (Table 3). The level of tocopherols in rye was in agreement with our previous report on vitamin E contents of other cereals (Zieliński, Ciska, & Kozłowska, 2001). The tocopherol and tocotrienol profiles of rye grain were also similar to the previously reported data (Holasowa, 1997; Holasowa, Velisek, & Davidek, 1995; Zieliński et al., 2001). Therefore, the differences noted in tocol contents may result from the genotype of rye grains and environmental effects (Holasowa et al., 1995; Paterson & Qureshi, 1993). The results provided in this study showed similar differentiation of tocopherol and tocotrienol concentrations within rye kernels and different morphological parts of each tested rye cultivar, and are in good agreement with reference data (Gasiorowski, 1994; Paterson & Qureshi, 1993; Piironen, Syvaoja, Varo, Salminek, & Koivistoinen, 1986). There-

Table 2
Content of reduced (GSH) and oxidized (GSSG) glutathione, GSH/GSSG ratio and activity of glutathione peroxidase (GPx) in the extracts of the whole grains and fractions of pericarb with testa and endosperm with embryo of selected rye cultivars[A]

Fraction/cultivars	GSH (nmol/g d.m.)	GSSG (nmol/g d.m.)	GSH/GSSG	GPx (U/g d.m.)
Whole grain				
Amilo	401 ± 6.04^{a}	157 ± 12.0^{a}	2.57 ± 0.24^{a}	0.41 ± 0.09^{a}
Warko	399 ± 0.35^{a}	168 ± 4.87^{a}	2.37 ± 0.07^{a}	0.68 ± 0.03^{b}
Dańkowskie Złote	324 ± 6.18^{b}	121 ± 4.54^{b}	2.69 ± 0.15^{ab}	0.55 ± 0.16^{ab}
Endosperm with embryo				
Amilo	419 ± 21.9^{a}	146 ± 3.78^{a}	2.88 ± 0.22^{a}	0.72 ± 0.09^{a}
Warko	458 ± 11.0^{b}	181 ± 0.81^{b}	2.53 ± 0.07^{b}	0.69 ± 0.12^{a}
Dańkowskie Złote	468 ± 2.97^{bc}	142 ± 9.57^{a}	3.31 ± 0.20^{c}	0.44 ± 0.14^{b}
Pericarb with testa				
Amilo	420 ± 7.57^{a}	230 ± 11.3^{a}	1.83 ± 0.12^{a}	0.53 ± 0.11^{a}
Warko	389 ± 3.23^{b}	181 ± 11.0^{b}	2.15 ± 0.15^{b}	0.49 ± 0.10^{a}
Dańkowskie Złote	429 ± 29.4^{ac}	182 ± 10.9^{bc}	2.37 ± 0.30^{bc}	0.54 ± 0.13^{a}

[A] Data expressed as means ± SD ($n = 3$). Within each column for whole grain and indicated grain fraction, means with the same letter are not significantly different ($P \leqslant 0.05$).

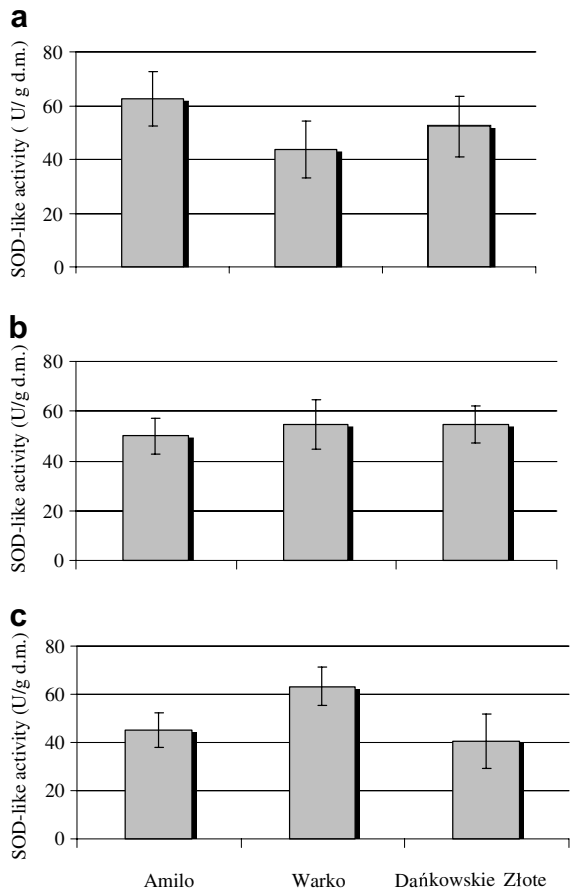

Fig. 2. Superoxide dismutase-like activities (U/mg of soluble protein) of the whole grain extract (a), endosperm with embryo extract (b) and pericarb with testa extract (c) of different rye cultivars.

fore, the tocopherol and tocotrienol profile is not sufficient for use as a quality index of rye cultivars. However, the ratio of tocotrienols to tocopherols (T3/T) could be considered supportive, since its high value, found mainly in cereal and cereal products, is beneficial for consumers, especially those with cardiovascular diseases (Andlauer & Furst, 1998).

3.6. Total phenolics (TPC) and total flavonoid (TF) content of whole grain and its morphological fractions

The contents of total phenolic compounds and total flavonoids in PBS and 80% methanolic extracts in the whole grain and its morphological fractions of different rye cultivars are shown in Table 4. A mixture of methanol/water (4:1, v/v) was found to be a better solvent for the extraction of phenolic compounds than were pure methanol or 50% methanol (data not shown). The pericarb with testa fraction was about twice richer in TPC, extractable by 80% methanol and PBS, than was the endosperm with embryo fraction or the whole grain. This finding is consistent with data reported by Liukkonen et al. (2003) who showed the highest TPC content in rye bran, which was related to the outer layer of grain. However, PBS extracts prepared from the whole rye grain and fractions of endosperm with embryo and pericarb and testa of each tested cultivar were found to be richer in phenolic compounds than were the 80% methanolic extracts by about 37%, 40% and 32%, respectively. In contrast, a different result was found when total flavonoid contents were compared between PBS and 80% methanolic extracts of the investigated material (Table 4). In this case, the 80% methanolic extracts prepared from the whole rye grain and fractions of endosperm with embryo and pericarb and testa of each cultivars were richer in flavonoids than were the PBS extracts by about 304%, 252% and 194%, respectively.

3.7. Superoxide dismutase (SOD)-like activity of whole grain and its morphological fractions

The superoxide dismutase (SOD)-like activity (U/g d.m.) of the phosphate-buffered saline extracts originating from the whole rye grain and a fraction of endosperm with

Table 3
The contents of tocopherols (T) and tocotrienols (T3) (in grains and their morphological fractions) of different rye cultivars (μg/g d.m.)[A]

Fraction/cultivars	T					T3				T3/T
	α	β	γ	δ	Total	α	β	γ	Total	
Whole grain										
Amilo	6.27 ± 0.52	1.49 ± 0.06	0.11 ± 0.01	–	7.87 ± 0.55^{a}	5.41 ± 0.20	4.13 ± 0.22	–	9.53 ± 0.41^{a}	1.21
Warko	8.73 ± 0.34	1.37 ± 0.13	0.08 ± 0.03	–	10.2 ± 0.49^{a}	6.87 ± 0.29	4.19 ± 0.33	–	11.07 ± 0.59^{b}	1.09
Dańkowskie Złote	8.05 ± 0.40	1.68 ± 0.07	0.09 ± 0.01	–	9.82 ± 0.47^{bc}	7.47 ± 0.17	6.49 ± 0.07	–	13.96 ± 0.14^{c}	1.42
Endosperm with embryo										
Amilo	5.29 ± 0.71	1.46 ± 0.19	0.07 ± 0.03	–	6.81 ± 0.87^{a}	4.92 ± 1.14	4.56 ± 0.84	–	9.84 ± 1.97^{a}	1.44
Warko	7.61 ± 0.23	1.30 ± 0.06	0.08 ± 0.01	–	8.99 ± 0.29^{b}	6.84 ± 0.52	4.60 ± 0.10	–	11.5 ± 0.62^{a}	1.27
Dańkowskie Złote	5.99 ± 0.21	1.39 ± 0.03	0.09 ± 0.01	–	7.46 ± 0.24^{a}	6.23 ± 0.30	6.00 ± 0.30	–	12.2 ± 0.59^{a}	1.64
Pericarb with testa										
Amilo	1.93 ± 0.06	0.47 ± 0.04	0.02 ± 0.01	–	2.43 ± 0.09^{a}	12.8 ± 0.64	7.20 ± 0.39	–	20.0 ± 1.03^{a}	8.21
Warko	2.38 ± 0.27	0.44 ± 0.06	0.02 ± 0.01	–	2.84 ± 0.32^{b}	13.5 ± 2.10	7.01 ± 1.16	–	20.6 ± 3.22^{a}	7.24
Dańkowskie Złote	2.34 ± 0.14	0.57 ± 0.05	0.03 ± 0.01	–	2.94 ± 0.18^{bc}	17.2 ± 0.93	11.61 ± 0.91	–	28.8 ± 1.84^{b}	9.79

[A] Data expressed as means ± standard deviation ($n = 3$). Within each column for whole grain and indicated grain fraction, means with the same letter are not significantly different ($P \leqslant 0.05$).

Table 4
The contents of total phenolic compounds (TPC) and total flavonoids (TF) in PBS and 80% methanolic extracts in the whole grain and its morphological fractions of different rye cultivars (mg/g d.m.)[A]

Fraction/cultivars	TPC		TF	
	PBS	80% MeOH	PBS	80% MeOH
Whole grain				
Amilo	2.16 ± 0.024^{a}	1.38 ± 0.035^{a}	0.082 ± 0.004^{a}	0.307 ± 0.010^{a}
Warko	2.37 ± 0.038^{b}	1.47 ± 0.047^{b}	0.080 ± 0.002^{a}	0.290 ± 0.009^{b}
Dańkowskie Złote	2.18 ± 0.003^{a}	1.35 ± 0.001^{a}	0.063 ± 0.001^{b}	0.300 ± 0.002^{ab}
Endosperm with embryo				
Amilo	2.37 ± 0.107^{a}	1.41 ± 0.003^{a}	0.096 ± 0.002^{a}	0.313 ± 0.004^{a}
Warko	2.63 ± 0.011^{b}	1.60 ± 0.023^{b}	0.091 ± 0.002^{b}	0.326 ± 0.009^{b}
Dańkowskie Złote	2.40 ± 0.051^{a}	1.41 ± 0.025^{a}	0.081 ± 0.004^{c}	0.301 ± 0.006^{c}
Pericarb with testa				
Amilo	4.01 ± 0.078^{a}	2.76 ± 0.019^{a}	0.177 ± 0.009^{a}	0.467 ± 0.028^{a}
Warko	4.01 ± 0.024^{a}	2.65 ± 0.063^{b}	0.142 ± 0.013b	0.424 ± 0.033^{a}
Dańkowskie Złote	3.78 ± 0.043^{b}	2.60 ± 0.0 6b[c]	0.134 ± 0.001^{bc}	0.432 ± 0.007^{a}

[A] TPC expressed as mg ferulic acid equivalents per gramme of rye whole grain or indicated grain fraction as means ± SD ($n = 3$). TF content is expressed as mg of catechin equivalents ($n = 3$). Within each column for the whole grain and indicated grain fraction, means with the same letter are not significantly different ($P \leqslant 0.05$).

embryo and pericarb and testa of each cultivar is shown in Fig. 2. Since an SOD standard with a known activity was used in this study, it was possible to calculate the enzyme activity directly from the standard curve. The results showed the highest SOD-like activities of the extracts prepared from the whole grain rye cv. Amilo. However the highest SOD-like activities of pericarb with testa fraction extracts of rye cv. Warko should be pointed out (Table 5). This fraction can be responsible for amelioration of the oxidative stress caused by environmental factors.

3.8. Trolox equivalent antioxidant capacity (TEAC) of the whole grain and its morphological fractions

Determination of the antioxidant capacity of the whole grain and its fraction was the next approach in this study. The term "antioxidant capacity" used in this study corresponds to the measure of the moles of a given free radical scavenged by a test solution, independently of the antioxidant activity of any antioxidant present in the mixture (Ghiselli, Serafini, Natella, & Scaccini, 2000). Hence, the measure of the trolox equivalent antioxidant capacity (TEAC) of food extracts considers the cumulative action of all the antioxidants present in the extract, including their chain-braking, scavenging and chelating effects, and thus providing an integrated parameter rather than the simple sum of measurable antioxidants (Nagah & Seal, 2005). The capacity of known and unknown antioxidants and their synergistic interaction is therefore determined, thus giving a tool for screening a wide range of foods for their antioxidant properties.

The estimated values of TEAC, based on the relative abilities of the PBS and 80% methanolic extracts to scavenge the $ABTS^{\cdot+}$ in comparison with trolox, showed higher values for the PBS extract of the whole grain and the fraction of endosperm with embryo of each investigated rye

Table 5
The contents of soluble proteins (SP) and SOD-like activities of the whole grain and its morphological fractions of different rye cultivars[A]

Fraction/cultivars	SP (mg/g d.m.)	SOD-like activity (U/mg of soluble protein)
Whole grain		
Amilo	47.8 ± 1.10^{a}	1.31 ± 0.22^{a}
Warko	61.5 ± 2.30^{b}	0.71 ± 0.18^{b}
Dańkowskie Złote	66.1 ± 1.00^{c}	0.79 ± 0.18^{bc}
Endosperm with embryo		
Amilo	54.7 ± 2.70^{a}	0.92 ± 0.16^{a}
Warko	70.6 ± 5.38^{b}	0.78 ± 0.18^{a}
Dańkowskie Złote	72.1 ± 2.49^{bc}	0.76 ± 0.12^{a}
Pericarb with testa		
Amilo	87.3 ± 0.90^{a}	0.52 ± 0.08^{a}
Warko	94.3 ± 1.60^{b}	0.67 ± 0.09^{b}
Dańkowskie Złote	101 ± 2.21^{c}	0.40 ± 0.11^{a}

[A] Data expressed as means ± standard deviation ($n = 3$). Within each column for whole grain and indicated grain fraction, means with the same letter are not significantly different ($P \leqslant 0.05$).

cultivar when compared to the respective 80% methanolic extracts. PBS and 80% methanolic extracts of the fraction of pericarb and testa had the highest TEAC values, ranging from 18.4 to 18.6 μmol trolox/g d.m. and from 18.5 to 22.1 μmol trolox/g d.m, respectively (Table 6). Statistically significant differences were found in TEAC of PBS extracts of the whole grain, endosperm with embryo and pericarb with testa fractions of the rye cultivars studied. Moreover, further differences were noted in TEAC of 80% methanolic extracts of endosperm with embryo and pericarb with testa fractions but not in respect to 80% methanolic extracts of the whole grains. For this reason, the selection of the rye cultivar for technological purposes, based only on TEAC of 80% methanolic extracts of the whole grain, cannot be taken for consideration. This conclusion was confirmed by TEAC values of the whole grain expressed at the same

Table 6
Trolox equivalent antioxidant capacities (TEAC) of PBS and 80% methanolic extracts of the whole grain and its morphological fractions of different rye cultivars[A]

Fraction/cultivars	PBS extracts		80% MeOH extracts	
	TEAC (μmol trolox/g d.m.)	TEAC (μmol trolox/mg ferulic acid)	TEAC (μmol trolox/g d.m.)	TEAC (μmol trolox/mg ferulic acid)
Whole grain				
Amilo	14.7 ± 0.03^{a}	6.80 ± 0.09^{a}	9.91 ± 0.17^{a}	7.21 ± 0.31^{a}
Warko	15.2 ± 0.02^{b}	6.42 ± 0.36^{a}	9.82 ± 0.81^{a}	6.69 ± 0.34^{a}
Dańkowskie Złote	14.3 ± 0.17^{c}	6.57 ± 0.09^{ab}	9.55 ± 0.21^{ab}	7.07 ± 0.16^{a}
Endosperm with embryo				
Amilo	14.3 ± 0.01^{a}	6.03 ± 0.27^{a}	10.01 ± 0.07^{a}	7.12 ± 0.03^{a}
Warko	15.7 ± 0.07^{b}	5.95 ± 0.01^{a}	11.08 ± 0.12^{b}	6.91 ± 0.02^{a}
Dańkowskie Złote	13.7 ± 0.37^{c}	5.73 ± 0.03^{ab}	9.97 ± 0.24^{a}	7.09 ± 0.30^{ab}
Pericarb with testa				
Amilo	18.6 ± 0.06a	4.63 ± 0.08^{a}	22.07 ± 0.16^{a}	7.99 ± 0.11^{a}
Warko	18.4 ± 0.04^{b}	4.58 ± 0.04^{a}	19.53 ± 0.46^{b}	7.37 ± 0.35^{b}
Dańkowskie Złote	19.0 ± 0.06^{c}	5.02 ± 0.04^{b}	18.54 ± 0.15^{c}	7.14 ± 0.04^{c}

[A] Data expressed as means ± standard deviation ($n = 3$). Within each column for whole grain and indicated grain fraction, means with the same letter are not significantly different ($P \leqslant 0.05$).

level of TPC which, for PBS and 80% methanolic extracts of the whole grain, were not statistically different and ranged from 6.42 to 7.21 μmol trolox/mg ferulic acid equivalents. The fractions of endosperm with embryo and pericarb and testa of the investigated rye cultivars had TEAC values ranging from 4.58 to 7.99 μmol trolox/mg ferulic acid equivalents (Table 5).

3.9. Correlation studies

The correlation coefficient between the total phenolic compound contents and antioxidant capacity of the whole grains, pericarb with testa fractions and endosperm with embryo fractions was 0.96 for PBS extracts and 0.99 for 80% methanolic extracts. Moreover, a high correlation coefficient was noted between TEAC of 80% methanolic extract of the whole grain and its fraction and the T3/T ratio ($r = 0.95$; $P \leqslant 0.05$). An interesting finding was noted among correlation coefficients between antioxidant capacity and SOD-like activity of the PBS extracts originating from the whole rye grain and the fraction of endosperm with embryo and pericarb with testa of each rye cultivar. The correlation coefficients accounted for −0.20, −0.14 and −0.98 for extracts originating from whole rye grain and the fraction of endosperm with embryo and pericarb with testa, respectively. Thus, the ability of the investigated rye extracts to scavenge ABTS radical cation seems to be different from their ability to scavenge superoxide radicals, since the total antioxidant status reflects the ability of hydrogen-donating antioxidants to scavenge the ABTS radical cation.

4. Conclusions

The profile and concentrations of antioxidants in the whole rye grain and its morphological fractions are determined by rye variety. The results obtained indicate the possibility of using antioxidant contents and antioxidant properties of pericarb with testa fraction, rather than these of the whole grain, for the selection of rye variety of high technological quality; however, other parameters of milling and baking quality have to be taken into account as well. This conclusion was supported by the complimentary effect observed between the antioxidant capacity of the fraction of pericarb with testa and SOD-like activity, by a twofold higher antioxidant capacity and total phenolic compounds content of the 80% methanolic extract of pericarb with a testa fraction when compared to the respective data for extracts of the whole grain and endosperm with an embryo fraction and by about seven times higher ratio of tocotrienols to tocopherols of the pericarb with testa fraction when compared to the ratio determined for the whole grain of each cultivar. The GSH/GSSG ratio of pericarp with testa fraction can be thus recommended as one among other parameters describing the technological quality of rye.

Acknowledgements

We gratefully acknowledge research Grant No. PBZ-KBN-094/P06/2003/13 from the Polish State Committee for Scientific Research. This article is a part of the Ph.D. thesis of A. Michalska.

References

Adams, J. F., & Engstrom, A. (2000). Dietary intake of whole grain vs. recommendations. *Cereal Foods World, 45*, 75–78.

Andlauer, W., & Furst, P. (1998). Antioxidative power of phytochemicals with special reference to cereals. *Cereal Foods World, 43*, 356–360.

Andlauer, W., & Furst, P. (1999). Does cereal reduce the risk of cancer? *Cereal Foods World, 44*, 76–78.

AOAC (1990). *Official methods of analysis* (15th ed., p. 777). Washington, DC: Association of Official Analytical Chemists.

Bushuk, W. (2001). Rye production and uses worldwide. *Cereal Chemistry, 46*(2), 70–73.

Edge, M. S., Jones, J. M., & Marquart, L. (2003). A new life for whole grains. *Journal of the American Dietetic Association*, 1856–1860.

EU-Air Concerted Action CT 94 2185. (1998). Nettox compilation of consumption data, report no. 4 (pp. 20–21). Published by Danish Veterinary and Food Administration, Denmark.

Franz, M., & Sampson, L. (2006). Challenges in developing a whole grain database: Definitions, methods and quantification. *Journal of Food Composition and Analysis, 19*, S38–S44.

Gasiorowski, H. (1994). Żyto – Chemia i technologia. *PWRiL* (pp. 106–107). Poznań.

Ghiselli, A., Serafini, M., Natella, F., & Scaccini, C. (2000). Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radical Biology and Medicine, 29*, 1106–1114.

Hissin, J., & Hilf, R. (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Analytical Biochemistry, 74*, 214–226.

Holasowa, M. (1997). Distribution of tocopherols and tocotrienols in the main products of wheat and rye milling. *Czech Journal of Food Sciences, 15*(5), 343–350.

Holasowa, M., Velisek, J., & Davidek, J. (1995). Tocopherol and tocotrienol contents in cereal grains. *Czech Journal of Food Sciences, 13*(6), 409–417.

Jia, Z., Tang, M., & Wu, J. (1998). The determination of flavonoid contents in mulberry and their scavenging effects on superoxides radicals. *Food Chemistry, 64*, 555–559.

Lang, R., & Jebb, S. A. (2003). Who consumes whole grains, and how much? *Proceedings of the Nutrition Society, 62*, 123–127.

Lantzsch, H. J. (1990). Untersuchungen uber ernahgungsphysiologische Effekte des Phytats bei Monogastrien (Ratte, Schwein). *Ubersuchung Tierenahrung, 18*, 197–212.

Li, W., Bollecker, S. S., & Schofield, J. D. (2004). Glutathione and related thiol compounds. I. Glutathione and related thiol compounds in flour. *Journal of Cereal Science, 39*, 205–212.

Liukkonen, K-H., Katina, K., Wilhelmsson, A., Myllymaki, O., Lampi, A-M., Kariluoto, S., et al. (2003). Process-induced changes on bioactive compounds in whole grain rye. *Proceedings of the Nutrition Society, 62*, 117–122.

Miller, H. E., Rigelhof, F., Marguart, L., Prakash, A., & Kanter, M. (2000). Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *Journal of the American College of Nutrition, 19*, 312S–319S.

Nagah, A. M., & Seal, C. J. (2005). *In vitro* procedure to predict apparent antioxidant release from wholegrain foods measured using three different analytical methods. *Journal of the Science of Food and Agriculture, 85*, 1177–1185.

Paterson, D. M., & Qureshi, A. A. (1993). Genotype and environment effects on barley and oats. *Cereal Chemistry, 70*, 157–162.

Pheifer, J. H., & Briggs, D. E. (1995). Thiols and disulphides in quiscent and germinating barley grains, both dormant and mature. *Journal of the Institute of Brewing, 101*, 85–93.

Piironen, V., Syvaoja, E. l., Varo, P., Salminek, K., & Koivistoinen, P. (1986). Tocopherols and tocotrienols in cereal products from Finland. *Cereal Chemistry, 63*, 78–81.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS Radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Salovaara, H., & Autio, K. (2001). Rye and triticale. In D. A. V. Dendy & B. J. Dobraszczyk (Eds.), *Cereals and cereal products* (pp. 391–410). Maryland: Aspen Publishers.

Sandberg, A. S., & Ahderinne, R. (1986). HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in food and intestinal contents. *Journal of Food Science, 51*, 547–550.

Sandberg, A. S., Carlsson, N. G., & Svanberg, U. (1989). Effects of inositol tri-, tetra-, penta-, and hexaphosphates on *in vitro* estimation of iron availability. *Journal of Food Science, 54*, 159–161.

Shahidi, F., & Naczk, M. (1995). Methods of analysis and quantification of phenolic compounds. In F. Shahidi & M. Naczk (Eds.), *Food phenolic: Sources, chemistry, effects and applications* (pp. 287–293). Lancaster, Pennsylvania: Technomic Publishing Company.

Shamsuddin, A. M. (1995). Inositol phosphates have novel anticancer function. *Journal of Nutrition, 125*, 725–732.

Slavin, J., Marquart, L., & Jacobs, D. Jr., (2000). Consumption of whole-grain foods and decreased risk of cancer: Proposed mechanisms. *Cereal Foods World, 45*, 54–58.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Macia, A. K., Gartner, F. H., Provenzano, M. D., et al. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry, 150*, 76–85.

Smith, I. K., Vierheller, T. L., & Thorne, C. A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Analytical Biochemistry, 175*, 408–413.

Steller, W. (1995). Consumer habits – Changes of rye products in the European Union. In: K. Poutanen, & K. Autio (Eds.), *VTT Symposium, international rye symposium: technology and products, Helsinki, 7–8 December, Espoo* (pp. 194–200).

Temple, N. J. (2000). Antioxidants and disease: More questions than answers. *Nutrition Research, 20*, 449–459.

Willcox, J. K., Ash, S. L., & Catignani, G. L. (2004). Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition, 44*, 275–295.

Zieliński, H. (2002). Low molecular weight antioxidants in the cereal grains. *Polish Journal of Food and Nutrition Sciences*(11/52), 3–9.

Zieliński, H., Ciska, E., & Kozłowska, H. (2001). The cereal grains: Focus on vitamin E. *Czech Journal of Food Sciences, 5*, 182–188.

Zieliński, H., Honke, J., Troszyńska, A., & Kozłowska, H. (1999). The reduced/oxidised glutathione status as a potential index of oxidative stress in mature cereal grain. *Cereal Chemistry, 76*, 944–948.