JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Analysis of Alkylresorcinols in Cereal Grains and Products Using Ultrahigh-Pressure Liquid Chromatography with Fluorescence, Ultraviolet, and CoulArray Electrochemical Detection

Alastair B. Ross*

Nestlé Research Centre, Vers chez les Blanc, 1000 Lausanne 26, Switzerland

Supporting Information

ABSTRACT: Alkylresorcinols are phenolic lipids, with homologues ranging from C17 to C25, found in high concentrations in whole grain wheat and rye, lower concentrations in barley, and negligible concentrations in refined wheat flour. The analysis of alkylresorcinols is of importance due to their potential as biomarkers of whole grain intake and emerging evidence for some biological effects. Present HPLC methods have insufficient resolution for accurately quantitating the mix of alkyl- and alkenylresorcinols found in rye. An ultrahigh-pressure liquid chromatography method was developed, and three detection methods (CoulArray (CAED), ultraviolet (UV), and fluorescence detection (FD)) were compared for cereal alkylresorcinol analysis. The lower limits of quantitation and detection were 50 and 20 pg injected, 5 pg and 2 pg injected, and 500 and 1250 pg injected for FD, CAED, and UV, respectively. FD and CAED provided similar results, with some bias for higher results with FD (<10% difference). UV detection generally resulted in overestimation of alkylresorcinol concentrations. The method was applied to cereal (15) and cereal product (90) samples mainly from the United States with results in the same range as previous methods. The improved resolution with this method allows facile analysis of alkylresorcinols from cereal products, including minor unsaturated homologues such as those found in rye.

KEYWORDS: *alkylresorcinols, cereals, whole grain, UPLC, fluorescence*

INTRODUCTION

Alkylresorcinols in cereals are long-chain phenolic lipids with side chains mainly ranging from C17 to C25, with saturated hydrocarbon chains being mainly present in wheat (95%) and rye (80%).[1,2] Unsaturated alkyl chains and other natural derivatives of alkylresorcinols are also found, with generally <5% in wheat and <20% in rye present as nonsaturated alkylresorcinols.[3,4] They are located only in the outer layers of wheat, rye, and barley[5] and as such have been suggested to be markers of the whole grain flour of these cereals in food products and biomarkers of whole grain intake when measured in plasma.[6] They are not found in the edible parts of other food plants with the exception of mango; low concentrations (approximately 90 μg/g DM) of shorter chain alkylresorcinols (mainly C15:0, C17:1, and C17:2) have been reported in mango flesh.[7]

Alkylresorcinols are of interest in nutrition science as they are promising biomarkers of whole grain intake in populations where this is largely based on wheat and rye.[6,8−11] There is also an emerging body of in vitro evidence that suggests that although they have limited antioxidant capacity,[12,13] they may have some bioactivities related to inhibition of enzymes.[6,14] In vitro they can inhibit lipolysis and accumulation of triglycerides in adipocytes possibly via inhibition of phosphorylation of hormone-sensitive lipase and via the inhibition of glycerol-3-phosphate dehydrogenase.[15,16] Alkylresorcinols may also induce apoptosis,[17−19] especially in colon cancer models.[20,21] Some models suggest that chain length and chain modifications such as unsaturation or keto groups have an impact on potential bioactivities, possibly related to differing solubility,[13,16] suggesting that quantitation of these homologues may be important. Conclusive studies on whether alkylresorcinols have any bioactivity in higher mammals remain to be carried out.

A wide range of analytical methods have been applied to the analysis of alkylresorcinols in cereals and cereal foods,[4] including spectrophotometric methods based on reactions with the dye Fast Blue B to measure total alkylresorcinols[22,23] and both gas chromatographic (GC)[2,24] and high-performance liquid chromatography (HPLC)[25−27] methods to measure the individual alkylresorcinol homologues. GC analysis has the advantage of providing very high resolution, important for analysis of rye, which contains a number of unsaturated homologues, although published methods for cereals tend to have longer run times (25−35 min)[23] than some HPLC methods (14 min).[26] Most published HPLC methods based on UV detection have run times in the range of 30−90 min[27−29] due to the lower resolution of HPLC and the relative nonspecificity of UV detection. Even with longer run times, adequate resolution of unsaturated homologues from saturated homologues remains an issue. Recent advances in liquid chromatography allowing the use of higher back pressures (>1000 bar) and consequently smaller silica particle sizes in columns (<3 μm) have the potential to improve resolution in liquid chromatography and may allow better resolution of

Received: March 29, 2012
Revised: August 7, 2012
Accepted: August 11, 2012
Published: August 11, 2012

saturated alkylresorcinol homologues from unsaturated homologues.

Previously we have successfully used CoulArray electrochemical detection (CAED) as a partially selective method to rapidly analyze alkylresorcinols by HPLC.[26] This method could separate the main alkylresorcinol homologues with a 14 min run time, although baseline separation was not achieved for unsaturated and saturated homologues, meaning that this method is best suited for wheat-based products (<5% unsaturated homologues), but less ideal for rye samples with a greater proportion of unsaturated homologues (~20%). In addition, fluorescence detection (FD), previously suggested to be a potential detection method for HPLC[4] and used as a standalone technique to analyze alkylresorcinols in bulk cereal extracts,[27,30,31] has yet to be tested for suitability as a detector for chromatographic analysis of alkylresorcinols.

The aim of this paper was to use UHPLC to improve the resolution of alkyl- and alkenylresorcinols in cereals and cereal grains and to compare different detection methods: CoulArray (CAED), ultraviolet (UV), and, for the first time, fluorescence detection (FD). Confirmation of peak identity of minor alkenylresorcinols was carried out using offline GC-MS. This new method was applied to the analysis of cereal samples from Europe and North America.

MATERIALS AND METHODS

Chemicals and Samples. Alkylresorcinol standards [heptadecylresorcinol (C17:0), nonadecylresorcinol (C19:0), eicosylresorcinol (C20:0), heneicosylresorcinol (C21:0), tricosylresorcinol (C23:0), and pentacosylresorcinol (C25:0)] were from Reseachem (Burgdorf, Switzerland) and were >95% pure. Ammonium acetate was from Sigma-Aldrich (Buchs, Switzerland). All solvents used were of HPLC grade from Merck (Darmstadt, Germany). Cereal samples were obtained from local supermarkets (Lausanne area, Switzerland) or were gifts (see the Acknowledgment).

UHPLC Method Development. This method was modified on the basis of a previously published HPLC method using CAED.[26] The following parameters were optimized: column, injection volume, temperature, gradient, and re-equilibration time. An extract from rye was used to determine the optimum conditions, as rye has the most complex homologue profile. The following columns were tested: Acquity BEH C18, 150 × 2.0 mm, 1.7 μm particle size (Waters, Milford, MA, USA); Acquity BEH-shield C18, 150 × 2.0 mm, 1.7 μm particle size (Waters; different selectivity for phenolic compounds compared to the BEH column); Zorbax Extend C18, 100 × 2.1, 1.8 μm (Agilent, Santa Clara, CA, USA); and a Kinetex core−shell C18, 150 × 2.0 mm, 1.7 μm particle size column (Phenomenex, Torrance, CA, USA). A C18-based precolumn (Phenomenex) was used for all analyses. Injection volumes from 1 to 10 μL were tested.

Column temperatures were 40, 50, and 60 °C, with temperature optimized for peak resolution and system back-pressure.

For CAED analyses, solvent A was MeOH/water/5 M ammonium acetate, pH 6 (89:10:1 v/v), and solvent B was MeOH/5 M ammonium acetate, pH 6 (99:1 v/v). For UV and fluorescence detection analyses, solvent A was MeOH/water (89:11 v/v) and solvent B was MeOH/water (99:1 v/v).

A Dionex UltiMate 3000 RS ultrahigh-performance liquid chromatography system was used for all analyses (Dionex, Sunnyvale, CA, USA) and included a DAD and fluorescence detector in series. The system was coupled to an eight-channel CAED detector (ESA Science, Chelmsford, MA) and connected directly to the column outlet when used. Electrodes were set to 0, 100, 200 400, 560 (predominant electrode), 780 (dominant electrode), 820 (postdominant electrode), and 850 mV. The thermal organizer housing the CAED cells was set to 37 °C.

UV data were collected at 276 nm. Optimal fluorescence detector conditions were determined using stop-flow analysis of standard alkylresorcinols. On the basis of the UV spectrum, the optimal excitation wavelength was 276 nm. Optimal emission was at 306 nm. Standard samples were run at wavelengths higher than 306 nm to ensure that, on the basis of peak area and signal-to-noise ratio, that this was the optimum emission wavelength. Samples were integrated using CoulArray Data Station (ESA Science) software and Chromeleon 6.8 (Dionex).

The final LC method had the following program: 0 min, 0% B; 1 min, 25% B; 6 min, 100% B; 8 min, 100% B; 10 min, 0% B; 14 min, 0% B. Flow rate was constant at 0.65 mL/min. Optimal column temperature was 60 °C.

Limit of detection (LOD) and limit of quantitation (LOQ) were determined in a cereal extract matrix naturally free of alkylresorcinols (white rice) by sequential dilution of a mix of alkylresorcinol standards (C15:0−C25:0) in 4 mL of white rice extract, followed by evaporation and resuspension in 1 mL of MeOH. LOD and LOQ were defined as signal-to-noise ratios of 3:1 and 5:1, respectively.

The UHPLC method with CAED, UV detection, and FD was compared to a previously published method using HPLC-CAED[26] on the UltiMate 3000 RS system described above and using the same column, solvents, and flow rate as previously published.

Extraction and Quantitation of Cereal Samples. Samples were extracted in duplicate using appropriate methods for unprocessed cereals[24] and processed cereals.[1] An internal standard, alkylresorcinol C20:0 (20 μg/sample, not present naturally), was added to each cereal sample prior to extraction. Unprocessed cereal flours (approximately 0.5 g) were extracted using 20 mL of ethyl acetate for 24 h and then centrifuged at 2000*g* to obtain a clear supernatant. Processed cereal samples (approximately 0.5 g) were extracted using 1-propanol and water (3:1 v/v) in a boiling water bath (3 × 10 mL for 2 × 2 h and 1 × 1 h), with extracts pooled and evaporated under N_2. For unprocessed samples, 4 mL of sample extract was evaporated under nitrogen and reconstituted in 1 mL of methanol. For processed samples, extracts were pooled and reconstituted in 1.5 mL of methanol. For samples estimated to contain >200 μg/g, it was necessary to dilute the samples by 2 times to bring the response into the range of the standard curve. All whole grain wheat and rye samples were diluted in this manner. Prior to injection, sample vials were centrifuged at 5350*g*. For UHPLC analyses, 2 μL of sample was injected onto the LC system. Standard curves were run with the samples with the following concentrations: 1, 2.5, 5, 10, and 25 μg/mL. Peak areas relative to C20:0 were used to determine the relative detector response for each alkylresorcinol. Peaks were identified by comparing retention times with authentic standards and offline analysis by GC-MS (see below). Samples with >10% difference between duplicate extracts were reanalyzed.

GC-MS Analysis. Confirmation of the identity of alkylresorcinols for which no standards were available (i.e., unsaturated alkylresorcinols) was performed using offline GC-MS analysis of LC fractions. An extract of rye was injected 30 times (5 μL injection volume; no discernible change in resolution compared to 2 μL injection volume), and fractions were collected every 15 s. Dried LC fractions were derivitized using MSTFA + 1% TMCS (60 min at 60 °C) and injected onto an Agilent-6890 GC system with an Agilent 5975A MS detector (Agilent) fitted with an DB-5MS 15 m column (0.25 mm i.d.; 0.25 μm film thickness; Agilent). Alkylresorcinols were separated using the following oven program: 150 °C (0 min); 150 °C (2 min); 230 °C (4 min); 290 °C (16 min); 290 °C (22 min); 300 °C (23 min); 300 °C (28 min); 320 °C (28.5 min); 320 °C (32.5 min). The injector was held at 300 °C. Spectra were collected in full-scan mode, scanning from m/z 100 to 750. TMS-derivitized AR were identified by their characteristic base peak at m/z 268[24] and the molecular ion (see ref 4 for a list).

Data Analysis. HPLC-CAED and UHPLC-CAED, -UV, and -FD results from a subset of 40 samples with concentrations ranging from 20 to 550 μg/g (based on CAED quantitation) were compared using Bland−Altman plots[32] and regression analysis (Excel; Microsoft Corp., Redmond, WA, USA) and Minitab (Minitab Inc., State College, PA, USA). Correlations are reported with the intercept included in the regression model.[23]

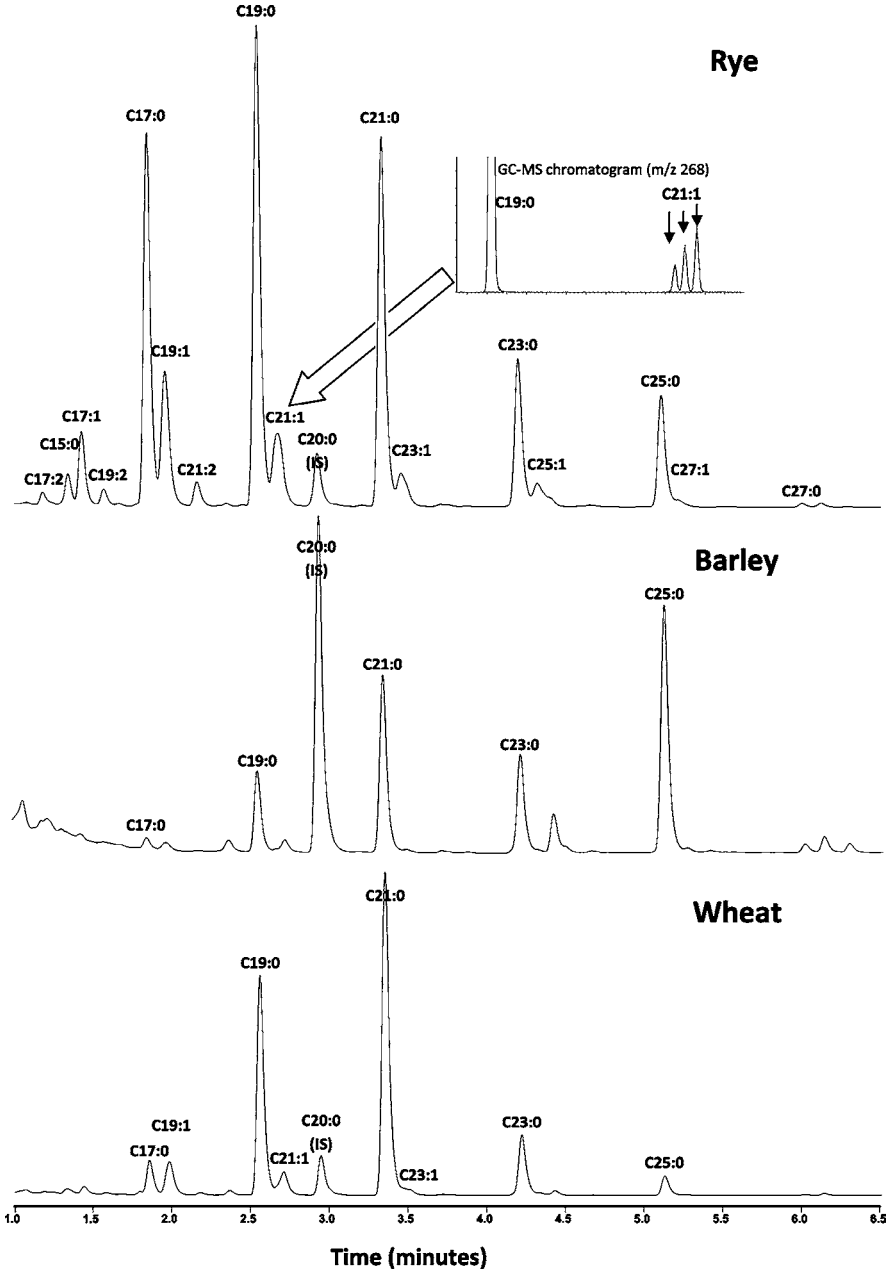

Figure 1. Fluorescence detection chromatograms of rye, barley, and wheat samples. *Y*-axes scales differ for each chromatogram (not shown), and the internal standard (alkylresorcinol C20:0) indicates the relative concentration. The inset with the rye chromatogram is from GC-MS analysis of a fraction collected containing both C19:0 and C21:1, indicating that the C21:1 peak consists of three different positional isomers of C21:1. The same pattern was found for the other monounsaturated peaks.

■ RESULTS AND DISCUSSION

In developing a previous method for the HPLC separation of alkylresorcinols, the objective was rapid separation of the main alkylresorcinol homologues. In this work, the objective was to focus on improving resolution between alkylresorcinols and alkenylresorcinols that had previously coeluted to some extent in the previous method[26] and also to assess if FD could be an alternative to CAED for high-sensitivity analysis of AR in cereals.

Testing of Different Columns. Four different columns were tested: Waters BEH C18, 150 × 2.0 mm, 1.7 μm column; Waters BEH-shield C18, 150 × 2.0 mm, 1.7 μm column; Agilent Zorbax Extend C18, 100 × 2.1 mm, 1.8 μm; Phenomenex Kinetex, 150 × 2.0 mm, 1.7 μm core shell column. The best resolution (based on a sample with the most complex AR homologue pattern (rye)) was achieved using the Kinetex column, and it was possible to achieve close to baseline resolution for all unsaturated alkylresorcinols from the saturated alkylresorcinols. This column was used for all further method development. Surprisingly, the 1.8 μm particle size Zorbax Extend column resulted in peak tailing, when the 3.5 μm variant did not for HPLC analysis.[26] An inherent improvement based on core−shell technology is unlikely as earlier tests on a 3 μm Kinetex core−shell column resulted in peak tailing (data not shown). The two Waters columns tested resulted in sharper peaks compared to HPLC, but did not result in greatly improved resolution between saturated and unsaturated homologues. Back pressures on the system tested

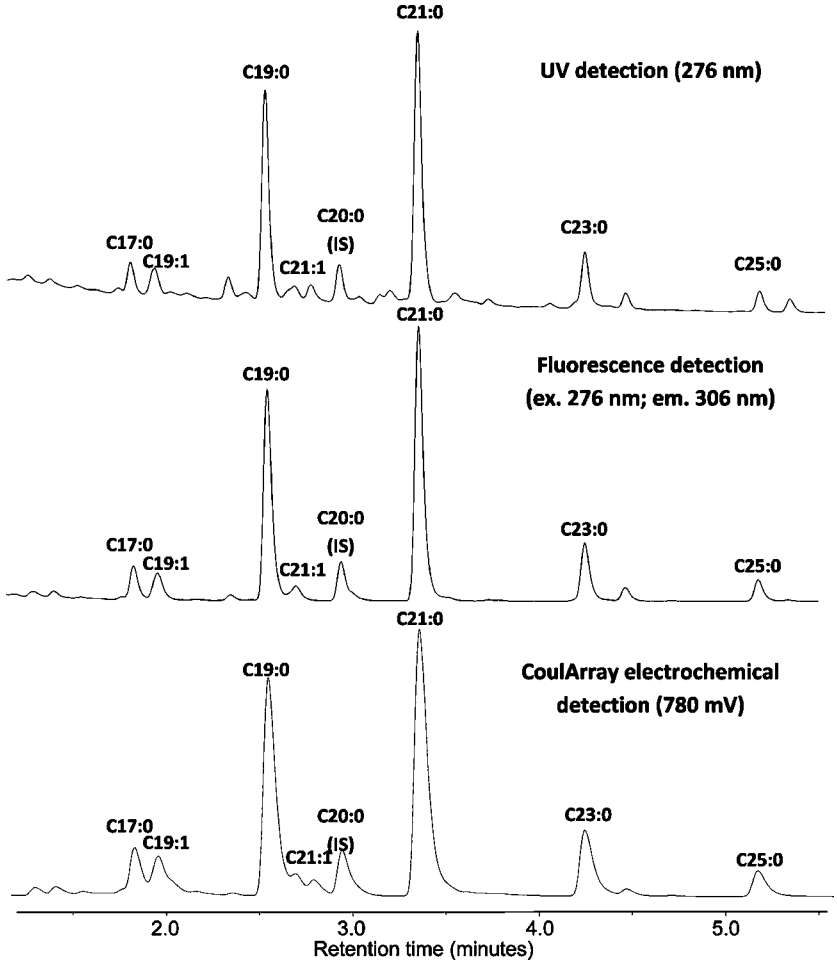

Figure 2. Chromatograms of the same whole grain wheat sample with ultraviolet, fluorescence, and CoulArray electrochemical detection.

were similar for the three 150 mm columns with a maximum pressure of around 900 bar with a flow rate of 0.65 mL/min at 60 °C. On the basis of the final chromatographic conditions, 37% less mobile phase solvent was used compared to the previous HPLC-CAED method,[26] and using the UV or fluorescence detector also avoided the need to add salt to the mobile phase.

Although possible adjustments to the method may have allowed a slightly shorter run time, a 14 min total run time was kept to allow a long re-equilibration time, while still being sufficiently high throughput for the analysis of over 40 samples in duplicate per day; at this point, sample preparation rather than chromatographic analysis is the bottleneck in cereal alkylresorcinol analysis. Testing with shorter re-equilibration times (2 and 3 min) resulted in deterioration of peak shape over 10 injections (data not shown).

Different injection volumes were tested, and it was found that irrespective of the concentration of alkylresorcinols in the sample, injection volumes >10 μL resulted in peak splitting. Large injection volumes were not required due to the sensitivity of both the CAED and FD, and 2 μL injections were found to be the best compromise between peak shape, sensitivity, and repeatability for the sample loop used (50 μL).

Comparison of CoulArray Electrochemical Detection with Fluorescence and Ultraviolet Detection. FD was found to be suitable for detecting alkylresorcinols and resulted in chromatograms without some of the interferences that were observed for UV detection, especially close to the C23:0 peak (Figures 1−3). FD resulted in low LOD and quantitation LOQ (20 and 50 pg injected, respectively) compared to UV detection at 276 nm (0.5 and 1.25 ng injected for LOD and LOQ, respectively). CAED was still the most sensitive of the detection methods with an LOD of 2 pg and LOQ of 5 pg injected. The improved peak shape compared to the previous rapid HPLC method meant that all three detectors tested had better sensitivity compared to HPLC-CAED tested previously (LOD = 1 ng and LOQ = 2.5 ng injected). Even though sensitivity with the UV detector was improved with UHPLC, in practice the general baseline of extracts detected with UV was bumpy, reflecting the relative abundance of compounds that absorb light around 280 nm (Figures 2 and 3). Peak shape was adversely affected by postcolumn dead volume, with peak shape decreasing in the same order as the postcolumn dead volume (UV-FD-CAED) (Figures 2 and 3). Using UV and fluorescence detectors in series is not recommended for routine quantitative analysis.

Calibration curve correlations were similar between detection techniques, as were relative responses. The pattern for an increasing response with decreased chain length was negated when molecular weight was accounted for (use of μmol/L rather than μg/mL) (Supporting Information, Supplementary Figure 1), confirming that for all detection techniques, the response is due to the 1,3-dihydroxybenzene "head" group of the alkylresorcinol.

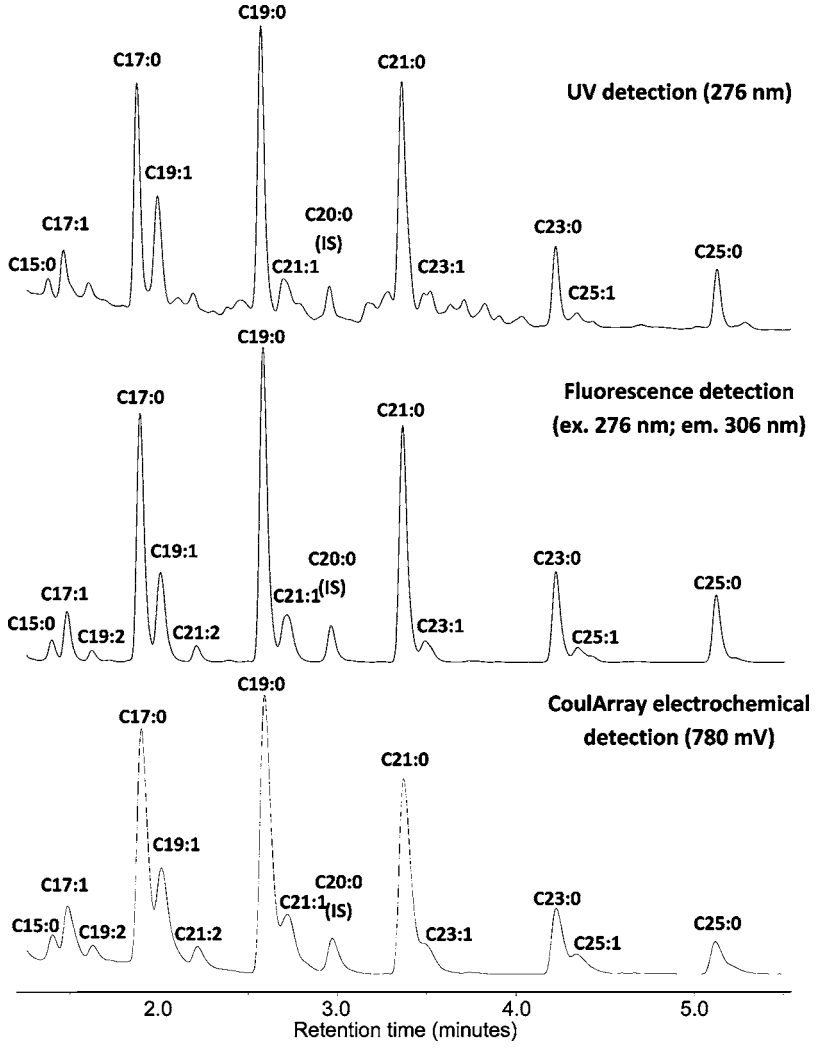

Figure 3. Chromatograms of the same whole grain rye sample with ultraviolet, fluorescence, and CoulArray electrochemical detection.

Table 1. Alkylresorcinol Concentrations in Different Cereals[a]

			% of each alkylresorcinol homologue					
	country of origin[b]	*N*	C17:0	19:0	C21:0	C23:0	C25:0	total (μg/g)
refined wheat	US	2	2.9 (2.5)	29.6 (4.9)	46.7 (3.2)	12.9 (1.3)	7.9 (2.8)	20.9 (13.8)
durum wheat semolina	US	2	0.85 (1.1)	12.2 (1.3)	56.2 (5.2)	21.6 (3.5)	9.2 (4.0)	71.3 (4.4)
whole grain hard wheat	US	4	3.6 (1.2)	31.7 (2.0)	49.5 (1.4)	13.8 (1.0)	4.6 (0.6)	376.2 (36.9)
whole grain durum wheat	US	4	0.2 (0.3)	12.3 (0.9)	55.8 (2.2)	23.1 (2.0)	8.7 (1.3)	444.2 (55.8)
whole grain buckwheat	US	1	0	0	0	0	0	0
whole grain einkorn	DE	1	0.2	14.7	43.5	28.4	13.1	391
whole grain Kamut	DE	1	0	10.1	54.2	26.1	9.5	255.5

[a]Data are based on fluorescence detection. Means and percentages of homologues are based on duplicate analyses of each sample with a difference of <10%. Numbers in parentheses are the standard deviation. [b]Country of origin: US, United States; DE, Germany.

There was excellent correlation between results from HPLC-CAED and UHPLC-CAED (R^2 = 0.99; P < 0.001; slope = 0.90) and from UHPLC-CAED and UHPLC-FD (R^2 = 1.0; P < 0.001; slope = 1.06), whereas correlations were lower for UHPLC DAD with UHPLC-CAED (R^2 = 0.95; P < 0.001; slope = 1.40) and UHPLC-FD (R^2 = 0.95; P < 0.001; slope = 1.32). When included in the regression model, the intercept for UHPLC-CAED versus UHPLC-FD (the main comparison of interest) was 6.7 μg/g (P = 0.02), indicating that there was a slight difference between these two detection methods, but of minimal importance (an average of 4% difference across all measurements). Below 20 μg/g, percentage differences were greater between CAED and FD, although <5 μg/g and thus of little practical importance. Bland–Altman comparisons indicated that there was a significant trend for higher values for FD compared to CAED (R^2 = 0.53; P = 0.026), although this trend was nonsignificant for samples below 300 μg/g (R^2 = 0.10; P = 0.095) (Supporting Information, Supplementary Figure 2). Comparison between CAED and FD with UV using Bland–Altman plots found that there was relatively good agreement between 20 and 200 μg/g and then a much greater propensity to overestimate the concentration. The average

Table 2. Alkylresorcinol Concentrations in Different Cereal Products[a]

	country of origin[b]	% WG[c]	% of each alkylresorcinol homologue					total (μg/g)
			C17:0	C19:0	C21:0	C23:0	C25:0	
Breakfast Cereals								
corn-based cereal	US	0	0	0	0	0	0	0
cornflakes 1	US	0	0	0	100	0	0	0.2
cornflakes 2	US	0	0	0	0	0	0	0
rice-based breakfast cereal 1	US	0	0	24.1	75.9	0	0	0.1
rice-based breakfast cereal 2	US	0	0	0	0	0	0	0
WG oat cold breakfast cereal	UK	92 O	4.3	34.5	45	13.1	3.1	4.6
porridge oats 1	AU	100 O	7.5	25.7	33.3	31.9	1.6	1.2
porridge oats 2	US	100 O	5.5	35.2	37.6	15	6.7	1.3
cream of wheat	US	0	5.2	33.3	47.8	9.7	3.9	52.6
refined wheat flakes + bran	FR	0	4	29.5	48.7	11.9	5.8	100.7
high-fiber breakfast cereal	US	43[d]	4.4	37.5	47.6	8.5	2.1	145.9
muesli 1 (WG oat and wheat)	DE	ND	4.4	28.9	47	15.1	4.6	199.3
muesli 2 (WG oat and wheat)	FR	18 W	5.2	32.2	45.4	11.7	5.4	141.8
WG wheat breakfast cereal 1	US	81 W	4.4	33	50.8	8.9	2.9	279.8
WG wheat breakfast cereal 2	US	70 W	4.6	32.2	49.1	9.5	4.5	412.4
WG wheat/rye breakfast cereal	IT	28 W + 25 R + WB	7.7	28.9	42.1	13.9	7.4	627.9
Breads and Crackers								
white wheat bread 1	US	0	5.2	35.7	44.7	10.3	4.1	19.8
white wheat bread 2	US	0	7.1	31.9	46.1	10.5	4.5	24.6
white wheat bread 3	FR	0	8.2	29	39.5	13.1	10.2	28
white wheat bread 4	FR	0	3.7	25.6	42.8	18.7	9.2	16.8
refined wheat bread roll	US	0	4.5	29.3	47.3	12.6	6.3	15.8
refined wheat hamburger bun	US	0	6.7	31.4	44.4	11.4	6.1	25.1
refined wheat tortilla	US	0	5.8	33.4	44.2	11.4	5.2	13.6
refined wheat pancakes (cooked)	US	0	6.2	32.8	42.9	12.6	5.5	15.4
refined wheat crackers 1	US	0	4.4	31.4	50	7.4	6.7	16.8
refined wheat crackers 2	US	0	9.4	22.5	44.1	15.9	8	15.9
refined wheat dry toast	IT	0	4	29.3	38	19.9	8.8	34.2
refined bagels 1	US	0	5	31.5	47.1	11.2	5.2	16
WG wheat bread 1	US	75 W[d]	4.8	36.2	48.6	7.9	2.5	277.5
WG wheat bread 2	US	73 W[d]	4.4	32	49.3	9.9	4.5	456
WG wheat roll	US	60 W[d]	4.7	32.7	49.7	9.2	3.7	467
WG wheat hamburger bun	US	80 W[d]	4.4	32	49	10.2	4.5	451.6
WG rye bread	FR	54 R	21.5	28.7	24.6	14.3	11	555.6
WG wheat and corn tortilla	US	80 W[d]	6.4	36.4	45.6	8.6	3	364.2
WG wheat pancakes (cooked)	US	29 W[d]	4.9	35.4	47.8	8.8	3.1	130.9
WG wheat bagel 1	US	80 W[d]	4.3	31.8	49.9	10	3.9	480.8
WG wheat bagel 2	US	80 W[d]	4.7	31.7	49.7	9.9	4	454.1
WG wheat muffin	US	51 W[d]	4.4	33.6	47.8	10	4.2	199.7
WG wheat banana muffin	US	51 W[d]	4.4	33.3	47.9	10.8	3.6	192.7
WG wheat crackers	US	90 W[d]	4	30.9	52.6	9.4	3.1	305
WG multigrain crackers	US	83 M[d]	7.3	31.3	45.5	10.8	5.2	373.7
WG wheat dry toast 1	IT	40 W[d]	4.5	30.5	45.6	13.8	5.7	193
WG wheat dry toast 2	FR	47 W	4.2	27.6	49.1	14.1	5.1	192.8
WG wheat dry toast 3	FR	11 W	4.5	30.2	47.7	12.1	5.5	93
multicereal dry toast 4	FR	7 W	7.3	29	38.8	15.2	9.6	57
rye crispbread 1	DE	58 R + WB	13.7	28.9	36.9	13.1	7.3	948.8
rye crispbread 2	DE	77 R	21.5	28	27.1	13.9	9.6	704
WG wheat rusks 1	SE	56 W	5	30.3	45.4	13.9	5.5	339.4
WG wheat rusks 2	SE	60 W	4.9	30.4	45.5	13.6	5.6	329.4
Pasta and Other Main Meal Foods								
refined wheat pasta 1	US	0	0.9	12.4	58	20.7	8	44
refined wheat pasta 2 (raw)	US	0	0.9	13.3	58.6	20	7.1	53.5
refined wheat pasta 2 (cooked)	US	0	1.1	13.9	59.7	19	6.3	58
refined wheat pasta 3	IT	0	0.2	13.3	52.9	22.9	10.7	49.5
refined wheat pasta 4	IT	0	0.3	7.3	58.4	23.5	10.5	40.7
egg noodles (raw)	US	0	1.1	12.8	57.2	21.1	7.8	33
egg noodles (cooked)	US	0	1	13.5	58.7	20	6.8	39.7

dx.doi.org/10.1021/jf301332q | J. Agric. Food Chem. 2012, 60, 8954−8962

Table 2. continued

	country of origin[b]	% WG[c]	% of each alkylresorcinol homologue					total (μg/g)
			C17:0	C19:0	C21:0	C23:0	C25:0	
Pasta and Other Main Meal Foods								
refined wheat grits	US	0	0	0	0	100	0	0
refined wheat couscous	FR	0	0.4	12	55.5	22	10.1	69.2
WG wheat pasta 1	IT	100 W	1.1	11.2	55.7	23.2	8.9	201.2
WG wheat pasta 2	IT	100 W	1.2	11.2	56	23.5	8.1	206.2
WG wheat pasta 3	US	100 W	0.5	13.4	60.2	19.2	6.6	237.5
WG wheat pasta 4 (raw)	US	100 W	0.5	11.8	59.5	20.7	7.5	348.9
WG wheat pasta 4 (cooked)	US	100 W	0.5	12	60.2	20.3	6.9	380.3
WG wheat pasta 5	NL	100 W	2	17.5	53.3	18.9	8.3	383.1
WG wheat pasta 6	IT	100 W	0.4	11.5	54.6	23.1	10.4	214.2
WG wheat pasta 7	IT	100 W	0.5	12.5	53.2	23.8	10.1	192.5
WG wheat pasta 8	IT	100 W	0.4	12.5	52.9	24.4	9.8	180.3
WG wheat pasta 9	IT	100 W	0.5	13.5	53	23.5	9.4	171.7
bulgur	FR	100 W	1.4	9.6	13	25.2	9	312.3
precooked wheat grains	FR	0[e]	0.4	9.5	14.2	23.5	8.4	260.7
WG couscous	FR	100	0.5	10.5	54.8	24.6	9.6	401.7
Snacks								
cookie 1	US	0	14.3	9.5	18.3	52.8	5.1	1.7
cookie 2	US	0	38.3	0	61.7	0	0	0.8
cookie 3	US	0	8.3	22.1	45.1	20.9	3.6	14.3
refined pretzels	US	0	4.7	25.6	45.9	16	7.9	21.8
WG multigrain cookie	US	30 W	8.1	27.2	34.4	25.8	4.5	133.2
mulitgrain muesli bar	US	15 W[d]	11.5	33.6	39.5	10.1	5.2	63.2
WG oat and wheat muesli bar 1	AU	28 O + 10 W	9.2	31.3	48.6	8.3	2.5	32.3
WG oat and wheat muesli bar 2	AU	33 O + 10 W	5.2	33.5	46	11.4	3.7	28.8
WG oat and wheat muesli bar 3	AU	33 + 10 W	4.7	33.3	48.1	10.5	3.4	50.4
WG oat and wheat muesli bar 4	PL	34 O + 6 W	2.8	23.1	39.5	29	5.6	59.4
WG oat and wheat muesli bar 5	PL	34 O + 6 W	2.8	23.3	37.4	30.4	6.2	54.5
WG oat and wheat muesli bar 6	PL	29 O + 7 W	2.9	22.9	39.5	28.1	6.6	54.4
WG wheat snack bar 1	PL	26 W	3.6	24.9	54.6	13.1	3.9	187.3
WG wheat snack bar 2	PL	25 W	4.4	31.7	48.7	12	3.3	161.1
WG wheat snack bar 3	PL	26 W	4.1	28.3	51.8	12.8	3	155
WG wheat snack bar 4	PL	26 W	4.4	31.8	46.7	12.9	4.2	151.4
WG wheat snack bar 5	PL	26 W	3.8	29.2	51.9	12.2	2.9	151.4
WG wheat cheese snacks	US	51 W[d]	4.4	30.8	43.5	15.8	5.5	178.5
WG corn/wheat/oat chips	US	19 W[d]	4.9	33.9	47.3	10.7	3.3	86.3

[a]Data are based on fluorescence detection. Concentrations and homologue percentages are means of two separate analyses with a difference of <10%. [b]Country of origin: USA, United States; UK, United Kingdom; AU, Australia; FR, France; DE, Germany; IT, Italy; SE, Sweden; NL, The Netherlands; PL, Poland. [c]Whole grain content (%) on a dry weight basis, assuming that manufacturers are using the AACC definition of a whole grain. The type of whole grain cereal contained in the product is indicated by a letter: W, wheat; R, rye; O, oat; M, mix of grains including wheat, rye, and barley. The addition of wheat bran is indicated by WB. ND, not determined. [d]Estimated whole grain content; actual whole grain content not declared on the label. Estimation based on other ingredients and recipes. [e]A large portion of the outer layers is lost during processing and thus not considered whole grain.

difference between UV and CAED and between UV FD was 70 and 59 μg/g, respectively, with UV detection generally overestimating the alkylresorcinol concentration (Supporting Information, Supplementary Figure 2). The difference in results between CAED, UV, and FD results may also have been partly due to different software packages being used. CoulArray Data Station and Chromeleon have very different algorithms for integrating peaks, which may have led to a bias, even though the same person did all integrations.

Fraction Collection and Offline Analysis by GC-MS. Analysis of collected fractions confirmed that the UHPLC method largely separates alkylresorcinols and alkenylresorcinols (Figure 1). Molecular ions from GC-MS analysis indicated that diunsaturated alkylresorcinols were separated from their monounsaturated counterparts. Three diunsaturated homologues were identified in rye: C17:2, C19:2, and C21:2, using GC-MS, and could be distinguished using both FD and CAED, but not UV detection (Figure 3). While diunsaturated homologues have been previously reported,[3] they are not regularly quantified. The UHPLC method with FD also allowed the facile detection of low concentrations of C27:0 and C27:1. Previous GC-MS analysis of alkylresorcinols in rye has suggested that there are two monounsaturated homologues for each chain length, based on finding different peaks with the same molecular ion.[4] In fractions collected from the UHPLC analysis, it was clear that there are three monounsaturated homologues for each chain length and that the third would normally be covered by the saturated homologue in a GC chromatogram. These are likely to be positional isomers, and three have been previously reported for C17:1.[33] Although reported in wheat and rye,[3,4,28,33] no keto- or oxo-alkylresorcinols were detected, and their detection may require

greater enrichment than used here, or their presence in significant quantities may be limited to certain cultivars.

Analysis of Alkylresorcinols in Cereal Samples. Alkylresorcinols in cereals and cereal products analyzed from both Europe and North America (Tables 1 and 2) were in the range of those previously reported in analyses of European cereals.[1,23,24,26,34−36] Although specific studies looking at genetic/geographic effects on the alkylresorcinol content of cereal grains would be needed to confirm this apparent similarity, on the basis of these results it would appear that there is negligible difference between the amounts of alkylresorcinols in the food supply between Europe and the United States.

Alkylresorcinols are known to be stable during baking[1] and pasta production,[35] but to our knowledge it had not been confirmed if boiling to cook pasta or noodles changes the alkylresorcinol concentration through possible leaching into the cooking water. No difference was found between the uncooked and cooked pasta samples, indicating that they are stable and retained in the food matrix during boiling.

It was previously suggested that alkylresorcinols could be a marker for gluten-containing cereals.[10] In some samples of oat products (not labeled as containing wheat, but not labeled gluten-free), small amounts of alkylresorcinols matching the profile for wheat were detected (<5 μg/g). This type of result suggests that a potential application of measuring alkylresorcinols in cereal products is to check non-gluten-containing cereal products for contamination from gluten-containing cereals. As in cereals, alkylresorcinols are found only in gluten-containing cereals (wheat, rye, barley, and triticale (a wheat × rye hybrid)); they could be a surrogate marker for the presence or consumption of these cereals. Further work specifically focusing on this potential application of alkylresorcinol analysis is needed before its use as an alternative for monitoring potential gluten contamination.

The sensitivity of the present method using CAED should make it sufficiently sensitive for detecting alkylresorcinols in plasma, under the conditions tested this was not the case. The main reasons for this were the low tolerance of the column to large injection volumes (>10 μL) and the need to greatly concentrate the sample (data not shown).

This improved resolution method for the analysis of alkylresorcinols in cereal products allows sufficient separation of alkyl- and alkenylresorcinols to make it suitable for the analysis of complex alkylresorcinol mixtures such as those found in rye. The work also demonstrates that the commonly available LC FD is well suited for the sensitive detection of AR in cereals but that caution should be exercised when using UV detection with this LC method.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Figures 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: alastair.ross@rdls.nestle.com. Phone: 00-41-21-785-8065. Fax: 00-41-21-785-9486.

Notes

The author works for Nestec SA, part of the Nestlé food and beverage company.

■ ACKNOWLEDGMENTS

The provision of samples used in this paper by Professor Nicola McKewon, Kristina Harris, Professor John Kirwan, Scott Howell, Dr. Karen Cooper, Dr. Frank Thielecke, and Dr. Laure Jolly is gratefully acknowledged.

■ REFERENCES

(1) Ross, A. B.; Shepherd, M. J.; Schupphaus, M.; Sinclair, V.; Alfaro, B.; Kamal-Eldin, A.; Åman, P. Alkylresorcinols in cereals and cereal products. *J. Agric. Food Chem.* **2003**, *51*, 4111−4118.

(2) Gohil, S.; Pettersson, D.; Salomonsson, A.-C.; Åman, P. Analysis of alkyl- and alkenylresorcinols in triticale, wheat and rye. *J. Sci. Food Agric.* **1988**, *45*, 43−52.

(3) Seitz, L. M. Identification of 5-(2-oxoalkyl)resorcinols and 5-(2-oxoalkenyl)resorcinols in wheat and rye grains. *J. Agric. Food Chem.* **1992**, *40*, 1541−1546.

(4) Ross, A. B.; Åman, P.; Andersson, R.; Kamal-Eldin, A. Chromatographic analysis of alkylresorcinols and their metabolites. *J. Chromatogr., A* **2004**, *1054*, 157−164.

(5) Landberg, R.; Kamal-Eldin, A.; Salmenkallio-Marttila, M.; Rouau, X.; Åman, P. Localization of alkylresorcinols in wheat, rye and barley kernels. *J. Cereal Sci.* **2008**, *48*, 401−406.

(6) Ross, A. B.; Kamal-Eldin, A.; Åman, P. Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutr. Rev.* **2004**, *62*, 81−95.

(7) Knödler, M.; Reisenhauer, K.; Schieber, A.; Carle, R. Quantitative determination of allergenic 5-alk(en)ylresorcinols in mango (*Mangifera indica* L.) peel, pulp, and fruit products by high-performance liquid chromatography. *J. Agric. Food Chem.* **2009**, *57*, 3639−3644.

(8) Landberg, R.; Kamal-Eldin, A.; Andersson, A.; Vessby, B.; Åman, P. Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. *Am. J. Clin. Nutr.* **2008**, *87*, 832−838.

(9) Ross, A. B.; Bourgeois, A.; Macharia, H. N.; Kochhar, S.; Jebb, S. A.; Brownlee, I. A.; Seal, C. J. Plasma alkylresorcinols as a biomarker of whole grain food consumption in a large population − results from the WHOLEheart intervention study. *Am. J. Clin. Nutr.* **2012**, *95*, 204−211.

(10) Ross, A. B. Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake. *J. Nutr. Metabol.* **2012**, *2012*, 1−12.

(11) Ma, J.; Ross, A. B.; Shea, M. K.; Bruce, S. J.; Jacques, P.; Saltzman, E.; Lichtenstein, A. H.; Booth, S. L.; McKeown, N. M. Relationship between plasma alkylresorcinols, whole grain intake, and metabolic risk factors in older adults. *J. Nutr.* **2012**, in press.

(12) Kamal-Eldin, A.; Pouru, A.; Eliasson, C.; Åman, P. Alkylresorcinols as antioxidants: hydrogen donation and peroxyl radical-scavenging effects. *J. Sci. Food Agric.* **2001**, *81*, 353−356.

(13) Parikka, K.; Rowland, I. R.; Welch, R. W.; Wahala, K. In vitro antioxidant activity and antigenotoxicity of 5-*n*-alkylresorcinols. *J. Agric. Food Chem.* **2006**, *54*, 1646−1650.

(14) Ross, A. B.; Chen, Y.; Frank, J.; Swanson, J. E.; Parker, R. S.; Kozubek, A.; Lundh, T.; Vessby, B.; Åman, P.; Kamal-Eldin, A. Cereal alkylresorcinols elevate γ-tocopherol levels in rats and inhibit gamma-tocopherol metabolism in vitro. *J. Nutr.* **2004**, *134*, 506−510.

(15) Rejman, J.; Kozubek, A. Inhibitory effect of natural phenolic lipids upon NAD-dependent dehydrogenases and on triglyceride accumulation in 3T3-L1 cells in culture. *J. Agric. Food Chem.* **2004**, *52*, 246−250.

(16) Andersson, U.; Dey, E. S.; Holm, C.; Degerman, E. Rye bran alkylresorcinols suppress adipocyte lipolysis and hormone-sensitive lipase activity. *Mol. Nutr. Food Res.* **2011**, *55*, S290−S293.

(17) Gasiorowski, K.; Szyba, K.; Brokos, B.; Kozubek, A. Antimutagenic activity of alkylresorcinols from cereal grains. *Cancer Lett.* **1996**, *106*, 109−115.

(18) Gasiorowski, K.; Brokos, B. DNA repair of hydrogen peroxide-induced damage in human lymphocytes in the presence of four

antimutagens. A study with alkaline single cell gel electrophoresis (comet assay). *Cell Mol. Biol. Lett.* **2001**, *6*, 897−911.
(19) Gasiorowski, K.; Brokos, B.; Kulma, A.; Ogorzalek, A.; Skorkowska, K. Impact of four antimutagens on apoptosis in genotoxically damaged lymphocytes in vitro. *Cell Mol. Biol. Lett.* **2001**, *6*, 649−675.
(20) Zhu, Y.; Conklin, D. R.; Chen, H.; Wang, L.; Sang, S. 5-Alk(en)ylresorcinols as the major active components in wheat bran inhibit human colon cancer cell growth. *Bioorg. Med. Chem.* **2011**, *19*, 3973−3982.
(21) Liu, L.; Winter, K. M.; Stevenson, L.; Morris, C.; Leach, D. N. Wheat bran lipophilic compounds with in vitro anticancer effects. *Food Chem.* **2012**, *130*, 156−164.
(22) Gajda, A.; Kulawinek, M.; Kozubek, A. An improved colorimetric method for the determination of alkylresorcinols in cereals and whole-grain cereal products. *J. Food Compos. Anal.* **2008**, *21*, 428−434.
(23) Landberg, R.; Andersson, A. A. M.; Åman, P.; Kamal-Eldin, A. Comparison of GC and colorimetry for the determination of alkylresorcinol homologues in cereal grains and products. *Food Chem.* **2009**, *113*, 1363−1369.
(24) Ross, A. B.; Kamal-Eldin, A.; Jung, C.; Shepherd, M. J.; Åman, P. Gas chromatographic analysis of alkylresorcinols in rye (*Secale cereale* L) grains. *J. Sci. Food Agric.* **2001**, *81*, 1405−1411.
(25) Mattila, P.; Pihlava, J. M.; Hellstrom, J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *J. Agric. Food Chem.* **2005**, *53*, 8290−8295.
(26) Ross, A. B.; Kochhar, S. Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC-Coularray-based electrochemical detection. *J. Agric. Food Chem.* **2009**, *57*, 5187−5193.
(27) Mullin, W. J.; Wolynetz, M. S.; Emery, J. P. A comparison of methods for the extraction and quantitation of alk(en)ylresorcinols. *J. Food Compos. Anal.* **1992**, *5*, 216−223.
(28) Knödler, M.; Kaiser, A.; Carle, R.; Schieber, A. Profiling of alk(en)ylresorcinols in cereals by HPLC-DAD-APcI-MSn. *Anal. Bioanal. Chem* **2008**, *391*, 221−228.
(29) Kulawinek, M.; Kozubek, A. Quantitative determination of alkylresorcinols in cereal grains: independence of the length of the aliphatic side chain. *J. Food Lipid* **2008**, *15*, 251−262.
(30) Evans, L. E.; Dedio, W.; Hill, R. D. Variability in the alkylresorcinol content of rye grain. *Can. J. Plant Sci.* **1973**, *53*, 485−488.
(31) Verdeal, K.; Lorenz, K. Alkylresorcinols in wheat, rye and triticale. *Cereal Chem.* **1977**, *54*, 475−483.
(32) Bland, J. M.; Altman, D. G. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1986**, *1*, 307−310.
(33) Kozubek, A.; Tyman, J. H. Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chem. Rev.* **1999**, *99*, 1−26.
(34) Chen, Y.; Ross, A. B.; Åman, P.; Kamal-Eldin, A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. *J. Agric. Food Chem.* **2004**, *52*, 8242−8246.
(35) Landberg, R.; Kamal-Eldin, A.; Andersson, R.; Åman, P. Alkylresorcinol content and homologue composition in durum wheat (*Triticum durum*) kernels and pasta products. *J. Agric. Food Chem.* **2006**, *54*, 3012−3014.
(36) Andersson, A. A.; Kamal-Eldin, A.; Fras, A.; Boros, D.; Åman, P. Alkylresorcinols in wheat varieties in the HEALTHGRAIN Diversity Screen. *J. Agric. Food Chem.* **2008**, *56*, 9722−9725.