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Analytical Methods

Antioxidant activity of rye bran alkylresorcinols and extracts from whole-grain cereal products

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ABSTRACT

The antioxidant properties of rye bran alkylresorcinols (C15:0–C25:0) and extracts from whole-grain cereal products were evaluated using their radical-scavenging activity on DPPH and the chemiluminescence method (CL). DPPH radical reduction varied from ~10% to ~60% for the alkylresorcinol homologues at concentrations from 5 to 300 μM and was not dependent on the length of the alkyl side chain of the particular homologue. Differences in the EC_{50} values for the studied compounds were not statistically significant, the values varying from 157 μM for homologue C23:0 to 195 μM for homologue C15:0. Moreover, values of EC_{50} for all the alkylresorcinol homologues were significantly higher than those for Trolox and α-, δ-, and γ-tocopherols, compounds with well-defined antioxidant activity and used as positive controls. CL inhibition was evaluated for all the tested alkylresorcinol homologues at concentrations of 5 and 10 μM and varied from ~27% to ~77%. Similar to the DPPH method, the slight differences in CL inhibition suggest that the length of the alkyl side chain had no major impact on their antioxidant properties. The extracts from whole-grain products were added to the DPPH and CL reaction systems and their antioxidant activities were tested and compared with the total amount of alkylresorcinols evaluated in the extracts. DPPH radical and CL reduction for the whole-grain products varied from ~7% to ~43% and from ~37% to ~91%, respectively. A clear relationship between DPPH radical and CL reduction levels and the amount of total alkylresorcinols was obtained for whole-grain breakfast cereals, in which the reduction level decreased in the order rye > wheat > mixed > barley. Therefore it may be considered that the antioxidant activity of alkylresorcinols could be of potential importance to the food industry, which is continuously searching for natural antioxidants for the protection of food products during their processing and storage.

1. Introduction

There is a growing demand for protecting food against fat oxidation and supplementing of the daily food intake with antioxidants. The oxidation of unsaturated fatty acids in edible oils and fats is a crucial problem in the food industry (Reische, Lillard, & Eintenmiller, 1998). There are many reasons why the addition of antioxidants is usually required to maintain the natural appearance, aroma, and taste of food during storage (Finley & Given, 1986). As a rule, synthetic antioxidants are used for this purpose. However, their use involves the risk of unpredictable negative side effects. Many reports on synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), suggest their toxic and carcinogenic properties in living organisms (Ames, 1983; Baardseth, 1989). Concern for the safety of food and the potential effects of synthetic additives on health resulted in demands to use other effective but natural antioxidants (Reische et al., 1998). Of the various systems protecting against peroxidation above the physiological level, lipid soluble chain-breaking antioxidants play a significant role. The best known natural lipophilic chain-breaking antioxidants are tocopherols (vitamin E), their analogs, and synthetic phenolic antioxidants (Barclay, Vinquist, Mukai, Itoh, & Morimoto, 1993; Fukuzawa & Fujii, 1992). Chemically, these compounds are phenolic derivatives with a long saturated isoprenoid side chain. Alkylresorcinols (also known as resorcinolic lipids), compounds which were demonstrated to occur in cereal grains and related materials such as whole-grain cereal products, are similar to tocopherols except that they have a straight aliphatic hydrocarbon side chain and a single phenolic ring (Fig. 1). The length of the alkyl side chain of alkylresorcinols in cereal grains varies from 13 to 27 carbon atoms. The side chain is usually saturated, but unsaturated and oxygenated chain analogues have also been reported. The chemistry, nutritional effects, and bioactivities of alkylresorcinols are described in more detail in reviews by Kozubek and Tyman (1999) and Ross, Kamal-Eldin, & Åman, 2004.

Abbreviations: CL, chemiluminescence; DM, dry matter; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; AAPH, 2,2′-azobis (2-methylpropionamidine) dihydrochloride.

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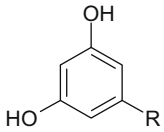

Fig. 1. Chemical structure of the alkylresorcinols. *R* – the alkyl side chain of the alkylresorcinols, *R* = 15, 5-*n*-pentadecylresorcinol (C15:0); *R* = 17, 5-*n*-heptadecylresorcinol (C17:0); *R* = 19, 5-*n*-nonadecylresorcinol (C19:0); *R* = 21, 5-*n*-heneicosylresorcinol (21:0); *R* = 23, 5-*n*-tricosylresorcinol (C23:0), *R* = 25, 5-*n*-pentacosylresorcinol (C25:0).

Several studies claim that alkylresorcinols exhibit an ability to protect cellular lipid components from oxidative processes (Hładyszowski, Zubik, & Kozubek, 1998; Kozubek & Nienartowicz, 1995; Nienartowicz & Kozubek, 1993; Struski & Kozubek, 1992; Winata & Lorenz, 1996). Compared with α-tocopherol, alkylresorcinols are very weak antioxidants *in vitro*; for example, homologue C15:0 was a much stronger antioxidant than resorcinol, but about 10-times weaker than α-tocopherol (Kamal-Eldin, Pouru, Eliasson, & Åman, 2001) and had a relatively lower antioxidant activity than the other alkylphenols tested (Sumino, Sekine, Ruangrungsi, Igarashi, & Ikegami, 2002). However, alkylresorcinols were effective antioxidants in phospholipid bilayers, indicating that the alkyl side chain may be important in this case (Hładyszowski et al., 1998). It has been demonstrated that long-chain alkylresorcinol homologues prevent Fe^{2+}-induced peroxidation of fatty acids and phospholipids in liposomal membrane as well as autoxidative processes in triglycerides and fatty acids (Struski & Kozubek, 1992; Hładyszowski et al., 1998; Nienartowicz & Kozubek, 1993). Long-chain alkylresorcinol mixtures also prevent the peroxidation of lipids in natural membranes. At micromolar concentrations, bacterial and cereal grain alkylresorcinols completely inhibited Fe^{2+}-ascorbic acid and Fe^{2+}-NADPH-induced peroxidation of liver microsomes and fragments of the sacroplasmic reticulum (Erin et al., 1987; Zubik, Hladyszowski, Czucha, & Kozubek, 1996). Long-chain alkylresorcinols isolated from rye grains have also been effective in protecting of the erythrocyte membrane against hydrogen peroxide-induced oxidation (Kozubek & Nienartowicz, 1995). Moreover, alkylresorcinols possess antigenotoxic and antioxidant activities in biological systems under *in vitro* conditions; they are, for example, able to significantly inhibit copper-mediated oxidation in human LDL (low-density lipoprotein) *in vitro* (Parikka, Rowland, Welch, & Wähälä, 2006).

The antioxidant properties of cereal alkylresorcinols could also be of potential importance to the food industry, which is continuously searching for natural antioxidants for the protection of food products during their processing and storage. It may be considered that the antioxidant activity of alkylresorcinols is responsible for the longer shelf-life of whole-grain bread in comparison with white bread, in which the total alkylresorcinol content is negligible. In this study we used two simple test models, the DPPH and luminescence methods, to evaluate the antioxidant activity of all major rye bran alkylresorcinol homologues (C15:0–C25:0). The effects of the alkylresorcinol homologues were compared with those of α-, δ-, and γ-tocopherol. We also studied the antioxidant activity of extracts from selected whole-grain breads and breakfast cereals commonly available on the Polish market. We tried to find correlation between their antioxidant properties and the total amount of alkylresorcinols determined in the extracts.

2. Materials and methods

2.1. Chemicals and reagents

Acetone, methanol, and 1-propanol were from Chempur (Piekary Slaskie, Poland). Water and methanol for preparative HPLC were purchased from POCH SA (Gliwice, Poland). Acetic acid was from Standard (Lublin, Poland). The diazotised salt Fast Blue B (BF_4 salt) was a gift from Chemapol (Prague, Czech Republic). 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), Tris(hydroxymethyl)aminomethan, (+)-α-tocopherol (purity of 97%), (+)-δ-tocopherol (purity of 90%) and (+)-γ-tocopherol (purity of 97%) were purchased from Sigma Aldrich (Poznan, Polska). Individual homologues of alkylresorcinols were isolated from rye bran as described by Kozubek (1985). The extracted alkylresorcinols were hydrogenated (catalytic hydrogenation with hydrogen and platinum over carbon) to obtain a mixture of saturated homologues. The hydrogenated homologues were subsequently subjected to preparative HPLC to obtain individual species with side chains of 17–25 carbon atoms in length. The HPLC system consisted of a model 600 HPLC pump with 996 PDA detector, all from Waters (Milford, MA, USA). The HPLC method employed a Silica RP 18.5 μm (25 × 250 mm) column (Knauer, Berlin, Germany) with water (A) and methanol (B) as the mobile phase at a flow rate of 4 ml/min and UV detection at 280 nm. For homologue separation, the mobile phase gradient was: 94 to 100% B in 90 min, hold at 100% B for 10 min, 100 to 94% B in 1 min, and finally hold at 94% B for 10 min. A Waters Millennium Version 3.20 processing module was used for recording and processing the chromatograms. Homologue C15:0 was isolated from hydrogenated Cashew Nutshell Liquid (CNSL) that was a gift from Cardolite B.V. (Ghent, Belgium). The purity of the isolated C15:0 homologue and the separated alkylresorcinol homologues was over 98% as confirmed by GC/MS analysis (Hewlett–Packard HP 6890 gas chromatograph coupled with an HP 5973 mass detector). GC separation was performed on an HP-5MS column (length: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 μm, Hewlett-Packard, Avondale, PA, USA) with the following temperature program: 130 °C (1 min), 230 °C (5 min), and 320 °C (14 min). The injector temperature was 250 °C. The extracts were analysed using a 1-μl injection at a split of 1:20. The mass spectrometer was set at an ionising voltage of 70 eV. Commercial whole-grain cereal products were purchased from a local supermarket (Wroclaw, Poland).

2.2. Extraction of alkylresorcinols from cereal grain material

One-gram samples of whole-rye and wheat-bran and whole-grain products were used. The samples were coarsely ground in a coffee grinder (Niewiadow, Poland) at a speed of 450 rpm for 5 min and then placed in 50-mL tubes and extracted by continuous shaking for 48 h at room temperature with 40 ml of acetone (Kozubek, 1985). The extracts were filtered through paper filters and evaporated to dryness at 45 °C in a vacuum pump (KNF Neuberger Laboport, Germany). The dry residues from all the extractions were then dissolved in methanol (1 ml). All values in this study are reported on a dry matter (DM) basis. The DM content was determined by drying the samples in an oven at 105 °C for 8 h followed by cooling and then weighing. All DM analyses were carried out in triplicate.

2.3. Colorimetric method for determining alkylresorcinols

Total alkylresorcinol content in the processed grain material was determined by the method involving Fast Blue B BF_4 (Tłuścik, Kozubek, & Mejbaum-Katzenellenbogen, 1981). Briefly, 10 μl of each extract sample was put into a glass tube and the solvent was then removed under a stream of gaseous nitrogen. The total content of alkylresorcinols in the cereal grain material was estimated using the appropriate calibration curve prepared with the alkylresorcinols from rye (for whole-rye products) and wheat (for

whole-wheat products) as the reference compound in the range of 1–10 μg.

2.4. DPPH radical scavenging or hydrogen-donating method

The effect of rye bran alkylresorcinols and extracts from the whole-grain cereal products on DPPH radical was estimated according to the procedure described by Brand-Williams, Cuvelier, and Berst (1995). Tocopherols alpha, delta, and gamma (final concentrations of 5, 10, and 50 μM) were used as control antioxidants. All the spectrophotometric data were acquired using a Schimadzu UV 2401 PC Probe spectrophotometer (Kyoto, Japan) in a 10-mm plastic cuvette. Briefly, 1 ml of freshly prepared 200 μM DPPH in methanol was added to the sample (final volume of 2 ml). An appropriate volume of homologues C15:0–C25:0 (stock solution of 1 mg/ml in methanol) was added to the sample to a final concentration of 5, 10, 30, 50, 100, or 300 μM. In the case of the extracts from whole-grain cereal products, 100 μl of a methanolic solution of the particular extract was added to the sample. The absorbance was read at 515 nm after 3600 s at 37 °C. Methanol was used as a blank for all the readings. The absorbance of the DPPH radical without any antioxidant (control) was measured before each test. The EC_{50} values were measured as the concentration of the compound which inhibited 50% of DPPH radical. The percentage of DPPH reduction was calculated using the equation:

$$\%\ \text{reduction} = 1 - \frac{A_t}{A_0} \times 100 \quad (1)$$

Where A_t represents the absorbance after 3600 s of measurement and A_0 the absorbance at the beginning of measurement.

2.5. AAPH-induced luminol chemiluminescence method

The chemiluminescence (CL) measurements were conducted according to the procedure described by Krasowska et al. (2001). Photons were counted in an EG&G Berthold LB96p microplate luminometer (Bad Wildbad, Germany) at 30 °C. The experiments were performed in a final volume of 250 μl on white microplates in 0.1 M Tris, pH 9.0. Twenty-five μl of freshly prepared AAPH was put into a microplate well. A 1-mM stock solution of luminol was diluted four times with distilled water. One hundred μl of the diluted solution was automatically injected into the sample at the beginning of the measurement and the tested compound 60 s later. A 40-mM stock solution of AAPH was prepared in distilled water. The 1-mM luminol stock solution was obtained by dissolving it in 10 mM NaOH. One-mg/ml stock solutions of homologues C15:0–C25:0 were prepared in methanol. The final concentrations of the particular homologues in the experiments were 5 and 10 μM. In the case of the extracts from cereal grain products, 100 μl of the particular extract was diluted in 10 mL of distilled water. Tocopherols alpha, delta, and gamma (final concentrations of 5 and 10 μM) were used as control antioxidants. The final concentrations of methanol were 0.025-0.0125%. These methanol concentrations did not affect luminescence (data not shown). The percentage of CL reduction was calculated using the equation:

$$\%\ \text{reduction} = \%k_0 - \%k \quad (2)$$

Where $\%k_0$ is the percentage of the control before adding antioxidant and $\%k$ the percentage of the control after 31 s of measurement.

2.6. Statistical analysis

Statistical analysis included Student's *t* test and was processed using the software program STATISTICA (Microsoft, Statsoft). All analyses were carried out in triplicate. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Antioxidant activity of alkylresorcinol homologues C15:0–C25:0

A suitable method for obtaining information about the ease and kinetics of hydrogen atom donation by an antioxidant is the reaction of the antioxidant with the DPPH radical. In our experiments the reaction with DPPH radical according to the procedure described by Brand-Williams et al. (1995), which is a relatively stable free radical, was used to determine the hydrogen-donating abilities of alkylresorcinol homologues with alkyl side chain lengths from 15 to 25 of carbon atoms and these were compared with those of α-, δ-, and γ-tocopherol. Also, the effect of extracts from whole-grain cereal products on DPPH radical was estimated. DPPH radical reduction was evaluated for all the tested compounds at various concentrations and resulted in tested compound/DPPH radical (mol/mol) ratios of 0.05, 0.1, 0.3, 0.5, 1, and 3 for the alkylresorcinol homologues and 0.05, 0.1, and 0.5 for the tocopherols, which were used as control antioxidants. As shown for the alkylresorcinol homologues (Fig. 2), the particular homologues had similar influence on the level of DPPH radical reduction. Some studies considered that, besides the phenolic ring, the length of the aliphatic side chain plays an important role in the antioxidant activity of resorcinolic lipids. For example, the antioxidant activity of orcinol (C1:0) occurs at a concentration at least one order of magnitude higher than that of the alkylresorcinol homologue C15:0 and higher homologues isolated from cereal grains (Hładyszowski et al., 1998; Zubik et al., 1996). In our study, regardless of the concentration, alkyl chain length seems to have had only a slight impact on the antioxidant activity of the alkylresorcinol homologues. Different experimental models and conditions could be the most likely explanation for the different results. Hładyszowski et al. (1998) obtained results in which the antioxidant activity of alkylresorcinols increased with increasing chain length due to increased incorporation in membranes with increased lipophilicity. In contrast to our experiment, they worked with liposomal emulsion of phosphatidyl choline. Thus in this case the observed antioxidant activities of dif-

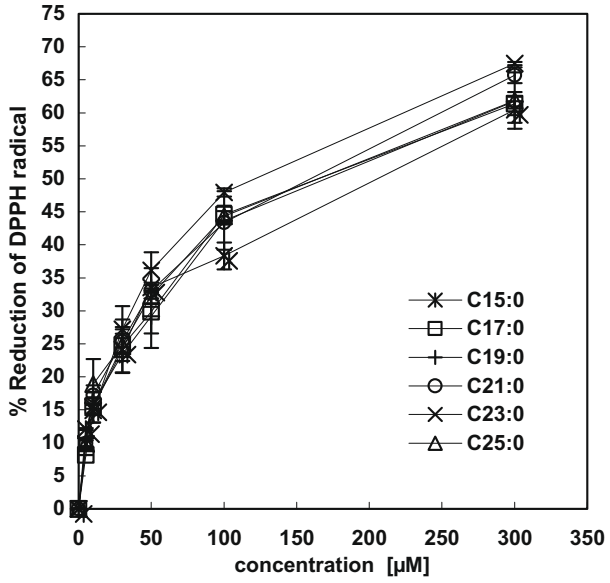

Fig. 2. Concentration-dependent relative percent reduction of DPPH radical for alkylresorcinol homologues C15:0–C25:0.

ferent alkylresorcinol homologues were corresponded also with their physicochemical properties, especially with respect to their ability to incorporate into lipid membranes. Our experimental model was simpler and did not contain any liposomal emulsion. In the case of the 5 μM concentration of homologue added to the experimental mixture, about 10% of the DPPH radical reduction of the control samples was obtained after 60 min of the experiments for all the tested homologues. At 60 min of the experiments with the 10 μM concentration of alkylresorcinols we observed ~15% DPPH radical reduction, except for homologue C25:0, in which we observed a 20% reduction. At 60 min of the experiments with the 30 and 50 μM concentrations of alkylresorcinols we obtained 20 and 30% reductions of DPPH radical, respectively. Exposure of the 100 μM alkylresorcinol homologues to DPPH radical resulted in a 15% reduction of DPPH radical at the first minute of the experiment and reached about 40% in 60 min. Using a 300 μM concentration of alkylresorcinol homologues, 30% DPPH radical reduction was obtained in the first minute and reached almost 60% compared with the control samples. As mentioned above, tocopherols were used as the positive control for these experiments. Application of a 5-μM concentration of the appropriate tocopherols resulted in about 10% reduction of DPPH radical. A higher concentration (10 μM) of α- and γ-tocopherol induced a 20% reduction and of δ-tocopherol about 16%. However, the highest concentration (50 μM) induced about 90%, 80%, and 70% reduction for α-, δ-, and γ-tocopherol, respectively. These findings were in agreement with the result reported by Kamal-Eldin et al. (2001), who found that α-tocopherol, in concentrations expressed as mol tocopherol mol^{-1} DPPH varying from 0.1 to 0.4, induced the reduction of DPPH radical from ~20% to ~70%. It should also be mentioned that in our experiments with tocopherols, substantial reduction was obtained in less than 5 min after mixing, while the alkylresorcinol homologues reacted slowly as antioxidants on DPPH radical. These results also corroborate those reported by Kamal-Eldin et al. (2001). Moreover, the tocopherols were characterised by significantly higher antioxidant activity than the alkylresorcinol homologues. On the other hand, our results from experiments with the homologues were quite different from those reported by Kamal-Eldin et al. (2001). They found that homologue C15:0 at a concentration of 309 expressed as mol C15:0 mol^{-1} DPPH radical induced only ~65% reduction. In our study a similar reduction level was obtained for homologue C15:0 at a concentration of 3 expressed as mol C15:0 mol^{-1} DPPH radical. A different source of homologue C15:0 could be the most likely explanation for the differences in the reduction levels determined in this study and those reported by Kamal-Eldin et al. (2001), but this needs further investigation.

A good parameter for comparing the antioxidant activities of the various compounds could be expressing the results as EC_{50}, which represents the antioxidant concentration necessary to decrease the initial DPPH concentration by 50%. In this paper we present for the first time the EC_{50} values for the alkylresorcinol homologues isolated from rye bran (Table 1) and compare them those for α-, δ-, and γ-tocopherol, and Trolox (a water-soluble vitamin E analog). The regression equations and correlation coefficients of the calibration curves indicated in the DPPH method for the particular alkylresorcinol homologues, tocopherols, and Trolox are also presented in Table 1. These curves were applied to determine the EC_{50} values. Sumino et al. (2002) reported that the EC_{50} values of the resorcinolic lipid homologues C15:0, C15:1, and C15:2 isolated from the fruits of *Ardisia colorata* were 90, 87, and 80 μM, respectively. In our experiment, with the alkylresorcinol homologues, the observed differences in EC_{50} value were not statistically significant, the values varying from 157 μM for homologue C23:0 to 195 μM for homologue C15:0. Lengthening the alkyl side chain of the alkylresorcinol homologues caused only a slight tendency of lowering the EC_{50} value. This observation was valid for homologues C15:0 to C23:0. The EC_{50} for homologue C25:0, however, differed from the above tendency and reached 185 μM. Moreover, the values of EC_{50} for all the alkylresorcinol homologues were significantly higher than those of Trolox and the tocopherols, compounds with well-defined antioxidant activity. This confirmed that the antioxidant properties of the native alkylresorcinols are significantly lower than those of tocopherols and Trolox, which is in good agreement with previous data (Kamal-Eldin et al., 2001; Nienartowicz & Kozubek, 1993; Struski & Kozubek, 1992).

Table 1
EC_{50} (μM) of alkylresorcinol homologues, tocopherols, and Trolox according to the DPPH method.

	Regression equation	R^2	EC_{50} (μM)
C15:0	$y = 86.322e^{-0.0028x}$	0.9187	195
C17:0	$y = 86.897e^{-0.0029x}$	0.9056	190
C19:0	$y = 86.919e^{-0.0029x}$	0.9225	191
C21:0	$y = 86.524e^{-0.0033x}$	0.9382	166
C23:0	$y = 85.589e^{-0.0035x}$	0.9162	157
C25:0	$y = 85.415e^{-0.0029x}$	0.9022	185
α-Tocopherol	$y = 116.35e^{-0.0534x}$	0.9894	16
δ-Tocopherol	$y = 107.29e^{-0.0348x}$	0.9917	22
γ-Tocopherol	$y = 100.37e^{-0.0254x}$	0.9999	27
Trolox	$y = -1.7319x + 96.05$	0.9844	27

Means of triplicates (CV < 5%).

Chemiluminescence is a potentially sensitive method and permits the observation of reaction kinetics. Moreover, lipid peroxidation causes CL to coincide with the decomposition of hydroperoxides, rather than the formation of secondary products (Krasowska et al., 2001). To enhance CL, which is an extremely weak process, an appropriate enhancer is crucial. In our study, luminol was used as the CL enhancer and AAPH was used as the source of hydrophilic radicals. The luminol-AAPH system generates strong and prolonged luminescence and can be used to test various antioxidants. All the tested alkylresorcinol homologues quenched CL (Table 2). This CL inhibition was not, however, stable and was rather short lasting. This was probably because of the lipophilic properties of alkylresorcinols. All the homologues were dissolved in methanol and then diluted in distilled water (the final concentration of methanol was 0.025–0.0125%). These methanol concentrations did not affect luminescence (data not shown). Luminescence inhibition was evaluated for all the tested alkylresorcinol homologues at concentrations of 5 and 10 μM, as shown in Table 2, and varied from 27% for C15:0 to 60% for C19:0 at the concentration of 5 μM, and from 61% to 77% for C21:0 and for C17:0 and C19:0 at the concentration of 10 μM. Similarly to the DPPH method, the slight differences in luminescence inhibition of the alkylresorcinol homologues suggest that the length of the alkyl

Table 2
Percent reduction of luminescence by alkylresorcinol homologues and tocopherols.

	% reduction of luminescence	
	5 (μM)	10 (μM)
C15:0	26.85	70.73
C17:0	30.44	77.59
C19:0	59.68	76.66
C21:0	28.07	61.32
C23:0	42.81	63.30
C25:0	41.56	72.30
α-Tocopherol	81.28	81.78
δ-Tocopherol	83.55	86.21
γ-Tocopherol	87.69	89.46

Means of triplicates (CV < 5%).

side chain had no major impact on their antioxidant properties. This observation could also be a result of the experimental conditions, and probably is. All the luminescence tests were carried in a hydrophilic environment (water) with a slight amount of methanol. Another consideration regarding the participation of the alkyl side chain of the alkylresorcinol homologues in their antioxidant activity may involve lipid partitioning, especially as alkylresorcinols can be inserted into membranes and form hydrogen bonds with neighbouring phospholipids. The effect of this ability is interference with the structure of these membranes (Bitkov, Nenashev, Pridachina, & Batrakov, 1992). This may also explain the correlation between increased chain length of alkylresorcinols and their increased antioxidant effect. It appears that alkylresorcinols could modulate the oxidative reaction in lipid oxidation tests in membranes, but alkylresorcinols themselves are not very effective antioxidants. This, as well as different configuration of hydroxyl groups in the ring structure, could also explain the approximately 10-times lower antioxidant activity of alkylresorcinols compared with tocopherols. The CL analysis also suggested differences in the mechanisms of the antioxidant actions of alkylresorcinols and tocopherols. In contrast to the alkylresorcinol homologues, CL inhibition of the tocopherols was stable and long lasting. Using a 5 or 10 μM concentration of tocopherols resulted in about 80% CL inhibition and this value was reached in all the experiments on a stable level. However, the alkylresorcinol homologues resulted in lower CL inhibition, which depended on the homologue concentration and was not as stable as that obtained with tocopherols.

3.2. Antioxidant activity of the extracts from whole-grain cereal products

The DPPH and CL methods were used to determine the antioxidant activity of extracts from whole-grain cereal products. In this study we also analysed alkylresorcinol content in four whole-grain breakfast cereal samples (rye, wheat, barley, and mixed) and in eight whole-grain bread samples commonly available on the Polish market. The amount of total lipids posted by the producers of the whole-grain breads and cereal were 1.77 ± 0.79 and 2.95 ± 1.4 g/100 g of products, respectively. The whole-grain breads used in the experiment were made from only whole-rye flour in the case of the whole-rye breads (1) and (2) and from a mixture of whole-rye and whole-wheat flour in the case of the whole-grain breads (1)–(6). The total alkylresorcinol concentrations determined in these breads varied from ~10 mg/kg DM to ~230 mg/kg DM. The extracts from all the above whole-grain products were added to the DPPH reaction system and the reduction of DPPH free radical was tested during a 60-min experiment similar to that for the alkylresorcinol homologues. All the extracts from whole-grain breads were characterised by a similar influence on the amount of DPPH radical and only slight differences were observed between the samples (Table 3). The highest decreases in DPPH radical, i.e. about 17% and 15%, were obtained for whole-grain bread (1) and whole-rye bread (1), respectively. About 10% reduction was observed for whole-rye bread (2) and whole-grain breads from (2) to (5) and a minor reduction in DPPH radical was obtained for whole-grain bread (6). Unfortunately, for the extracts from whole-rye and whole-grain breads we could not obtained a clear relationship between the level of DPPH radical reduction and the amount of alkylresorcinols used in the experiment, which varied from 1 μM in whole-rye bread (1) and whole-grain breads (3), (4), and (6) to 30 μM in whole-grain bread (2). Similar results were obtained by the CL method. Therefore a relationship between total alkylresorcinol amount used in the experiment (from ~0.1 to ~2.5 μM) and reduction of CL was not observed. The highest luminescence reduction was obtained for whole-grain breads (3) and (1) and whole-rye bread (1), being 77%, 74%, and 71%, respectively.

Table 3
Percent reduction of the DPPH radical and luminescence of the extracts from whole-grain cereal products.

	Total alkylresorcinol[a]	% Reduction[b]	% Reduction[c]
Whole-rye bread (1)	7	15.16	71.45
Whole-rye bread (2)	212	11.37	59.71
Whole-grain bread (1)	82	17.17	74.19
Whole-grain bread (2)	234	11.37	60.00
Whole-grain bread (3)	10	10.95	77.37
Whole-grain bread (4)	10	10.17	47.82
Whole-grain bread (5)	71	10.11	37.06
Whole-grain bread (6)	10	7.58	53.66
Rye cereals	840	42.98	90.63
Wheat cereals	420	30.83	74.9
Mixed cereals	240	24.25	79.17
Barley cereals	8	12.17	42.18

[a] Total alkylresorcinol content according to the method with diazonium Fast Blue B BF_4 salt, mg/kg DM, means of triplicates (CV < 5%).
[b] Percent reduction of the DPPH radical by the extracts from whole-grain cereal products, means of triplicates (CV < 5%).
[c] Percent reduction of luminescence by the extracts from whole-grain cereal products, means of triplicates (CV < 5%).

For whole-rye bread (2) and whole-grain breads (2) and (6), the CL reduction varied from 60% to 54%. The lowest CL reduction (Table 3) was observed for whole-grain breads (4) and (5).

A relationship between alkylresorcinol concentration and DPPH radical and CL reduction was clearly observed for the extracts from the breakfast cereals. In our study the highest levels of total alkylresorcinols were found in the whole-rye, whole-wheat, and mixed breakfast cereals (~840, ~420, and ~240 mg/kg DM, respectively), whereas very low levels of alkylresorcinols could be detected in barely breakfast cereals (~10 mg/kg DM). This finding supports earlier studies in which alkylresorcinols present in the lipid extracts from whole-grain cereal were the most active fraction showing antioxidant properties in protecting of lipids against air-induced oxidation (Kozubek & Tyman, 2005). DPPH radical and CL reduction levels were highest for the whole-rye breakfast cereals and reached about 40% and 91%, respectively. These reduction levels very clearly correspond to the amounts of alkylresorcinols used in the DPPH method (~110 μM) and the CL method (~8 μM). Lower DPPH radical and CL reduction were observed for the whole-wheat and mixed breakfast cereals (about 30% and 20% by the DPPH method and 75% and 79% by the CL method). In this case the alkylresorcinol concentrations in the DPPH and CL experiments were ~50 and ~4 μM for whole-wheat cereals, and ~30 and ~2.5 μM for whole-mixed cereals, respectively. Only a 10% DPPH radical reduction and a 42% CL reduction were obtained for the whole-barley breakfast cereals. The amounts of alkylresocinols used in this case were ~1 and ~0.08 μM in the DPPH and CL method, respectively. The observed relationship between DPPH radical and CL reduction and alkylresorcinol concentration were not, however, proportional. Nevertheless, we observed an interesting correlation in the DPPH reduction levels obtained for alkylresorcinol homologues (C15:0–C25:0) and the extracts from different breakfast cereals. In the case of the extracts from whole-rye cereals, the amount of alkylresorcinols used in the experiment (~100 μM) resulted in ~40% reduction of DPPH radical. This reduction level was very similar to that obtained for alkylresorcinol homologues (Fig. 2). The above observations confirmed that there is a direct relationship between the concentration of alkylresorcinols at which they could protect lipids completely (10% w/w) and their concentration in the total lipid extract from cereal grain (Kozubek & Tyman, 1999; Nienartowicz & Kozubek, 1993). This could be especially important when the potential role of alkylresorcinols in the protection of food against oxidation during their processing and storage is considered.

Although alkylresorcinols might be among the many antioxidant compounds found in extracts from whole-grain products, they should be considered beneficial constituents of a whole-grain (high-fiber) human diet. To investigate the antioxidant properties of alkylresorcinol homologues (C15:0–C25:0) and extracts from whole-grain products, characterised by different amounts of total alkylresorcinols, we presented two simple methods based on the reduction of DPPH radical and the reduction of CL. We obtained a very clear relationship between the antioxidant activity of the extract from breakfast cereals and their total amounts of alkylresorcinols, which may suggest the involvement of these lipids in both protection against non-enzymatic food oxidative processes as well as protection of the consumer of the food against the harmful effects of processes and products such as the non-enzymatic and enzymatic oxidation of biomolecules.

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