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Alkylresorcinols in Wheat Varieties in the HEALTHGRAIN Diversity Screen

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The contents of alkylresorcinols (AR) were analyzed in 131 winter wheats, 20 spring wheats, 10 durum wheats, 5 spelt wheats, and 10 early cultivated forms of wheat (5 diploid einkorn and 5 tetraploid emmer), which are part of the HEALTHGRAIN diversity screen. AR were analyzed by gas chromatography (GC), which provides both total contents and relative homologue compositions, as well as with a Fast Blue colorimetric method that provides only total contents but which is fast and easily screens a large number of samples. There was considerable variation in the total AR content analyzed with GC: winter wheat (220−652 μg/g of dm), spring wheat (254−537 μg/g of dm), durum wheat (194−531 μg/g of dm), spelt (490−741 μg/g of dm), einkorn (545−654 μg/g of dm), and emmer wheat (531−714 μg/g of dm). The relative AR homologue composition was different for different types of wheat, with a C17:0 to C21:0 ratio of 0.1 for winter, spring, and spelt wheats, 0.04 for einkorn and emmer wheat, and 0.01 for durum wheat. The total AR content analyzed with the Fast Blue method was lower than that analyzed with GC but there was a good correlation between the two methods ($R^2 = 0.76$).

KEYWORDS: Alkylresorcinols; wheat; wholegrain; biomarker

INTRODUCTION

Alkylresorcinols (AR) represent one of the major groups of phenolic compounds in wheat and rye cereals. They are amphiphilic 1,3-dihydroxybenzene derivatives with an odd-numbered alk(en)yl chain at position 5 of the benzene ring (**Figure 1**). The alk(en)yl chain is 15−25 carbon atoms long and is mainly saturated, but some AR have a modified alkyl chain that can be mono-, di-, and triunsaturated or may have a hydroxyl or keto group substituted on the alkyl chain (*1*−*4*). AR are found in high levels in the outer layers of wheat and rye and in low levels in barley but not in other commonly consumed foods. They are suggested as markers for wholegrain wheat and rye in food (*5*) and as a biomarker for estimating human intake of wholegrain wheat and rye (*6*). In vitro studies suggest that AR also may have biological and physiological effects as, for example, antimicrobial effects and effects on biological membranes (*6, 7*). A strong antimutagenic activity of AR isolated from rye bran has also been found (*8*).

The contents of AR in wheat have earlier been reported to vary between 300 and 1000 μg/g, which is lower than in rye [360−3200 μg/g of dry matter (dm)] (*9*) but much higher than in barley (41−210 μg/g of dm) (*10*). Because AR are located in the outer layers of the kernel, the AR content is higher in bran than in wholegrain flour, whereas there are almost no AR found in refined flour (*5, 11*). AR content was previously investigated in Swedish wheat samples and was found to vary between 227 and 639 μg/g of dm (*5*). The relative homologue composition of AR in the same wheat samples was 3−6% C17:0, 31−42% C19:0, 47−55% C21:0, 4−10% C23:0, and 1−3% C25:0. The ratio of C17:0 to C21:0 is generally about 0.1 for common wheat (*5*), whereas in durum wheat and rye it has been shown to be 0.01 and 1.0, respectively (*9, 12*). Thus, the ratio of C17:0 to C21:0 may be a tool to distinguish between the different cereals.

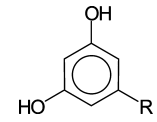

Figure 1. Structure of alkylresorcinols in wheat grains. R is a straight-chain hydrocarbon with 17−25 carbon atoms.

The aim of this study was to investigate the content of AR in a large number of wheat samples grown in Europe including spring and winter wheat, durum wheat, spelt wheat, and early cultivated forms of wheat (diploid einkorn and tetraploid emmer). These samples are part of the HEALTHGRAIN diversity screen, which was established to explore the extent of variation in phytochemicals and other bioactive components

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Table 1. Alkylresorcinols Content (Micrograms per Gram of Dry Matter) and Relative Homologue Composition (Percent) in Winter, Spring, Durum, Spelt, Einkorn, and Emmer Wheat Grain Samples Grown in Hungary, Analyzed with the Fast Blue Method and with GC[a]

		Fast Blue method	GC analysis						
				relative homologue composition (%) (mean ± SD)					ratio of
wheat type	*n*	total AR (mean ± SD)	total AR (mean ± SD)	C17:0	C19:0	C21:0	C23:0	C25:0	C17:0 to C21:0
winter	131	340 ± 57a	410 ± 93a	5 ± 1a	38 ± 3a	46 ± 3a	9 ± 1a	3 ± 1a	0.10 ± 0.02a
spring	20	341 ± 50a	416 ± 73a	4 ± 1a	34 ± 3a	48 ± 3a	10 ± 2b	4 ± 1b	0.09 ± 0.02a
durum	10	327 ± 66a	399 ± 109a	0.4 ± 0.01b	12 ± 2b	61 ± 4b	21 ± 2c	6 ± 2c	0.01 ± 0.001b
spelt	5	427 ± 71b	605 ± 103b	4 ± 0.5a	32 ± 3a	50 ± 2ac	11 ± 1b	4 ± 1b	0.09 ± 0.01a
einkorn	5	399 ± 56b	595 ± 50b	2 ± 1c	14 ± 1b	47 ± 2a	27 ± 1d	10 ± 1d	0.04 ± 0.01c
emmer	5	444 ± 45ab	581 ± 76b	2 ± 1c	16 ± 8b	53 ± 2c	21 ± 6c	7 ± 1e	0.05 ± 0.02c

[a] Values with different letters within a column are significantly different from each other ($p < 0.05$).

in the gene pool available for plant breeders (*13, 14*). AR were analyzed by gas chromatography (GC), which provides both total contents and homologue composition, as well as by the Fast Blue method that provides only total contents but that may be useful for screening large numbers of samples when a fast method is needed.

MATERIALS AND METHODS

Cereals. All cereal samples were supplied by Dr. Zoltan Bedő at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary. They were all grown in the same field at Martonvásár in Hungary and harvested in 2005. The study included 131 winter wheats, 20 spring wheats, 10 durum wheats, 10 early cultivated forms of wheat (5 diploid einkhorn and 5 tetraploid emmer), and 5 spelt wheats, the description of which and their cultivation conditions are given in detail by Ward et al. (*13*).

Analysis of Alkylresorcinols by Gas Chromatography. AR were extracted with ethyl acetate from intact cereal grains and analyzed by gas chromatography (GC) essentially according to the method of Ross et al. (*11*) with slight modifications. Briefly, 200 μL of 0.5 mg/mL methyl behenate (C22:0, fatty acid methyl ester, Larodan Fine Chemicals AB, Malmö, Sweden) was added as an internal standard to wholegrain samples (1 g) that were extracted with 40 mL of ethyl acetate for 24 h with continuous shaking at 20 °C. Portions of the extract (4 mL) were then evaporated to dryness under vacuum using a centrifuge evaporator (Speedvac concentrator, Savant Instruments Inc., Farmingdale, NY). Ethyl acetate (200 μL) was thereafter added, and samples were analyzed by GC as described previously (*11*) but with a modified temperature program to speed the analysis [250 °C (0 min), 320 °C (20 min), 320 °C (22 min), 330 °C (30 min)]. Dry matter content of whole grains was determined by oven-drying of crushed grains (coffee-type mill, Janke and Kunkel, IKA-WERK, Germany) at 105 °C for 16 h. All samples were analyzed in duplicate.

Analysis of Alkylresorcinols by the Fast Blue Method. AR were extracted from the whole grains with acetone and analyzed by a rapid colorimetric micromethod with Fast Blue B salt (*15*). Standard solution was prepared by dissolving 100 mg of 5-*n*-pentadecylresorcinol (Aldrich, 85% purity), purified by recrystallization, in 100 mL of *n*-propanol.

Wholegrain samples (4 g) were weighed in 30 mL screw-cap tubes and mixed with 16 mL of acetone. Thereafter, the tubes were shaken for 4 h at 50 °C and placed in the dark overnight. A working solution of Fast Blue B BF_4 salt (Chemapol, Prague, Czech Republic) was prepared by mixing 1 volume of this salt dissolved in 5% of acetic acid (0.5 mg/mL) with 4 volumes of methanol. This solution had to be prepared daily in the needed quantity. Then, 4 mL of the working solution was transferred into glass test tubes together with 20 μL of the acetone extract of samples. After mixing, the test tubes were placed in the dark for 60−120 min for color development. The absorbance was read at 520 nm for each sample against the reagent blank (working solution). A calibration curve was prepared from the standard solution containing 1 mg of purified 5-*n*-pentadecylresorcinol in 1 mL of *n*-propanol, of which 5, 10, 15, and 20 μL were used.

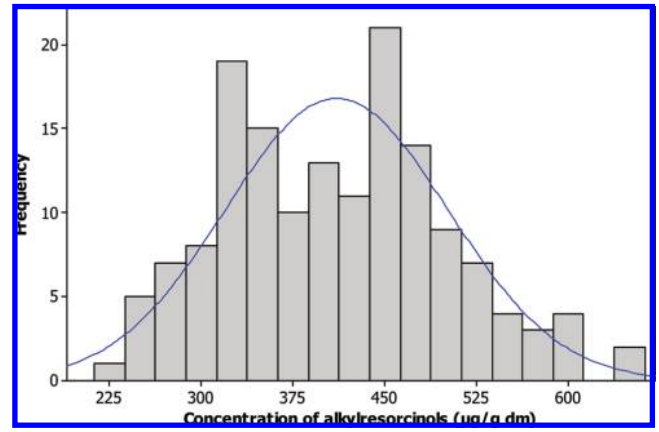

Figure 2. Distribution of total alkylresorcinol content analyzed with GC in European winter and spring wheat grain samples.

Statistical Analysis. The results were analyzed statistically with ANOVA, the GLM procedure, and Tukey's pairwise comparison (Minitab 15) to investigate differences between different types of wheat.

RESULTS AND DISCUSSION

Content and Homologue Composition of Alkylresorcinols in Wheat Grain Samples Analyzed by GC. The AR contents in European varieties of winter wheat, spring wheat, durum wheat, spelt wheat, and early cultivated forms of wheat (einkorn and emmer) were determined (**Table 1**). There was a wide variation in total AR content of the different wheats. The AR content in winter and spring wheats was not significantly different from a normal distribution (**Figure 2**) and ranged from 220 to 652 μg/g of dm (mean = 410 μg/g of dm) and from 254 to 537 μg/g of dm (mean = 416 μg/g of dm), respectively. The total mean is thus very similar to what has been reported by Chen et al. (*5*) for 32 Swedish wheat varieties (412 μg/g of dm). Chen et al. (*5*) found a difference between Swedish winter and spring wheat (295 and 548 μg/g of dm, respectively), which was not found in this study. Our results were also comparable to the content in North American wheat samples (300−700 μg/g of dm) (*16*) but lower than in western European wheat samples analyzed earlier (595−1429 μg/g of dm) (*9*). Among the winter wheats, most of the cultivars (10 of 14) with low AR content (<300 μg/g of dm) originated from Russia, Romania, and Serbia, whereas most of the cultivars (15 of 22) with high AR content (>500 μg/g of dm) were from Germany, France, and the United Kingdom. Correlations between different components and agronomic characteristics of different wheats are discussed by Ward et al. (*13*).

The AR contents in durum, spelt, einkorn, and emmer wheat varieties were 194−531 (mean = 399), 490−741 (mean = 605),

545−654 (mean = 595), and 531−714 (mean = 581) μg/g of dm, respectively, but only a few samples of these varieties were analyzed (**Table 1**). The AR content of durum wheat was in the same range as common wheat, whereas the contents of spelt, einkorn, and emmer wheats were significantly higher ($p < 0.05$). The content of AR in durum wheat has earlier been reported to be slightly higher than in common wheat (*9, 12*). In an earlier study, the contents of AR in one spelt and one emmer grown in Sweden were found to be 819 μg/g of dm (*9*), which were also higher than in any of the spelt and emmer wheats samples in this study. The variation in results between different studies may be due to different cultivars and different environmental conditions (*5, 17*).

The relative distribution of AR homologues showed only a small variation between different winter and spring wheat samples (**Table 1**), as has been reported earlier (*5, 17*). The dominant AR homologues in winter and spring wheat were C19:0 (about 36%) and C21:0 (47%), whereas C17:0, C23:0, and C25:0 were found in smaller amounts. The AR homologue pattern of durum wheat was completely different from common wheat, which has also been shown earlier (*9, 12*). Durum wheat contained less of C17:0 (∼0.4%) and more of the longer homologues, especially C23:0 (∼21%). As for common wheat, the predominating homologue in durum wheat was C21:0, but this was higher than in common wheat (60 and 50%, respectively). In common wheat, the ratio of the homologues C17:0 to C21:0 was ∼0.1, whereas in durum wheat it was ∼0.01. In rye, the ratio has been shown to be ∼1.0 (*2, 6*). Thus, the ratio of C17:0 to C21:0 can be used to distinguish between the three cereals. Spelt wheat showed a distribution of AR homologues similar to that of common wheat, in agreement with an earlier study (*9*). The AR homologue composition of einkorn and emmer wheat was more like durum wheat, with lower relative contents of C17:0 and C19:0 (2 and 15%, respectively) and higher content of C23:0 (24%). The relative content of C21:0 was, however, similar to that of common wheat. The ratio of the homologues C17:0 to C21:0 was ∼0.04, which is different from other cereals.

Content of Alkylresorcinols in Wheat Grain Samples Analyzed by the Fast Blue Method. For this analysis, extraction was performed with some modifications of the original method (*15*). Sample size, extraction solvent volume, and extraction time were changed. Extraction with acetone was previously found to provide the same yield of AR as extraction with ethyl acetate in GC analysis (*11*). The total content of AR analyzed by the Fast Blue method showed the same wide variation between samples as the content analyzed by GC, but it was lower. The content in winter and spring wheat ranged from 193 to 486 μg/g of dm (mean = 340 μg/g of dm) and from 250 to 420 μg/g of dm (mean = 341 μg/g of dm), respectively. The result from GC analysis, with no difference between winter and spring wheats, was thus confirmed. The AR content in durum wheat was 182−432 μg/g of dm (mean = 327 μg/g of dm) and in spelt and wild wheat, 350−518 μg/g of dm (mean = 427 μg/g of dm) and 353−495 μg/g of dm (mean = 421 μg/g of dm), respectively. These results also showed the same trends as for the GC analysis. There was a good correlation between the two methods ($R^2 = 0.76$), but as mentioned earlier, the content was lower with the Fast Blue method compared to the GC method (**Figure 3**). The Fast Blue method gives no information about the homologue composition of AR, but it is a fast, easy, and simple method for the analysis of a large number of samples. There is, however, a need to evaluate and validate the method further with other cereals (including rye and barley) to give a wider range of AR contents and compositions.

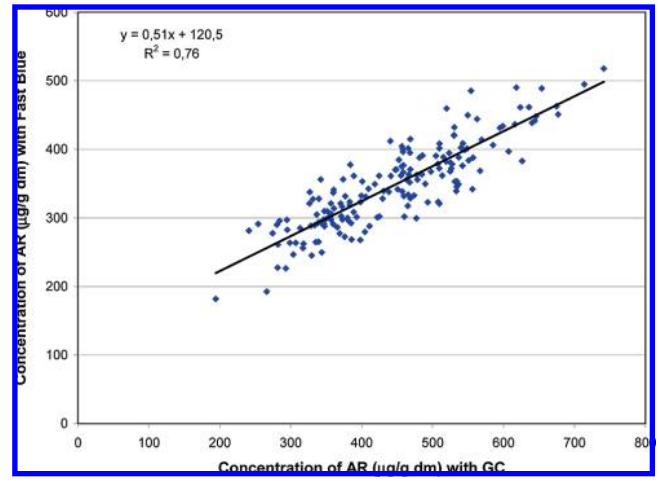

Figure 3. Correlation of alkylresorcinol (AR) concentration in wheat samples between the GC method and the Fast Blue method.

Conclusion. This is the largest study so far on alkylresorcinol content and composition in different wheat samples. The results showed that there was a wide variation in AR content in this diverse material, which is in agreement with earlier studies on smaller numbers of wheat samples from different countries (*5, 9, 16*). The wide variability provides potential to be used in plant breeding as has been done earlier to increase or decrease the content of AR (*18*). The wide variation can have both positive and negative implications. It is positive for the use of AR as a bioactive component because this makes it possible to breed/select toward higher contents. For the use of AR as a biomarker, a wide variation is of course negative. However, the variation in these samples is probably larger than in common wheat samples used for food production.

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