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# Alkylresorcinols in Selected Polish Rye and Wheat Cereals and Whole-Grain Cereal Products

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The alkylresorcinol content and homologue composition in selected Polish rye and wheat cultivars and selected whole-grain cereal products were determined in this study. Cereal grains and whole-grain cereal products were extracted with acetone, whereas bread types were extracted with hot 1-propanol. The average alkylresorcinol content in tested rye (~1100 mg/kg DM) and wheat (~800 mg/kg DM) grains harvested in Poland was within the range previously reported in Swedish and Finnish samples. The total alkylresorcinol content in tested cereal products available on the Polish market varied from very low levels in barley grain-based foods up to 3000 mg/kg DM in wheat bran. The total alkylresorcinol content in 14 bread samples extracted with hot 1-propanol varied from ~100 mg/kg DM in whole bread made with honey up to ~650 mg/kg DM in whole-rye bread. Calculated ratios of C17:0 to C21:0 homologues, a useful parameter previously used to distinguish between rye and wheat cereals and their derived products, was about 1.2−1.4 in rye products, about 0.2 in wheat products, and varied between 0.2 and 0.6 in cereal−derived products containing a mixture of whole rye and/or wheat. The data set obtained were subsequently compared using cluster and principal component analysis, which allowed the tested cereal products to be classified into two major groups consisting of whole-rye or whole-wheat products, respectively. On the basis of that approach, mixed cereal products containing rye and wheat bran or whole rye and wheat flour were grouped between those two well-defined clusters. Our work not only provides a detailed examination of alkylresorcinols in selected Polish rye and wheat cultivars and selected whole-grain cereal products, but also demonstrates that this type of analysis accompanied by the use of proper statistical algorithms offers an objective way to evaluate the quality of whole-grain rye and/or wheat and their derived products.

**KEYWORDS: Alkylresorcinols; whole grain; rye; wheat; cereal products; biomarker**

## INTRODUCTION

According to the Food and Drug Administration's definition, whole-grain foods are made from the entire grain seed (usually called the kernel), which consists of the bran, the germ, and the endosperm. In addition, this type of product must contain at least 51% of whole-grain ingredients by weight per reference amount and be low in fat and cholesterol (*1*). In refined grain products, bran and germ are missing and therefore whole-grain products contain more B vitamins, iron, dietary fiber, and other important nutrients. Most refined grain products are actually enriched in certain nutritious substances, such as certain vitamins of the B group (thiamin, riboflavin, niacin, folic acid) and iron, which are added back after processing (*2–4*). Numerous reports indicate that the bioactive compounds added to foods as artificial supplements are not as beneficial as those naturally occurring in grains (*5*). This aspect is especially important since epidemiological studies strongly correlate the consumption of whole-cereal products with a decreased risk of chronic diseases, including obesity, diabetes, coronary heart disease, stroke, hypertension, and some cancers (*6–9*). Unfortunately, it is not always possible to establish a quantitative measurement of either benefits or risks of diet in health and disease. The use of specific markers is, therefore, a tempting idea to resolve this issue (*10, 11*). Regarding whole-grain cereal products, many approaches have been proposed to determine the whole-grain content in flour based on total dietary fiber content, ferulic acid content (*12*), enterolactone content (*13*), or ash percentage (*14*). However, these substances are also present in the grain's endosperm, as well as in food additives, some vegetables, and fruits, which disqualifies them from being used as potential biomarkers specific solely to cereal products.

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Alkylresorcinols (aka resorcinolic lipids) have been recently shown to be a potential biomarker of whole-grain cereals and cereal products (*14–16*). Alkylresorcinols are phenolic lipids, 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 of the benzene ring. The alkyl side chain of alkylresorcinols isolated from cereal grains is usually saturated and varies from 13 to 27 carbon atoms (*17–19*). Unsaturated chain analogues or derivatives with keto or hydroxyl groups on the alkyl chain have also been reported in rye and wheat grain samples (i.e., 15−30% and 5% of total alk(en)ylresorcinols, respectively) (*18, 20, 21*). Interestingly, 99% of resorcinolic lipids occur only in an intermediate layer of caryopsis, including the hyaline layer, inner pericarp, and testa of rye and wheat grains (i.e., 0.03−0.15% of dry kernel weight) and are not detected in other parts of the grain, including the endosperm and germ (*22, 23*). The specific location of alkylresorcinols suggests a promising application of those phenolic lipids as selective markers in cereal fractions (*23*). Actually, several studies report on the presence of alkylresorcinols in the different rye and wheat cultivars grown in North America (range: 300−700 mg/kg in wheat kernel) (*24*), western European countries (595−1429 mg/kg in wheat kernel) (*15*), Sweden (549−1022 mg/kg in rye kernel, 200− 1480 mg/kg in wheat kernel) (*14, 15, 25*), and Poland (*26*). Resorcinolic lipids are present in small amounts in barley (40−100 mg/kg) (*27, 28*), but not in other cereals or in vegetables and fruits (*17*). Alkylresorcinol content was also established in many cereal products available in Scandinavian markets (*14, 25, 29*).

The biosynthesis of alkylresorcinols and their resulting content in cereal grains and cereal products can be altered by many biotic (*30*) and abiotic factors within different cultivars and also even within the same ecological niche (*31–33*). This is of particular importance since there are certain differences in demographic characteristics, dietary patterns, and other lifestyle factors that distinguish Western, Central, and Eastern European countries. These differences are why extensive analyses of alkylresorcinol content in cereal foods could be an objective way to verify the idea of using these particular compounds as biomarkers of whole-grain rye and/or wheat products commonly available in many different markets. In this study, we investigated variations in alkylresorcinol content in selected rye and wheat grain samples and selected cereal products available on the Polish market. Cereal grains and whole-grain cereal products were extracted with acetone, various bread samples were extracted with hot 1-propanol, and the data obtained were compared statistically using cluster analysis (CA) and principal component analysis (PCA). This new approach provides not only a detailed examination of alkylresorcinols in selected Polish rye and wheat cultivars and selected whole-grain cereal products, but also a means to resolve questions concerning the quality of whole-grain rye and/or wheat and their derived products.

## MATERIALS AND METHODS

**Chemicals and Reagents.** Acetone, ethanol, methanol, and 1-propanol were from Chempur (Piekary Slaskie, Poland). Acetic acid was from Standard (Lublin, Poland). Diazonium salt Fast Blue B $BF_4$ was a generous gift from Chempol (Prague, Czech Republic). Olivetol (5-pentylresorcinol) was purchased from Sigma-Aldich (Poznan, Poland). Total alkylresorcinols were isolated from rye and wheat bran as a mixture of homologues (C15−C25) as described by Kozubek (*34*).

**Cereals and Cereal Products.** Rye (*Secale cereale*) and wheat (*Triticum aestivum*) grains analyzed for their content of total alkylresorcinols were provided by the Kernel-Agricultural Institute (Kopaszewo, Poland) and Plant Breeding Institute (Tulce, Poland). Commercial whole rye and wheat products were purchased from a domestic supermarket located in Wrocław, Poland.

**Extraction of Alkylresorcinols from Cereal Grain Materials.** One-gram samples of whole rye and wheat grains, ground rye and wheat grains (coarsely ground in a coffee grinder), whole rye and wheat bran, and flour and whole-grain products were placed in 50-mL tubes and extracted by continuous shaking for 48 h at room temperature with 40 mL of acetone (*34*). Bread samples were extracted with 10 mL of 1-propanol/water (3:1 v/ v) with three extractions in a boiling water bath (1 × 2 h, 2 × 1 h) (*35*). Fresh solvent was used each time. The extracts were filtered through paper filters and evaporated to dryness at 45 °C in a vacuum pump (KNF Neuberger Laboport, Germany) with the help of absolute ethanol to remove remaining water. The dry residues from all the extractions were then dissolved in ethyl acetate (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.

**Colorimetric Method for Determining Alkylresorcinols Using Diazotized Fast Blue B $BF_4$ Salt.** Total alkylresorcinol content in the cereals and processed grain material was determined by the method involving Fast Blue B $BF_4$ (*36*). Briefly, 10 $\mu$L 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 $\mu$g.

**HPLC Analysis of Alkylresorcinols.** The HPLC system consisted of a model 600 HPLC pump with a model 717 plus Autosampler and 996 PDA detector, all from Waters (Milford, MA). The relative composition of alkylresorcinols extracted from the cereal grain materials was determined by RP-HPLC according to a modified method reported elsewhere (*29*). The method employed a 250 mm × 4.6 mm i.d., 5 $\mu$m, XTerra RP18 column from Waters with methanol (A) and water (B) as the mobile phase, a flow rate of 1 mL/min, and UV detection at 280 nm. Olivetol (C5:0) was used as a reference compound. Ten microliters of each ethyl acetate sample was injected into the chromatographic column and separated at room temperature. The mobile phase gradient was as follows: 86−88% B in 3 min, 88−94% B in 5 min, hold at 94% B for 40 min, and finally 94−86% B in 2 min. A Waters Millennium version 3.20 processing module was used for recording and processing the chromatograms. Response factors for alkylresorcinols homologues were calculated by taking into account their molecular weights versus olivetol. According to Mattila et al. (*29*), the responses do not depend upon the length of the resorcinolic lipid alkyl side chain.

**Statistical Analysis.** Statistical analysis used Student's *t* test, CA, and PCA and was processed using the software program STATISTICA (Microsoft, Statsoft). Differences were considered to be significant at $P < 0.05$. CA was used to classify the selected objects examined into groups (clusters), and a resulting dendrogram was constructed using Ward's amalgamation algorithm with Euclidean distances as a distance measure. PCA was used to classify the selected objects examined into groups, and three-dimensional score plots were created on the basis of calculated Varimax-rotated principal component (PC) loadings. In both cases, the proper matrices were constructed on the basis of the original data set reflecting alkylresorcinol concentrations, homologue composition, and C17:0/C21 ratios in the respective acetone extracts and on the patterns of alkylresorcinol homologues identified in the respective acetone extracts. Alternatively, the matrices reflecting solely alkylresorcinol concentrations and homologue composition were also considered.

## RESULTS AND DISCUSSION

**Content of Alkylresorcinols in Selected Rye and Wheat Cereals and Whole-Grain Cereal Products Available on the Polish Market.** In this study, we analyzed alkylresorcinol content in whole and ground wheat and rye grains, cereal products made from whole grains, and grains with added wheat

**Table 1.** Alkylresorcinol Content in Rye and Wheat Grains and Selected Polish Cereal Products Extracted with Acetone

| cereal grain material | total AR[a] | total AR[b] | % of each homologue | | | | | | ratio of C17:0 to C21:0 |
|---|---|---|---|---|---|---|---|---|---|
| | | | C15:0 | C17:0 | C19:0 | C21:0 | C23:0 | C25:0 | |
| whole-grain rye (Gradan) | 1152 | 821 | 8 | 27 | 28 | 18.5 | 9.5 | 9 | 1.48 |
| whole-grain rye (Nawid) | 1136 | 894 | 3 | 31 | 33 | 16 | 9 | 8 | 1.89 |
| whole-grain rye (Amilo) | 1058 | 912 | 6 | 25 | 28 | 20 | 11 | 10 | 1.23 |
| whole-grain rye ground (Gradan) | 1122 | 895 | 8 | 26 | 27 | 19.5 | 10 | 9.5 | 1.33 |
| whole-grain rye ground (Nawid) | 1105 | 888 | 3.5 | 30 | 33 | 17 | 8.5 | 8 | 1.70 |
| whole-grain rye ground (Amilo) | 1117 | 910 | 7 | 26 | 28 | 19 | 10 | 10 | 1.39 |
| rye bran | 2466 | 2034 | 4 | 26.5 | 30.5 | 19 | 11 | 9 | 1.40 |
| whole-rye flour (1) | 1284 | 1060 | 5 | 25 | 29 | 19 | 11 | 11 | 1.34 |
| whole-rye flour (2) | 899 | 788 | 3.5 | 25.5 | 29 | 21 | 11 | 10 | 1.23 |
| breakfast rye cereals | 879 | 721 | 4 | 24 | 30 | 20 | 11 | 11 | 1.21 |
| breakfast barley cereals | 18 | nd[c] | nd | nd | nd | nd | nd | nd | nd |
| breakfast mixed cereals (muesli) | 286 | 299 | 3 | 13.5 | 38 | 27 | 11.5 | 7 | 0.51 |
| whole-grain wheat (Mewa) | 943 | 775 | 2 | 9.5 | 32 | 45 | 8.5 | 3 | 0.21 |
| whole-grain wheat (Nadobna) | 851 | 809 | 1 | 8 | 32 | 43 | 11 | 5 | 0.19 |
| whole-grain wheat (Legenda) | 790 | 712 | 1.5 | 9 | 32.5 | 44 | 9.5 | 3.5 | 0.20 |
| whole-grain wheat (Griwa) | 695 | 642 | 1 | 8.5 | 34 | 46 | 8 | 2.5 | 0.18 |
| whole-grain wheat (Naridana) | 672 | 586 | 2 | 10 | 32 | 44 | 9 | 3 | 0.23 |
| whole-grain wheat ground (Mewa) | 736 | 627 | 2 | 9 | 32 | 45 | 9 | 3 | 0.21 |
| whole-grain wheat ground (Nadobna) | 877 | 879 | 1 | 9 | 31.5 | 44 | 10 | 4.5 | 0.20 |
| whole-grain wheat ground (Legenda) | 690 | 703 | 1.5 | 9 | 32.5 | 44.5 | 9 | 3.5 | 0.20 |
| whole-grain wheat ground (Griwa) | 693 | 687 | 1 | 8 | 33 | 47.5 | 8 | 2.5 | 0.17 |
| whole-grain wheat ground (Naridana) | 631 | 565 | 2 | 9 | 33 | 44 | 9 | 3 | 0.21 |
| wheat bran (1) | 3186 | 2608 | 1 | 9 | 31 | 45 | 10 | 4 | 0.20 |
| wheat bran (2) | 2737 | 2388 | 0.5 | 9 | 31 | 45 | 10.5 | 4 | 0.20 |
| whole-wheat flour | 269 | 258 | 3 | 8 | 26 | 40.5 | 16 | 6.5 | 0.21 |
| breakfast wheat cereals (1) | 671 | 574 | 2 | 9 | 31.5 | 43.5 | 10 | 4 | 0.20 |
| breakfast wheat cereals (2) | 203 | 194 | nd | 6.5 | 38 | 41 | 11 | 3.5 | 0.14 |
| breakfast wheat cereals (3) | 155 | 131 | 1 | 11 | 31 | 41 | 11 | 4 | 0.27 |
| soft rye bread (1) | 669 | 614 | 5 | 24 | 30 | 19 | 12 | 10 | 1.29 |
| soft rye bread (2) | 386 | 300 | 2 | 15 | 24.5 | 30.5 | 18 | 10 | 0.49 |
| soft wheat bread | 23 | nd | nd | nd | nd | nd | nd | nd | nd |
| soft mixed bread (three grains) | 416 | 408 | 5 | 17 | 37 | 27 | 8 | 6 | 0.63 |
| soft mixed bread (seven grains) | 157 | 154 | 2 | 9 | 20 | 39.5 | 21.5 | 8 | 0.24 |
| soft mixed bread (muesli) | 57 | nd | nd | nd | nd | nd | nd | nd | nd |
| whole-rye bread (1) | 462 | 382 | 3 | 24 | 31 | 21 | 10 | 11 | 1.13 |
| whole-grain bread (1) | 350 | 311 | 4.5 | 24.5 | 29.5 | 19.5 | 10.5 | 11 | 1.26 |
| whole-grain bread (2) | 237 | 174 | 2 | 16 | 26 | 18 | 11 | 27 | 0.90 |

[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] Total alkylresorcinol content according to HPLC, mg/kg DM, means of triplicates (CV < 5%). [c] Not detected (detection limit ≈ 3 mg/kg DM).

or rye bran, including flours, brans, breakfast cereals, muesli, crisp breads, soft breads, and baked breads commonly available on the Polish market. The varieties of rye and wheat tested in this study belong to a representative group widely cultivated in Poland. This fact makes it, therefore, quite likely that these particular cereals might be found in the analyzed cereal grain-based products; however, this assumption could not be verified, because cereal-food manufacturers keep all information about wheat and rye cultivars in their products confidential.

The methods for extracting resorcinolic lipids from grains and cereal products tend to be simplified and usually involve polar organic solvents (*15, 34, 37*); we used acetone in the present study. This approach, however, is not useful in the complete recovery of alkylresorcinols from baked cereal products (*14, 25*), which can be efficiently extracted with hot 1-propanol as reported by Ross et al. (*14*). The total alkylresorcinol concentrations determined in selected rye (from ∼1050 to ∼1150 mg/kg DM) and wheat grains (from ∼670 to ∼940 mg/kg DM) were within the range of those reported previously (*14, 15*). Similar amounts of total alkylresorcinols were found in ground rye and wheat grain samples (**Table 1**). Similarly to GC−MS analysis (*14, 15, 25*), HPLC chromatograms (**Figure 1**) show that the alkylresorcinol homologue compositions of the rye and wheat grain samples differ significantly. The C17:0, C19:0, and C21:0 homologues were predominant in rye (**Figure 1A**), constituting about 26, 28, and 19% of the alkylresorcinol pool in the acetone extracts (**Table 1**). As expected (*14, 25*), the predominant homologues in the wheat grain sample were C19:0 and C21:0 (**Figure 1B**) and were found to be about 32 and 45% of the total alkylresorcinol homologue composition in the acetone extracts (**Table 1**). Since the total content of resorcinolic lipids in rye and wheat has been shown to be very similar, different homologue compositions and homologue C17:0 and C21:0 ratios are especially useful in distinguishing between these cereal species (*14, 25, 29*). Indeed, Chen et al. (*25*) reported C17:0/C21:0 ratios in rye and wheat samples to be 1.0 and 0.1, respectively. However, Mattila et al. (*29*) obtained C17:0/C21:0 ratios in rye products of 1.3−1.5 and 0.2−0.6 in wheat products, respectively. Our results reported here are different from those reported by Chen et al. (*25*) but corroborate those reported by Mattila et al. (*29*) (**Table 1**). These ratios were ∼1.2−1.4 in rye products and ∼0.2 in wheat products and varied between 0.2 to 0.6 in cereal products containing a blend of whole-grain rye, wheat, or their bran. The application of HPLC-UV/DAD for alkylresorcinol determination in this study had certainly some limitations, as no complete separation of saturated alkylresorcinols from corresponding unsaturated homologues could be achieved under the conditions used. In fact, the saturated alkylresorcinol homologues were coeluted with the ensuing unsaturated alkylresorcinol homologues (e.g., C17:0 with C19:1, C19:0 with C21:1, etc.). To overcome these problems, extensively prolonged run times,

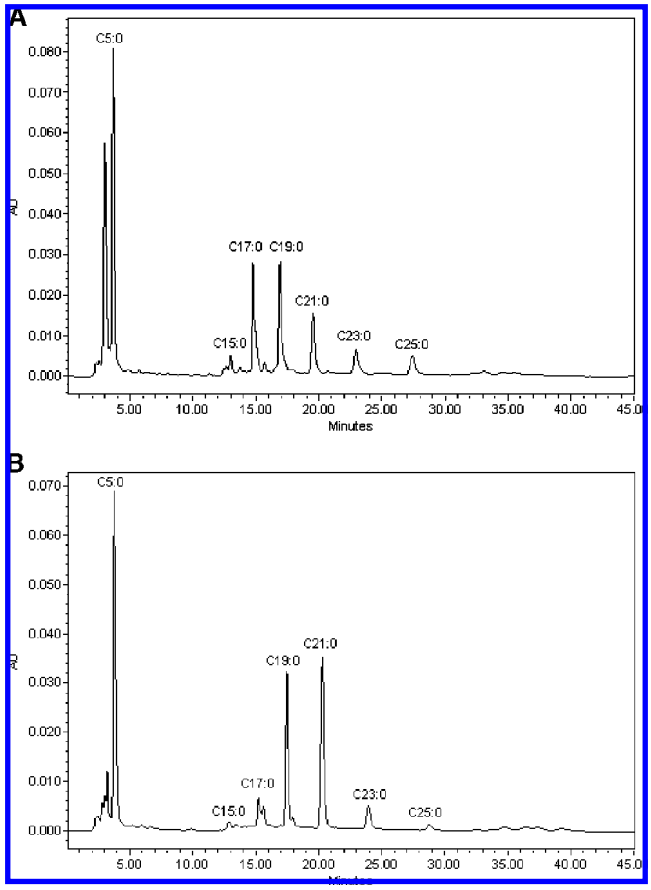


**Figure 1.** Representative chromatograms of the acetone extracts of rye (**A**) and wheat (**B**) grains. Olivetol and the main alkylresorcinol homologues are marked. Retention times of the particular homologues were 3.7 min (C5:0), 12.8 min (C15:0), 14.9 min (C17:0), 17.2 min (C19:0), 19.9 min (C21:0), 23.9 min (C23:0), and 28.2 min (C25:0).

complex gradients, and more advanced and sensitive detectors (e.g., mass detectors) should be used (*38*). Incomplete separation obtained with HPLC analysis could be also the most likely explanation of differences between the C17:0/C21:0 ratios determined in this and Mattila et al. (*29*) studies and those reported by Chen et al. (*25*), who used GC−MS. However, the differences between C17:0/C21:0 ratios in rye and wheat products were significant irrespective of the analytical methods applied. These two methods, therefore, could probably have practical application to distinguish between whole-rye and wheat-cereal products.

Total alkylresorcinol content was determined in Polish commonly used plain whole rye and wheat flour, organic whole-rye flour, in 15 different cereal products that contained rye, wheat, and/or barley, and also in 14 commercially available breads (**Tables 1** and **2**). Because the location of most alkylresorcinols has been ascribed to the intermediate layer of grains, containing hyaline layer, inner pericarp, and testa (*23*), resorcinolic lipids could be detected only in products considered as "whole grain". The highest levels of total alkylresorcinols were found in the whole rye and wheat products, including breakfast rye cereals, breakfast wheat cereals (1), and soft rye bread (1) (∼870, ∼670, and ∼670 mg/kg DM, respectively), whereas only very low levels of alkylresorcinols could be detected in breakfast barely cereals (∼20 mg/kg DM). The mixed cereal products were, however, characterized by decreased alkylresorcinol content. Higher levels of total resorcinolic lipids were found in the breakfast mixed cereals (muesli) and soft mixed bread (three grains) (∼280 and ∼410 mg/kg DM, respectively), whereas very low levels of alkylresorcinols were detected in the soft wheat and mixed (muesli) breads (∼20 and ∼50 mg/kg DM, respectively). This finding was in agreement with the common observation that grain products are usually not whole grain if labeled as multigrain, seven-grain, three-grain, or the like. The total alkylresorcinol content (**Table 1**) measured in rye bran was about 2400 mg/kg DM and was similar to that reported by Chen et al. (*25*) but lower than that reported by Mattila et al. (*29*). We determined alkylresorcinols in two kinds of wheat bran at levels similar to those reported previously (∼3000 and 2700 mg/kg DM) (*25, 29*). We also analyzed plain whole-rye flour (∼1200 mg/kg DM), organic whole-rye flour (∼900 mg/kg DM), and plain whole-wheat flour (∼270 mg/kg DM). Our results are comparable to those obtained from Swedish and Finnish cereal products (*14, 25, 29*).

**Table 2.** Alkylresorcinol Content in Selected Breads Commonly Available on the Polish Market Extracted with Hot 1-Propanol

| | total AR[a] |
|---|---|
| whole-grain bread (1) | 497 ± 28 |
| whole-grain bread (2) | 348 ± 22 |
| whole-grain bread (3) | 378 ± 19 |
| whole-grain bread (4) | 277 ± 7 |
| whole-grain bread (5) | 298 ± 9 |
| whole-grain bread (6) | 140 ± 9 |
| whole-rye bread (1) | 656 ± 44 |
| whole-rye bread (2) | 306 ± 21 |
| soybean bread (1) | 508 ± 5 |
| soybean bread (2) | 114 ± 5 |
| sunflower bread | 340 ± 7 |
| spelt bread | 390 ± 18 |
| whole-bread made with honey | 107 ± 8 |
| pumpkin bread | 128 ± 6 |

[a] Total alkylresorcinol content according to the method with diazonium Fast Blue B $BF_4$ salt, mg/kg DM. Results are means ± SD ($n = 3$).

**Alkylresorcinol Levels in Breads Available on the Polish Market.** According to the literature, we compared the acetone and hot 1-propanol extracts from bread samples. As expected, the latter extraction system was much more effective than acetone (data not shown). The detected alkylresorcinol concentrations (∼100−650 mg/kg DM) (**Table 2**) corroborated those reported by Ross et al. (*14*), Chen et al. (*25*), and Mattila et al. (*29*). The highest alkylresorcinol amounts were detected in whole-rye bread (1) (∼650 mg/kg DM) and whole-grain bread (1) (∼500 mg/kg DM), and a lower alkylresorcinol content was obtained in whole-grain breads (2), (3), (4), (5) and whole-rye bread (2) (i.e., ∼340, ∼370, ∼270, ∼290, and ∼300 mg/kg DM, respectively). The lowest alkylresorcinol content was found in whole-grain bread (6) (∼140 mg/kg DM). Not surprisingly, the presence of resorcinolic lipids was detected in the hot 1-propanol extracts from soybean bread (1) at a high level (∼500 mg/kg DM) and also in spelt, sunflower, pumpkin, soybean (2) breads, and whole bread made with honey (∼390, ∼340, ∼120, ∼110, and ∼100 mg/kg DM, respectively).

**HPLC Analysis of the Extracts from the Selected Rye and Wheat Cereals and Whole-Grain Cereal Products.** We used chromatographic analysis to confirm the results obtained using the colorimetric method as well as to examine alkylresorcinol profiles of the analyzed extracts from the cereals and cereal grain materials. It is well-known that certain types of phenolics or similar natural products (especially glycolipids) present in the acetone extracts could cross-react with Fast Blue B salt used in the colorimetric-based alkylresorcinol determination assay. To avoid this possibility, we measured the total alkylresorcinol content in the selected cereals and cereal products using the HPLC

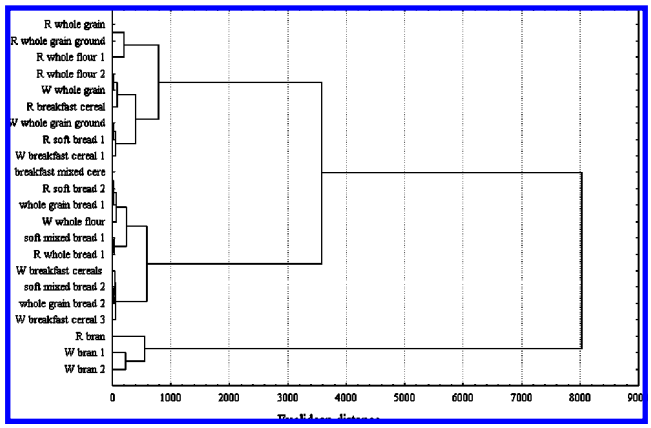


**Figure 2.** Grouping of selected rye and wheat products available on the Polish market using cluster analysis. Ward's amalgamation algorithm-based dendrogram reflects the variability in alkylresorcinol concentration, alkylresorcinol homologue patterns, and C17:0/C21:0 ratios in acetone extracts obtained from the respective products. Simple Euclidean distances were used as a distance measure. W and R refer to wheat and rye, respectively.

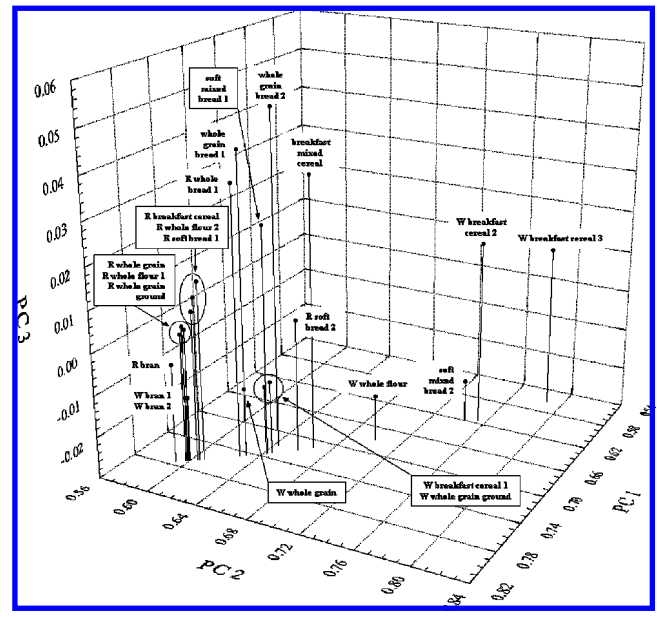


**Figure 3.** Grouping of selected rye and wheat products available on the Polish market using PCA. The proper matrix was constructed on the basis of the original data set reflecting alkylresorcinol concentrations, homologue compositions, and C17:0/C21:0 ratios in the respective acetone extracts. The three-dimensional score plot was created on the basis of calculated Varimax-rotated PC loadings. Values in parentheses refer to percentage variations observed in the original data. PC1 (53.11%), PC2 (41.85%), and PC3 (0.45%). W and R refer to wheat and rye, respectively.

method. We obtained results comparable to these obtained from spectrophotometric analysis (**Table 1**). The acetone extracts from exemplary whole rye and wheat grains, ground whole rye and wheat grains, and whole-grain cereal products were separated on an RPC18 column in a methanol/water mobile phase gradient. The response factors were calculated for different resorcinolic homologues, provided that the molar responses were not dependent on the alkyl side chain (*29*). The highest alkylresorcinol content was found in the acetone extracts from wheat bran (1) and (2), rye bran, whole and ground rye and wheat grains, and conventional and organic whole-rye flour (from ~2600 to ~620 mg/kg). Products made from rye or wheat grains, for example, breakfast rye cereals, breakfast wheat cereals (1), soft rye bread (1), and soft mixed bread (three grains), had alkylresorcinol content at high levels (from ~800 to ~400 mg/kg DM). Other products contained low or no alkylresorcinols and were probably made from sifted rye or wheat flour.

**Cluster and Principal Component Analysis of Selected Rye and Wheat Cereals and Whole-Grain Cereal Products.** The HPLC analysis allowed us to determine the alkylresorcinol homologue composition of rye and wheat grains and cereal products. We further applied statistical analysis to discriminate and compare cereal grains and whole-grain cereal products. Because of similar characteristics observed in the tested rye and wheat cultivars, we included only single wheat and rye samples as outgroups. Statistical analysis is a powerful device for representing large sets of variables and detecting differences between them that account for much of the variance among the sets of original data. Initially, we used cluster analysis employing Ward's amalgamation algorithm with Euclidean distances (**Figure 2**). The dendrogram reflects the variability in alkylresorcinol concentrations, alkylresorcinol homologue patterns, and C17:0/C21:0 ratios in the acetone extracts obtained from the respective products. Cluster analysis revealed a clearly distinguished cluster consisting of rye and wheat bran. There were also two large clusters grouping rye and wheat cereal products, whereas breakfast mixed cereals (muesli) were located between these two clusters. The matrices reflecting solely alkylresorcinol concentrations and homologue composition were also considered in this study, but this approach yielded similar clustering patterns (data not shown). Interestingly, we observed a low similarity between soft breads, especially those such as soft mixed breads (1) and (2), which had completely different grain compositions. As shown, rye soft bread (2) was produced from whole wheat rather than from whole rye, indicating that some products may not contain the kind of bran or flour their producers claim.

The selected rye and wheat products available on the Polish market were also compared using PCA. The proper matrix was constructed on the basis of the original data set reflecting alkylresorcinol concentrations, alkylresorcinol homologue composition, and C17:0/C21:0 ratios in the respective acetone extracts (**Figure 3**), or solely on the basis of alkylresorcinol homologue patterns identified in the respective acetone extracts (**Figure 4**). Alternatively, we also processed the matrices reflecting solely alkylresorcinol concentrations and homologue composition, but this approach again yielded similar grouping patterns (data not shown). As shown in **Figure 3**, rye and wheat bran, rye grains and products, and wheat grains and products were classified into three separate groups. Similarly, the results presented in **Figure 4** were ideally grouped into rye and wheat products. Soft mixed bread (2) contained more than wheat bran, rye bran, or soft mixed bread (1), which was rather a blend of both grains. Breakfast mixed cereals also contained rye and wheat components. As mentioned above, rye soft bread (2) was rather a whole-wheat product than the whole-rye one. Interestingly, whole-grain bread (2) contained some other cereal different from rye or wheat (e.g., barley).

We conclude that the high discriminative power of alkylresorcinol profiling accompanied by the use of proper statistical algorithms allows the differentiation of Polish rye and wheat cultivars and whole-grain cereal products. The average total alkylresorcinol content in whole grains and cereal products

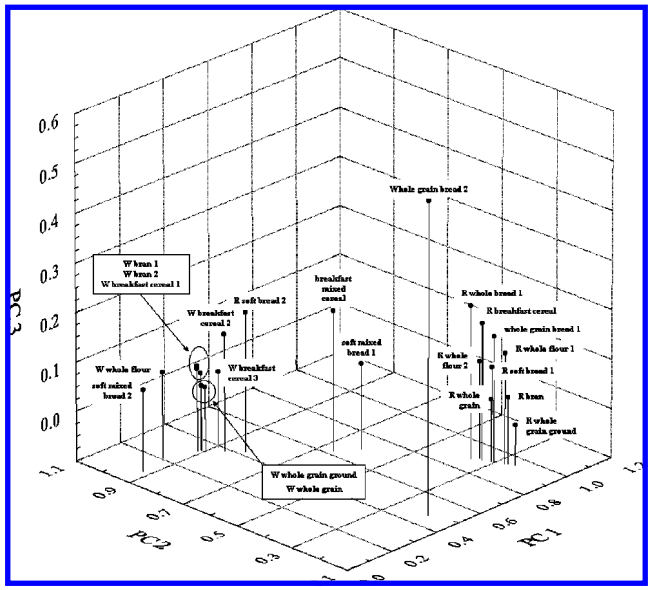


**Figure 4.** Grouping of selected rye and wheat products available on the Polish market using PCA. The proper matrix was constructed on the basis of patterns of alkylresorcinol homologues identified in the respective acetone extracts. The three-dimensional score plot was created on the basis of calculated Varimax-rotated PC loadings. Values in parentheses refer to percentage variations observed in the original data. PC1 (44.38%), PC2 (44,63%), and PC3 (2.43%). W and R refer to wheat and rye, respectively.

commonly available on the Polish market was generally similar to those reported in previous studies (*14, 25, 29*). The ratio of the C17:0 and C21:0 homologues was ~1.2−1.4 in rye and below 0.2 in wheat, indicating that this particular parameter can be used to distinguish between these two cereals. The C17:0/C21:0 ratio was altered in cereal products containing a mixture of whole-grain rye and wheat or their bran and varied between ~0.2 and ~1.4. The application of both CA and PCA in representative acetone extracts allowed detecting differences in the multivariable data set, which resulted in a clear-cut grouping of cereal products into basic groups containing whole rye and whole-wheat products, and mixed cereal products prepared from rye and wheat and their bran or flour. To recapitulate, our study not only provides a detailed examination of resorcinolic lipids in selected Polish rye and wheat cultivars and selected whole-grain cereal products, but also describes a valuable tool that will be useful for all issues related to the evaluation of the quality of whole-grain rye and wheat and their derived products.

## ABBREVIATIONS USED

CA, cluster analysis; DM, dry matter; PCA, principal component analysis.

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