

Reduced-Oxidized Glutathione Status as a Potential Index of Oxidative Stress in Mature Cereal Grain

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ABSTRACT

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The purpose of this study was to examine the reduced and oxidized glutathione status of selected cereal grains as a potential index of balance between oxidative stress and antioxidant systems, and the contribution of reduced glutathione to the total antioxidant status in cereal grain extracts. Wheat cultivars Almari and Henika, barley cultivars Gregor and Mobek, rye cultivar Dańkowskie Złote, oat cultivar Sławko, and buckwheat cultivar Kora were used. Total antioxidant status (TAS) was measured by the ABTS (2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulphonate)) method. Contents of total phenolic compounds were also determined. Reduced (GSH) and oxidized glutathione (GSSG) (γ -glutamyl-cysteinyl-glycine) were assayed using the spectrofluorimetric method, and results were confirmed by the enzyme recycling method. Correlation coefficient for the GSH/GSSG ratio

was $r = 0.79$. Correlation between TAS and the total phenolic compound content was $r = 0.81$. Correlation between GSH/GSSG ratio and TAS values was $r = 0.46$, depending on the extraction system used. The GSH/GSSG ratio may indicate a hierarchy among different cultivars and variance of cereal grains against damage caused by reactive oxygen species. For the main water-soluble antioxidants, our data indicate a potential hierarchy of resistance in investigated cereals against oxidative stress (buckwheat > wheat > barley \approx rye > oat). This hierarchy was confirmed by the ability of investigated cereal extracts to scavenge superoxide anion radicals in vitro. The reduced-oxidized glutathione status in different cereal grains can be applied as a potential index of balance between oxidative stress and antioxidant systems.

Thiol antioxidants are of increasing interest to the medical and food sciences. They act as antioxidants, participate as substrates in antioxidant reaction, recycle other antioxidants, interact with protein sulfhydryls, and are increasingly recognized as affecting gene expression. From the medical point of view, glutathione (γ -glutamyl-cysteinyl-glycine) is an ubiquitous antioxidant, known to react nonenzymatically with free radicals and nonradical reactive oxygen species, and being a substrate of glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase, thus participating in the removal of hydrogen peroxide and organic peroxides. It reacts with amino acid and protein peroxides. The reaction proceeds nonenzymatically but is accelerated by glutathione peroxidase. Moreover, glutathione is conjugated to reactive electrophiles, catalyzed in reaction by glutathione S-transferases. While the role of glutathione as an important cellular antioxidant is generally known, several aspects of the functions of this compound remain debatable (Bartosz 1996). Many pathological conditions cause a decrease in intracellular glutathione concentration and then its antioxidant role can be maintained by the cell when oxidized glutathione (GSSG) is reduced to glutathione (GSH) through the activity of glutathione reductase (GR). It is also a logical therapy to increase intracellular glutathione through supplementation. But glutathione is not readily absorbed in a usable form from the diet and, for this reason, alternative strategies have been developed to increase intracellular levels of glutathione (Packer 1995).

In plants, glutathione, together with ascorbate, forms the antioxidant system with defense enzymes such as superoxide dismutases (SOD), peroxidases, and catalases that protect them against damage caused by reactive oxygen species generated during photosynthesis, photorespiration, respiration, and other reactions of cellular metabolism (Asada 1992). Glutathione is also included in the most prominent antioxidant system naturally present in food which contains ascorbic acid, tocopherols, flavonoids, selenium, and carotenoids (Andlauer and Furst 1998). Although the antioxidant mechanism of action is extremely complex, it is important to note that some of the substances mentioned above are water-soluble and some are fat-soluble. These characteristics give each of them a

relative medium specificity; relative only, since synergism has been observed between both groups. In the absence of antioxidant mechanisms, when more complex oxidation reaction occurs with the production of toxic radicals, the vital elements of the cell would soon be destroyed. The accumulation of hydroperoxides, for instance, requires the intervention of catalase, tocopherol, selenium, or GSH (Bermond 1990). As a result of these mechanisms, a balance between prooxidant reactions and antioxidant systems should be established. The efficiency of the integrated antioxidant system in plants has an important effect on the status of the remaining constituents in food of plant origin.

The purpose of this study was to examine the GSH/GSSG ratio as a potential index of balance between oxidative stress and antioxidant systems of selected cereal grains and to show the contribution of GSH to total antioxidant status (TAS) activity. The potential hierarchy of resistance of different cereal grains against oxidative stress was determined and confirmed by the ability of the cereal grain extracts to scavenge superoxide anion radicals in vitro.

MATERIALS AND METHODS

Samples

Single cereal grain samples grown in 1997 were obtained from local plant breeding station in northeastern Poland. The samples included two cultivars of wheat (*Triticum aestivum* L.) (winter cv. Almari and spring cv. Henika); two cultivars of barley (*Hordeum vulgare* L.) (winter cv. Gregor and spring cv. Mobek); one cultivar of rye (*Secale cereale* L.) (cv. Dańkowskie Złote), one cultivar of oat (*Avena sativa* L.) (cv. Sławko), and one cultivar of buckwheat (*Fagopyrum esculentum* Moench) (cv. Kora). Samples from two replicates were chosen for the analyses. Whole-grain samples were ground in a laboratory mill type WZ-1. Ground samples were stored at 4°C no longer than 24 hr. All samples were analyzed in duplicate for dry matter, nitrogen, and ash using the standard methods (AOAC 1980). The hull content of the oat, barley, and buckwheat was determined by hulling seed samples (5 g) by hand and subtracting hull weight from total weight. Results are given as the means of triplicates with the standard deviation.

Extraction Methods

Extraction was conducted as described in Smith et al (1988). Cereal grains (3 g) were ground in an electric coffee mill. The flour was transferred to a centrifuge tube and mixed with phosphate buffer (15 mL, 0.2M, with EDTA, 1 mM, pH 7.5) and potassium

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chloride (KCl, 0.33 g). The mixture was homogenized for 30 sec using a polytron homogenizer at full speed. Polyvinylpyrrolidone (PVPP, 0.25 g) was added, and after thorough mixing, the mixture was centrifuged (2,000 × g for 10 min at 4°C). After recentrifugation, the supernatant was kept on ice and assayed for soluble protein, total phenolic compounds, GR activity, GSH and GSSG content, and TAS of the extracts.

The cold-water extracts of ground cereal grains were prepared according to standard methods (AOAC 1990). Water (100 mL) was gradually added to 10 g of sample at ≈0°C and the mixture was continuously shaken. Then, the sample was left to stand for 40 min at 0°C, shaking occasionally, centrifuged at 4000 rpm with centrifuge type MPW-360. Finally, the supernatant was filtered rapidly, filtered again until clear, and then the filtrate was lyophilized. The filtrate was used to authenticate the TAS and GSH/GSSG ratio values as well as to check for any trace amounts of vitamin C.

Analytical Procedures

TAS was measured by the ABTS (2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate)) method (Miller and Rice-Evans 1996), which is based on the relative ability of antioxidants to scavenge the radical cation of ABTS (ABTS⁺), a green chromophore generated from interaction between activated metmyoglobin, hydrogen peroxide, and ABTS. The extent of the quenching of ABTS⁺ absorption at 600 nm by antioxidants in the medium under investigation was compared with standard amounts of the Trolox equivalents. Trolox is a water-soluble analogue of vitamin E. Assays were conducted at 37°C using the total antioxidant status kit (cat. no. NX2332, Randox Laboratories, Ltd., UK). This test requires 20 µL of sample with a read time of 3 min on a Beckman spectrophotometer DU 7500.

GSH and GSSG were determined according to the spectrofluorometric method of Hissin and Hilf (1976). This method was based on the reaction of *o*-phthalaldehyde (OPT) as a fluorescent reagent with GSH at pH 8.0 and GSSG at pH 12.0. GSH was complexed to N-ethylmaleimide (NEM) to prevent interference of GSH with measurement of GSSG. To determine GSH content, 60 µL of the extract was mixed with 1.84 mL of 0.1M sodium phosphate and 0.005M EDTA buffer (pH 8.0), and 100 µL of the OPT solution, containing 100 µg of OPT in reagent-grade absolute methanol. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette. Fluorescence at 420 nm was determined with the excitation at 350 nm. A series of GSH standards were prepared in phosphate and EDTA buffer, pH 8.0, ranging from 0.033 to 6.51 nmol/60 µL. To determine GSSG content, 200 µL of extract was incubated at room temperature with 200 µL of 0.04M NEM for 30 min to interact with GSH in the sample. Then 1.8 mL of 0.1M NaOH and 100 µL of the OPT solution were added to 100 µL of the incubation mixture. The same procedure for GSH assay was used for the measurement of GSSG. A series of GSSG standards were prepared in 0.1M NaOH, ranging from 0.008 to 0.816 nmol/100 µL. The assays were performed using a Perkin-Elmer LS 50 B luminescence spectrometer. The data were calculated as cereal grains (g) on a wet basis.

GR was assayed with a glutathione reductase kit (cat. no. GR 2368, Randox). The assay was based on the fact that GR catalyzes the reduction of GSSG in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is oxidized to NADP⁺. From the change in absorbance at 340 nm, the amount of GSSG reduced to GSH was calculated. GR (1U) reduces 1 µmol of GSSG/min at 30°C, pH 7.5. The detection limit was 0.05 U/g of grounded cereal grain. The data were recalculated as cereal grains (g) on a wet basis.

Total phenolic compounds content was determined according to Naczk and Shahidi (1989). The data were calculated on (±) catechin equivalents. The concentration of protein in extracts was determined by the dye-binding method of Bradford (1976). Bovine serum albumin (BSA) was used as the standard. The assays were performed using a Beckman spectrophotometer DU 7500. The data were calculated as cereal grains (g) on a wet basis.

Vitamin C (ascorbic acid, AH₂) was determined in the lyophilized cold-water extracts using the HPLC method of Iriyama et al (1984). Lyophilized samples were diluted with 2% metaphosphoric acid to obtain final concentration of exactly 1 mg/mL. Samples (500 µL) were extracted with 100 µL of HPLC-grade heptane. After mixing and centrifuging at 13,000 × g for 5 min, the heptane layer was carefully removed. If the supernatant remained opaque, an additional 100 µL of heptane was added and the procedure was repeated. This lipid extraction step was repeated until the sample was clear. The analysis was done with a 25-cm Apex II

TABLE I
Means of Hull Content, Protein Content, Crude Ash, and Dry Matter in Total Grain of Selected Cereals

Cultivar	Hull (%)	Protein (%)	Crude Ash (g/kg, dwb)	Dry Matter (%)
Wheat				
Almari	...	15.4	19.4	86.6
Henika	...	14.6	17.0	87.2
Barley				
Mobek	7.6	12.2	20.6	86.9
Gregor	9.8	14.8	20.8	85.5
Rye				
Dańkowskie Złote	...	9.6	17.9	86.1
Oat				
Sławko	23.8	12.1	23.7	87.3
Buckwheat				
Kora	23.6	14.9	23.6	87.9

TABLE II
Content of Reduced (GSH) and Oxidized (GSSG) Glutathione, GSH/GSSG Ratio, and Activity of Glutathione Reductase (GR) in Extracts of Selected Cereal Grains^a

Cultivar	GSH (nmol/g)	GSSG (nmol/g)	GSH+GSSG (nmol red. eq/g)	GSH/GSSG Ratio	GR (U/g)
Wheat					
Almari	387.7 ± 7.2	56.7 ± 0.7	501.1	6.84	1.28 ± 0.04
Henika	390.5 ± 5.5	51.8 ± 0.3	494.1	7.54	1.31 ± 0.04
Barley					
Mobek	185.9 ± 3.9	40.5 ± 0.1	266.9	4.59	1.72 ± 0.06
Gregor	186.6 ± 0.9	33.8 ± 0.2	254.2	5.52	1.73 ± 0.03
Rye					
Dańkowskie Złote	268.5 ± 3.2	56.1 ± 0.9	380.7	4.79	1.74 ± 0.09
Oat					
Sławko	155.7 ± 4.9	81.5 ± 1.6	318.7	1.91	1.11 ± 0.11
Buckwheat					
Kora	529.6 ± 5.7	47.6 ± 0.2	624.8	11.13	3.96 ± 0.16

^a Determined by spectrofluorimetry.

ODS column and a mobile phase of 0.2 mol/L of K_2HPO_4 , 0.25 mmol/L of octanesulphonic acid (pH 2.1). Electrochemical detection used a current sensitivity of 0.1 nAmps, and concentration was determined with reference to a standard curve of 0–6.3 μM of AH_2 .

To determine the content of GSH and GSSG by the enzymatic method, the lyophilized cold-water extract samples were diluted with 0.01M PBS (phosphate buffered saline, pH 7.4) to obtain a final concentration of exactly 1 mg/mL. Total glutathione (GSx) was determined by the enzyme recycling method (Tietze 1969) modified for use in a microplate reader. The assay reaction mixture contained 2.8 mL of 1 mmol/L 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), 3.75 mL of 1 mmol/L reduced β -nicotinamide adenine dinucleotide phosphate (NADPH), 20 units of GR, and 5.85 mL of 100 mmol/L Na_2HPO_4 and 1 mmol/L of EDTA, pH 7.4. Standards (50 μL of 0–165 pmol/50 μL of GSSG) or samples were placed in a microtitre plate and 100 μL of reaction mixture was added. The level of glutathione was calculated by following the formation of 2-nitro-5-thiobenzoic acid (TMB) at 405 nm, over a 2-min period at 30°C using an EF 340 microplate reader (Biotek Instruments). Concentrations in test samples were calculated from the GSSG standard curve. The levels of GSSG were assayed as above, however, the GSH in solutions was conjugated before the assay. Conjugation was achieved by adding 5 μL of 2-vinyl pyridine (Aldrich Chemical Co.) to 130 μL of test sample or standard and incubating for 1 hr at room temperature. Following the assay, the amounts of GSH were calculated by subtracting GSSG from GSx concentrations.

The lyophilizates of cold-water extract from cereal grains were also used to determine the TAS followed by dilution with PBS (1 mg/mL) as described previously. Thus, all data reflecting the cold-

water extracts were recalculated on milligrams of initial lyophilizate weight.

To evaluate the SOD scavenging activity of cereal grain extracts, the ground samples were extracted in duplicate with 0.01M phosphate buffer, pH 7.0 (5 mL/g of sample) during 2 hr of shaking at 37°C. They were then centrifuged at $12,000 \times g$ with a Beckman GS-15 R centrifuge, and the fresh supernatants were used for further determinations. The SOD scavenging activity of the extracts was measured using a commercial kit (RANSOD). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD scavenging activity of the investigated extracts was then measured by the degree of inhibition of this reaction at 505 nm by using UV/VIS spectrophotometry. The SOD with the activity of 5.4 U/mL was used as a standard and was supplied as a part of reagent kit. In general, one unit of the 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%. Extrapolation of 50% inhibition values in samples allows calculation of enzyme activity. The percent of reaction inhibition was plotted against \log_{10} of different SOD activities (SOD/mL), giving a standard curve, and then SOD activity of the sample was calculated as SOD unit/mL of investigated extract. The results were finally recalculated on milligrams of soluble protein assayed according to the dye-binding method of Bradford (1976). The assays were performed at 37°C using a Beckman spectrophotometer DU 7500. The test requires 50 μL of sample and a read time of 3 min.

Statistical Analysis

Correlation analyses between data for two different assays of GSH and GSSG and between TAS values from two different methods of sample preparation was performed. The Pearson correlation coefficients were calculated. Correlation analysis was also made between the GSH/GSSG ratio obtained for cold-water extracts of cereal grains and the ability of these extracts to scavenge the superoxide anion radicals in vitro.

RESULTS

The means of hull content, protein content, crude ash (g/kg), and dry matter in total grain of selected cereals are shown in Table I. GSH, GSSG, and GSH/GSSG ratio determined by the spectrofluorimetric method, and GR activity are shown in Table II. The activity of GR was investigated in cereal grain extracts to check whether alterations in its activity could account for the changes in the GSH/GSSG ratio. Correlation between GSH/GSSG ratio and GR activity was $r = 0.36$.

TABLE III
Total Antioxidant Activity (TAS) and Content of Total Phenolic Compounds and Soluble Proteins of Cereal Grains

Cultivar	TAS (μmol Trolox ^a /g)	Total Phenolic Compounds (mg of catechin/g)	Total Soluble Proteins (mg of proteins/g)
Wheat			
Almari	1.64 ± 0.03	1.59 ± 0.11	17.5 ± 0.8
Henika	0.43 ± 0.04	1.18 ± 0.02	18.0 ± 0.3
Barley			
Mobek	1.48 ± 0.05	1.99 ± 0.02	17.2 ± 0.5
Gregor	1.92 ± 0.06	2.06 ± 0.02	19.5 ± 0.4
Rye			
Dańkowskie Złote	1.92 ± 0.02	1.18 ± 0.02	16.3 ± 0.1
Oat			
Sławko	4.33 ± 0.05	1.34 ± 0.01	11.7 ± 0.5
Buckwheat			
Kora	8.46 ± 0.07	3.61 ± 0.04	29.5 ± 0.8

^a Water-soluble analogue of vitamin E.

TABLE IV
Content of Reduced (GSH) and Oxidized (GSSG) Glutathione, GSH/GSSG Ratio, and Values of Total Antioxidant Status (TAS) in Lyophilized Cold-Water Extracts of Cereal Grains^a

Cultivar	GSH (nmol/mg)	GSSG (nmol/mg)	GSH + GSSG (nmol/mg)	GSH/GSSG Ratio	TAS (μmol of Trolox ^b /mg)
Wheat					
Almari	0.735	0.287	1.309	2.56	0.027
Henika	1.594	0.389	2.372	4.09	0.023
Barley					
Mobek	1.080	1.156	3.392	0.93	0.059
Gregor	1.729	1.126	3.981	1.54	0.064
Rye					
Dańkowskie Złote	1.239	0.084	1.407	4.75	0.003
Oat					
Sławko	0.534	1.366	2.434	2.56	0.032
Buckwheat					
Kora	4.366	0.318	5.002	13.73	0.138

^a Determined by enzymatic method.

^b Water-soluble analogue of vitamin E.

The TAS values for extracts from investigated cereal grains and the contents of total phenolic compounds and soluble proteins in total grain are shown in Table III. The total phenolic compounds in investigated extracts ranged from 1.18 to 3.61 mg of catechin/g of cereal grain, although the total soluble protein was about tenfold higher and ranged from 11.7 to 29.5 mg/g of cereal grain. The total soluble proteins include cytoplasmic protein. The soluble proteins contain also all enzymes of endosperm origin and some amounts of insoluble protein. According to the extraction procedure by Smith et al (1988), the final extract should contain albumin and globulin. Globulin contains high amounts of thiol groups and disulfide groups, ≈50 and 20% of total participation of this group in flour proteins. In contrast, water-soluble extract, in general, contains only albumin.

Correlation between TAS values and the content of total phenolic compounds and total soluble protein were $r = 0.81$ and 0.64 , respectively. Correlation between GSH/GSSG ratio and TAS values was $r = 0.46$, depending on the extraction system used.

The efficiency of water extraction was 5.7, 5.0, 5.2, 4.6, 9.9, 5.3, and 7.8 % (w/w), respectively, for Almari, Henika, Gregor, Mobek, Dańkowskie Złote, Sławko, and Kora. The TAS values of cold-water extracts calculated on milligrams of lyophilized extracts, the content of GSH, GSSG, and total glutathione, and values of GSH/GSSG ratio determined by the enzymatic method are shown in Table IV. Correlation between GSH/GSSG ratio and TAS values was $r = 0.70$.

The values of items indicated in Table IV are different from those listed in Table II. because we used different extracts to determine the content of GSH and GSSG and different analytical methods. Using the extraction system of Smith et al (1988), the extract may contain albumin, globulin, and a wide spectrum of enzymes. When we used the spectrofluorimetric method for estimating the level of GSH and GSSG, the thiol groups originating from globulin may have interfered with this assay and, as a result, they could have increased the values of GSH and GSSG, giving higher values of GSH/GSSG ratio when compared with water extracts containing mainly albumin and enzymes (Fig. 1). Thus, it may be suggested that the more specific enzymatic method for the determination of GSH and GSSG levels using only water extract is a better method for establishing the GSH/GSSG ratio in selected cereals. However, the correlation between values of GSH/GSSG ratio obtained by this assay and by the spectrofluorimetric assay was $r = 0.79$. Moreover, the correlation between values of TAS determined for cereal grain extracts obtained according to the method of Smith et al (1988) and for cold-water extracts was also $r = 0.79$.

Using a high-sensitivity assay, no trace amounts of vitamin C were detected in cold-water soluble extracts of investigated cereal grain samples.

The activity of SOD in extracts of investigated cereals grains is shown in Fig. 2. Correlation between GSH/GSSG ratio determined for cereal grain extracts obtained according to the method of Smith et al (1988) and for cold-water extracts versus the activity of SOD was $r = -0.51$ and $r = -0.85$, respectively.

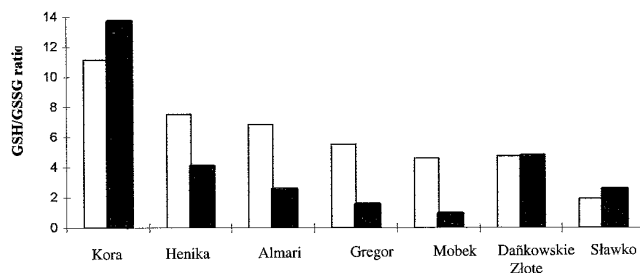


Fig. 1. Potential hierarchy of selected cereal grains against oxidative stress. □ = Reduced and oxidized (GSSG) glutathione (GSH/GSSG) ratio determined by spectrofluorimetry; ■ = GSH/GSSG ratio determined by enzymatic method.

Unfavorable environmental conditions such as low temperatures, high light intensities, drought stress, and air pollution, as well as an increased CO₂ concentration in the atmosphere can increase production of reactive oxygen species in plant tissues. Recently, the question has been raised whether high antioxidant levels increase stress resistance. The hypothesis is supported by the observed linkage between stress factors and a rise in reactive oxygen species and components of the antioxidative defense system such as glutathione and ascorbate. The antioxidative defense system is thus used as an indicator of oxidative stress (Foyer et al 1994).

In plant cells within different cell compartments, including the cytoplasm, chloroplast, mitochondria, and apoplast, antioxidative substrates such as glutathione and ascorbate together with antioxidative enzymes such as SOD, peroxidases, and catalases interact in a series of reactions to remove the toxic oxidants and to regenerate the reduced (functional) state of the antioxidants (Foyer and Halliwell 1976, Asada 1992). The best-studied phenomenon in plant cells on the participation in an oxygen-free-radical-scavenging pathway in the chloroplast is known as the Halliwell-Asada cycle. This pathway ensures the protection of the chloroplast organelle from oxidative damage during photosynthesis and involves ascorbate, glutathione, and several ascorbate-dependent enzymes. In this pathway, SOD scavenges superoxide anion radicals and produce hydrogen peroxide. The detoxification of H₂O₂ involves ascorbate (Groden and Beck 1979) and specific ascorbate peroxidases (APOD) that, in turn, produce monodehydroascorbate radicals (Asada 1992). Monodehydroascorbate radicals are reduced to ascorbate by the activity of monodehydroascorbate radical reductase (MDARR) by the use of NADH or NADPH as reductant or undergo self-disproportionation yielding ascorbate and dehydroascorbate (Hosain et al 1984). Dehydroascorbate is reduced by glutathione in both enzymatic and nonenzymatic reactions. Reduction of glutathione disulfide is achieved by GR activity by the oxidation of NADPH (Foyer and Halliwell 1976). Other systems with the ability to detoxify activated oxygen species are catalases located in the peroxisomes which reduce H₂O₂ generated during photorespiration, and nonspecific peroxidases located in the cell walls and in the cytosol (Mejnartowicz 1984). Because no trace amounts of vitamin C were detected in cold-water soluble extracts of investigated cereal grain samples, we concluded that GSH may play an important role in the antioxidant defense system in cereal grains. An increase in the GSSG/GSH ratio leads to the formation of mixed protein-disulfides (protein thiolation). We also concluded that the Halliwell-Asada cycle (Asada 1992) does not work in mature cereal grains and other biochemical systems, and synergism that may occur between components of the cereal grain antioxidant screen, play an important role in establishing the balance between prooxidant reactions and antioxidant mechanism of cereal grains. The lack of correlation between GSH/GSSG ratio and GR suggests that there were no indications that changes in enzyme activity

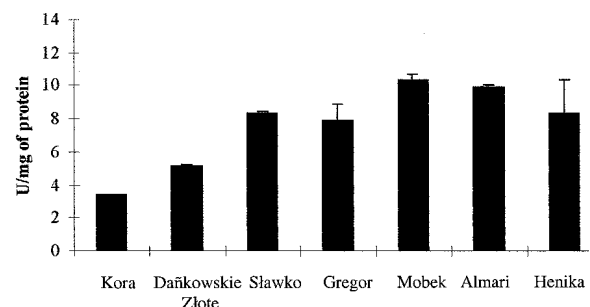


Fig. 2. Superoxide dismutase (SOD) activity in selected cereal grain extracts. Error bars ± standard deviation.

could explain the alteration in GSH/GSSG ratio between different cereal mature grains. However, there are limited conditions for GR functioning in the dry grain. In contrast, a progressive increase was observed during germination of barley grains aging (Pheifer and Briggs 1995). There is little information related to cereal grain antioxidants and their contribution to the defense system.

In this study, the level of glutathione and its oxidized form was quantified as a constituent part of the radical scavenging system of mature cereal grains. The cereal grains contain a small amount of peptides. GSH is a water-soluble, biologically active, tripeptide of increasing interest. Cereal grains contain a small amount of glutathione (0.01–0.05% of dry matter). The contents of GSH (recalculated for dry matter) were 0.005–0.019% for investigated cereal grains, giving values similar to those reported in literature (Grzesiuk and Kulka 1981). We were not able to show any trace amounts of ascorbic acid in mature cereal grains, so GSH appears to play an important role in many oxidative-reduction processes, especially as a coenzyme of some dehydrogenases. Because it has been possible to measure both forms of glutathione (GSH and GSSG), it is also possible to indicate how far the oxidative stress is developed in mature grain and what the resistance is of grains against damaging oxidative conditions. Given this, GSH/GSSG ratios may indicate a hierarchy among different cultivar and variance of cereal grains against damages caused by reactive oxygen species. Our data indicate a potential hierarchy of resistance of investigated cereals against oxidative stress as: buckwheat > wheat > barley ≈ rye > oat (Fig. 1) for the content of the main water-soluble antioxidants. This resistance was strongly negative correlated with the ability of the investigated extracts to scavenge the superoxide radical anions *in vitro*, which may suggest that the resistance of cereal grain against damages caused by reactive oxygen species is strongly connected with GSH and GSSG levels in the grains. O₂⁻ reacts with many thiols, but the rate constants for these reactions are low, and very high thiol concentrations are required to compete with nonenzymic dismutation and achieve significant scavenging (Winterbourn and Metodiewa 1994). Our findings indicate that the GSH/GSSG ratio may be a useful tool for monitoring the storage conditions of cereal grains. It may be also useful in researches concerning the problems of dormancy, germination, and grain aging (Pheifer and Briggs 1995). Our data point out a need of further studies on polyphenol content in cereal grains (Lasztity 1998, Watanabe 1998) currently ongoing in our laboratory.

CONCLUSIONS

The GSH/GSSG ratio can be used as a potential index of oxidative stress in different cereal grains. It may also reflect the resistance of different cereal cultivars against oxidative damage.

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