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# Rapid and Sensitive Analysis of Alkylresorcinols from Cereal Grains and Products Using HPLC−Coularray-Based Electrochemical Detection

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Alkylresorcinols (AR) present in the bran fraction of wheat, rye, and barley grains are of current interest as biomarkers of wholegrain cereal intake. This paper reports the development of a rapid quantitative HPLC method allowing sensitive analysis of AR in cereals in 14 min using coularray (CA) detection. The LOD was 1 ng injected and the LOQ 2.5 ng injected, whereas the intrarun and inter-run CVs were 0.8 and 6.4%, respectively. Analytical recovery determined by spiking ranged from 98 to 107% for AR homologues C15:0−C25:0. The method was used to analyze 79 flour and cereal products, with results in the same range as previous studies using GC analysis. Analysis of wholegrain white wheat were in the same range as other wheat varieties (420−556 μg/g). Use of the CA detector allowed detection of low concentrations of AR in white wheat flour (13−51 μg/g) that had not been reliably detected using previous methods. This method allows rapid throughput analysis of cereal samples, required for validation studies on the use of AR as biomarkers of wholegrain cereal intake.

**KEYWORDS: Alkylresorcinol; whole grain cereal; coularray detector; wheat flour**

## INTRODUCTION

Alkylresorcinols (AR; 1,3-dihydroxy-5-alkylbenzene derivatives) are phenolic lipids present in the bran fraction of wheat, rye, and barley kernels, but not in detectable concentrations in other plant foods (*1*−*4*). They exist predominantly as a series of long-chain, odd-numbered homologues from C15 to C25, with a distinct pattern for each cereal (*5*). As AR are stable during processing (*1*), absorbed by humans (*6*), and consequently found in plasma (*7*−*9*), they appear to be ideal candidates as biomarkers of wholegrain wheat, rye, and barley intake (*5*). A number of studies have now demonstrated that people eating a high amount of wholegrain wheat or rye have significantly higher concentrations of plasma AR (*9*−*12*). People who do not regularly consume wholegrain cereals have low concentrations of AR in their plasma (*10*, *11*), which raises a question about the origin of these AR. In vitro studies have suggested that AR may have some biological effects related to membranes (*13*), enzyme inhibition (*14*, *15*), and weak antioxidant activity (*16*, *17*), although any efficacy at relevant biological concentrations in vivo remains to be found, and acute effects from even high wholegrain cereal intake in humans are unlikely (*5*).

To quantify AR intake to validate their use as biomarkers, it is necessary to be able to analyze AR concentrations in native cereals and processed cereal products. Currently, a number of methods exist, using GC (*18*), HPLC (*19*, *20*), and spectroscopy using the reaction of AR with the dye Fast Blue B (*21*−*23*). Fast Blue B methods are the most rapid, but do not give any information about the homologue composition. The AR homologue composition in cereals (*2*) is reflected in plasma samples, giving information about the predominant type of wholegrain (wheat, rye, or barley) that a person has been eating (*11*). Sample preparation for both GC-FID and HPLC-UV is fairly simple (*1*, *18*−*20*) and does not require derivitization or other sample pretreatment. GC-FID provides good resolution between different homologues but does require a run time of around 30 min per sample, and accumulation of high boiling point compounds at the injector port can cause erratic baselines. The HPLC method most frequently used at present based on that of Mullin et al. (*19*) requires a 100 min run time, and there is no reported validation with spiking or inter- and intraday repeatability.

Current advancements in HPLC column technology make it possible to decrease sample runtime, while maintaining resolution. HPLC can also be coupled to a wider range of detectors than GC, making the use of HPLC attractive for rapid, routine analysis of AR in cereal grains. Recent use of coularray (CA) detection to analyze AR metabolites in urine (*24*) and plasma (*25*) highlights the possibility that this detector could also be used for analyzing intact AR.

Here, we present a rapid, validated HPLC method for the analysis of AR using CA detection, discuss its application to analysis of cereals and cereal products, and comment on its suitability for plasma AR analysis.

## MATERIALS AND METHODS

**Chemicals and Samples.** Samples were bought from local supermarkets (Lausanne, Switzerland; Pondecherry, India; Huskvarna,

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Sweden) or obtained from the University of Newcastle or Nestlé SA (Worldwide). AR standards [pentadecylresorcinol (C15:0), 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. All solvents used were of HPLC grade from Merck (Darmstadt, Germany).

**HPLC Method Development.** All previously published HPLC methods (*26*) have been based on water and methanol gradients and C18 columns. For the use of the CA detector, it is necessary to add salt to the mobile phase. We tested the following salts: lithium acetate, lithium perchlorate, and ammonium acetate at 25 and 50 mM concentrations in both water and methanol so that there was no salt gradient during the run. The following columns were tested: Zorbax C8 150 × 4.6 mm, 3.5 μm (Agilent SA, Basel, Switzerland); Zorbax Extend C18 150 × 4.6 mm, 3.5 μm (Agilent); Zorbax Extend C18 150 × 3.0 mm, 3.5 μm (Agilent); Zorbax C18 50 × 2.1 mm, 1.8 μm (Agilent); and Waters C18 Aquity 150 × 2.1 mm, 3 μm (Waters SA, Montreux, Switzerland).

The following method parameters were tested with a solution of mixed AR standards (C15:0−C25:0) in methanol (approximately 50 μg/mL of each homologue) to check for peak separation and peak shape: starting gradient (70, 80, 89, 99% MeOH); gradient time (2.5, 5, 7.5, 10, 15 min); column temperature (30, 40, 50, 60 °C); flow rate adjusted according to the column and resulting back pressure.

Method development and analysis were carried out on an Alliance 2975 HPLC with a 2995 PDA detector (Waters) scanning between 250 and 350 nm and an eight-channel CA detector (ESA Science, Chelmsford, MA). Electrodes were set to 0, 100, 200 400, 560 (predominant electrode), 780 (dominant electrode), 820 (postdominant electrode), and 850 mV. The thermal organizer temperature was set to 30 °C.

Samples were integrated using CoulArray Data Station (ESA Science) software.

The optimum conditions for resolution and peak shape were the following: Zorbax Extend C18 150 × 3.0 mm, 3.5 μm column kept at 50 °C. A 12 × 4.6 mm precolumn based on the same stationary phase was used for all analyses. Solvent A was MeOH/water/5 M ammonium acetate, pH 6 (90:10:1 v/v), and solvent B was MeOH/5 M ammonium acetate, pH 6 (99:1 v/v). The gradient program was from 0 to 100% B over 5 min, hold at 100% B for 5 min, return to 0% B over 2 min, hold at 0% B for 2 min. The flow rate was 1.0 mL/min from 0 to 8 min, 1.2 mL/min from 8 to 10 min, and 1.0 mL/min from 10 to 14 min. Injection volume could be varied depending on sample concentration, although 5 μL was sufficient for all cereal analyses. Limit of detection (LOD) and limit of quantification (LOQ) were determined by sequential dilution of a mix of alkylresorcinol standards (C15:0−C25:0), and LOD and LOQ were defined as signal-to-noise ratios of 3:1 and 5:1, respectively.

**Extraction and Quantification of Cereal Samples.** Samples were extracted in triplicate using the method of Ross et al. (*18*) for unprocessed cereals and that of Ross et al. (*1*) for processed cereals. An internal standard, AR C20:0 (20 μg/sample, not present naturally), was added to the cereal sample prior to extraction. Four milliliters of sample extract was evaporated under nitrogen and reconstituted in 1 mL of methanol, and 5 μL of sample was injected onto the HPLC. 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 AR. Peaks were identified by comparing retention times with authentic standards and offline analysis by GC-MS (see below). Samples with a coefficient of variation (CV) of >10% were reanalyzed.

To determine the analytical recovery of this method, maize flour (no AR) or empty tubes were spiked in triplicate with 50, 100, 500, and 750 μL of a 100 μg/mL mixed AR (C15:0−C25:0) standard and extracted according to the method of Ross et al. (*18*). Recovery was determined as the concentration in the cereal matrix compared to no matrix and expressed as a percentage. Interday repeatability was determined by extracting and analyzing in triplicate the same brown wheat flour sample 5 times over a 3 weeks. Intraday repeatability was determined by injecting the same brown wheat extract 10 times during one run, interdispersed with other sample extracts.

**GC-MS Analysis.** Confirmation of the identity of the peaks and whether samples did not contain AR was performed using offline GC-MS analysis of HPLC fractions. Dried sample extracts and HPLC fractions were derivitized using MSTFA + 1% TMCS (60 min at 60 °C) and injected onto an HP-6890 GC system with an Agilent 5975A MS detector (Agilent) fitted with a BP1 12 m column (0.25 mm i.d.; 0.22 μm film thickness; SGE Limited, Melbourne, Australia). AR were separated using the following oven program: 150 °C (0 min); 150 °C (1 min); 230 °C (3 min); 290 °C (9.7 min); 290 °C (12.2 min); 300 °C (13 min); 300 °C (15 min); 320 °C (15.5 min); 320 °C (17.5 min). The injector was held at 300 °C. Spectra were collected in full-scan mode, scanning from $m/z$ 100 to 650. TMS-derivitized AR were identified by their characteristic base peak at $m/z$ 268 (*1*) and the molecular ion (see ref (*26*) for a list).

**Plasma Analysis.** A plasma sample (Blood Transfusion Service, Lausanne, Switzerland) was used to test if a CA detector with this HPLC method could detect AR in plasma. Aliquots were extracted (*7*), and one was dried and resuspended in 100 μL of methanol and a second extract was purified according to a previously published method (*27*) to remove cholesterol. This sample was also diluted in 100 μL of methanol without derivitization. Fifty microliters of the plasma extract was injected onto the HPLC. The same sample has been previously analyzed using GC-MS and has a concentration of 405 nmol/L.

## RESULTS AND DISCUSSION

In developing this method, we wanted to exploit the recent improvements in HPLC column technology to reduce run time while maintaining separation between peaks. Using the Zorbax C18 50 × 2.1 mm column did result in shorter run times (elution of all AR within 6 min), but peak tailing was an issue, and baseline resolution between AR C19:0, C20:0, and C21:0 was marginal. Similar problems were observed for the Aquity C18 150 × 2.1 mm column. The Zorbax C8 column provided adequate resolution, although with some peak tailing, which was improved using the Zorbax C18 Extend columns. Use of lithium acetate and lithium perchlorate salts with the mobile phase caused an unstable baseline, and these were not extensively tested. Optimal resolution was seen with the C18, 150 × 4.6 mm Zorbax Extend column, but to get elution of all AR within 10 min, flow rates of 2.5 mL/min were needed. This problem was solved using a 150 × 3.0 mm column with the same stationary phase, which allowed a decrease in flow rate to 1.0 mL/min with similar back pressure (150−220 bar during a run) and resolution of AR in 8 min (**Figure 1**). We were able to perform 500+ injections on one column without any loss of separation, although occasional back flushing with methanol and water was used to clean the column and HPLC system when not in extended use.

Analytical recovery of the reported method ranged from 98.4 to 107.5% (**Table 1**), with a tendency for slightly lower recoveries at the lowest spiking concentration and higher at the highest concentration spiked. There was no apparent discrimination according to homologue chain length. Intraday repeatability of a brown wheat flour sample was good (**Table 2**), with a coefficient of variation (CV) for the total AR concentration of 0.8%. The longer chain homologues C23:0 and C25:0 had the greatest variation (1.5 and 3.6%, respectively). Interday repeatability was greater (CV, 6.4%; **Table 2**), but reflects variation in both the sample extraction and the instrument method. The interday variation for C23:0 and C25:0 was high (>10%) relative to the other homologues. This is possibly due to variation in the baseline increase as the gradient reaches 100% of mobile phase B. Relative retention times (RRT) compared to the internal standard determined over 3 months with two different columns of the same type varied only slightly, with RRT (% CV) for the different homologues C17:0, C19:0, C21:0, C23:0, and C25:0 of 0.72 (1.33), 0.91 (0.45), 1.09 (0.46), 1.27 (1.17), and 1.44 (1.27), respectively. The retention time of C20:0 varied between 5.65 and 5.75 min, but remained consistent within runs.

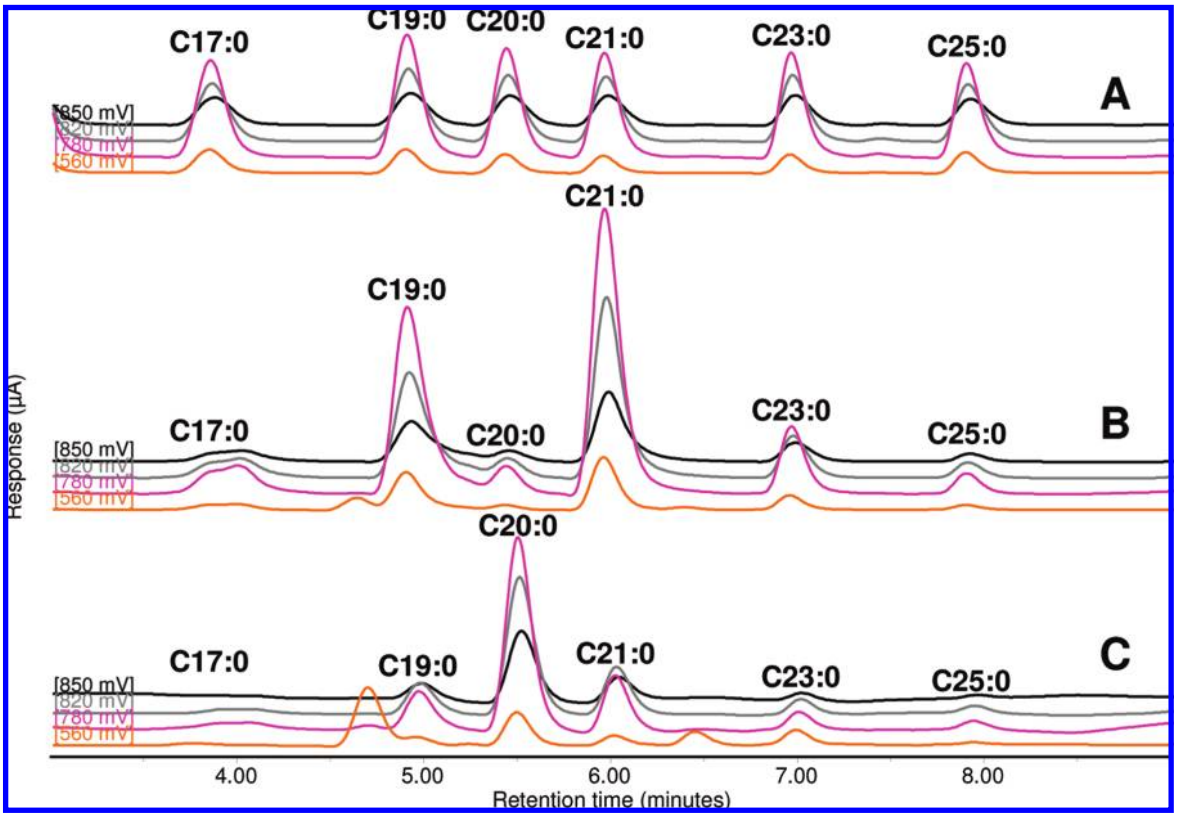


**Figure 1.** HPLC−coularray detection of alkylresorcinols: **A**, mix of synthetic standard alkylresorcinols; **B**, wholegrain wheat flour; **C**, white wheat flour. Quantification is based on the response at 780 mV. See text for HPLC conditions.

**Table 1.** Analytical Recovery of Alkylresorcinols from Spiked Maize Flour Using HPLC−Coularray Detection ($N = 3$ per Different Spiking Concentration)

| | analytical recovery (%) at | | | |
|---|---|---|---|---|
| homologue | 15 μg/g of maize | 30 μg/g of maize | 150 μg/g of maize | 200 μg/g of maize |
| C15:0 | 98.4 ± 4.27 | 100.4 ± 1.07 | 101.4 ± 1.51 | 104.6 ± 0.84 |
| C17:0 | 99.8 ± 5.14 | 103.2 ± 0.41 | 101.6 ± 1.29 | 104.3 ± 0.95 |
| C19:0 | 98.7 ± 2.19 | 99.6 ± 0.81 | 101.5 ± 1.48 | 104.8 ± 0.98 |
| C21:0 | 98.6 ± 2.85 | 100.8 ± 0.81 | 101.8 ± 1.45 | 105.1 ± 1.15 |
| C23:0 | 100.4 ± 2.07 | 101.8 ± 0.14 | 102.1 ± 1.06 | 104.6 ± 4.42 |
| C25:0 | 99.0 ± 1.86 | 100.9 ± 0.54 | 102.3 ± 1.20 | 107.5 ± 1.14 |
| total | 99.3 ± 2.55 | 101.3 ± 0.47 | 101.9 ± 1.28 | 105.5 ± 1.60 |

**Table 2.** Inter- and Intraday Repeatability of Alkylresorcinol Concentration in a Wholegrain Wheat Sample Analyzed Using HPLC−Coularray Detection

| | repeatability | |
|---|---|---|
| | intraday instrumental[a] | interday method[b] |
| C17:0 | 27.3 ± 1.7[c] | 24.7 ± 10.6 |
| C19:0 | 235.2 ± 0.8 | 215.6 ± 5.4 |
| C21:0 | 324.1 ± 0.9 | 300.3 ± 5.0 |
| C23:0 | 67.7 ± 1.5 | 59.3 ± 15 |
| C25:0 | 21.4 ± 3.6 | 18.6 ± 29.3 |
| total | 675.7 ± 0.8 | 618.4 ± 6.4 |

[a] Based on 10 injections of the same sample over one sequence including a total of 30 samples. [b] Based on 4 separate triplicate extractions and injections of the same sample analyzed over 1 month. [c] μg/g of DM ± % CV.

AR peaks were identified by comparison of their retention times with those of synthetic standards and the ratio of the pre- and postdominant channels. For the unsaturated AR, no standards are available, so these were identified by offline GC-MS analysis of HPLC fractions. As observed by Knödler et al. (*28*), unsaturated homologues coeluted with the saturated homologues with a chain length minus two (e.g., C19:1 coeluted with C17:0; **Figure 2**). A number of minor AR present in rye were identified as coeluting with the major saturated and monounsaturated AR, including C27:1 and C27:2, although the apparent concentrations were minute (< 1% of total GC-MS area).

Several methods have been previously published for HPLC analysis of AR in cereals and cereal foods (*19*, *20*, *28*−*31*). Unfortunately, none have been validated as quantitative methods, probably due to the previous lack of commercially available AR standards. Total analysis times have varied from 30 to 90 min (*19*, *20*, *28*, *29*, *31*), normally due to the need for a long gradient to separate the many homologues present in rye samples. In those publications where chromatograms were presented, separation of the minor homologues (mostly unsaturated) from the major homologues (saturated) in rye was always incomplete, and their exact identification unclear unless using MS detection (*28*) or offline MS analysis. As the dominant AR-containing cereal of interest worldwide is wheat (with the notable exceptions of northern and eastern Europe), we did not consider the separation of the unsaturated AR homologues from the saturated homologues as a necessary goal as wheat generally contains < 5% unsaturated AR homologues (*1*). As for previous methods, there was no complete separation of saturated and unsaturated homologues. For rye, it is still possible to report total AR concentration, although exact quantification of the minor homologues should be treated with caution. Analysis of rye samples did yield results and homologue compositions similar to those previously published using GC (*1*, *18*) (**Tables 3** and **4**). Given the importance of wheat as a wholegrain cereal, this limitation is acceptable given the greatly shorter run time achieved. If precise quantification of different

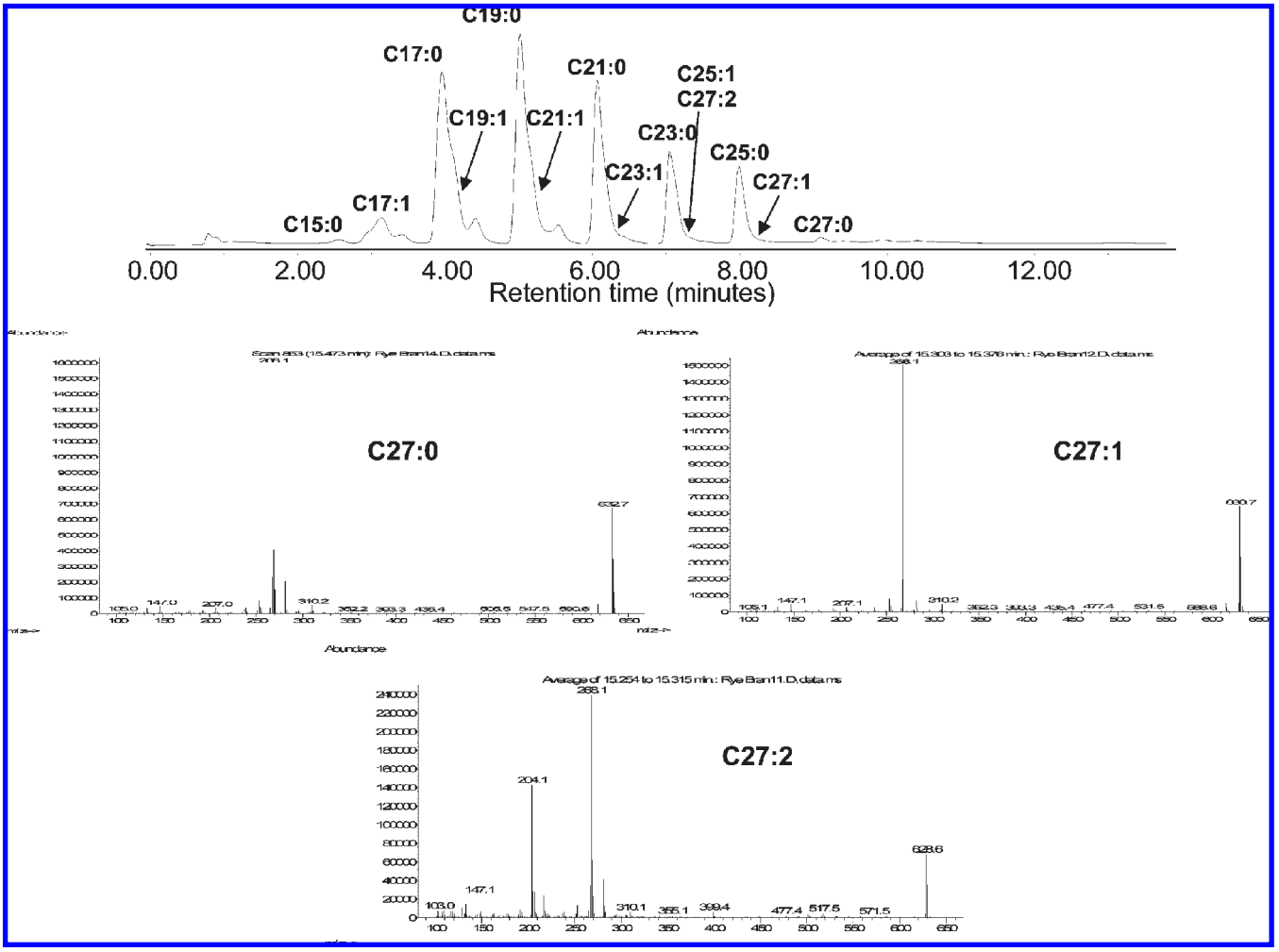


**Figure 2.** Chromatogram of alkylresorcinols from a rye bran sample analyzed by HPLC−coularray detection (dominant channel only). Alkylresorcinol homologues with a chain length of C27 were identified by fraction collection and GC-MS.

minor homologues of rye is desired, then a GC-based method is more appropriate.

As for the GC-MS analysis of plasma (*7*), this method used C20:0 AR, which is not found naturally, as an internal standard. This has the advantage over previously used internal standards [olivitol (C5:0 AR) and methyl behenate (C22:0 fatty acid methyl ester) for GC-FID] as it is much closer in terms of properties of the AR of interest and elutes in the same time frame, avoiding the need to adjust the gradient to allow for quantification of an internal standard with a different retention time. Absolute response of AR on the CA detector was variable over time, but relative response of the different homologues compared to C20:0 was consistent from run to run, with an average relative response over 2 months of 1.02 (C17:0) through to 0.84 (C25:0) (CV % 0.6−8.8).

Along with greatly decreased sample analysis time, the major advantage of this method is that using a CA detector allows easy quantification of AR in food samples at low concentrations ( < 50 $\mu$g/g of total AR) due to the greater sensitivity of the detector and fewer interfering peaks detected using the detector (**Figure 1**). The LOD and LOQ for all AR were 1 and 2.5 ng injected, respectively, although this does not factor in possible matrix effects. The LOD for GC-FID is reported as 0.2 $\mu$g/g (*23*). During method development, it was found that it was possible to quantify low concentrations of AR in refined wheat flour samples (**Table 3**). Low concentrations of AR were previously reported (*20*) in two samples of white wheat flour (44 and 47 $\mu$g/g), although the AR homologue ratio was very different from the characteristic wheat profile (*2*). Other studies have not detected AR in white wheat flour, possibly as they have been just below the limit of detection for the GC-FID, HPLC-UV, and colorimetric methods used (*18*, *20*, *21*, *23*) or due to the many minor compounds that can interfere at low concentrations with unspecific detection methods. CA has some degree of specificity as the ratio of the predominant, dominant, and postdominant responses is characteristic for each class of compound.

The AR concentrations found in the refined wheat flour samples in this study (13−47 $\mu$g/g) are minute compared to those in wholegrain flours (489−660 $\mu$g/g) (**Table 3**), but may explain why low concentrations of AR are found in the plasma of subjects who do not eat wholegrain wheat or rye (*11*, *12*). Even though AR have been definitively located to the outer layers (*4*) of wheat and rye, low-level contamination of bran in milling streams could lead to these concentrations in flour. Given the low but easily detectable amount of AR in white wheat flour, it could be possible to use analysis of AR as a way of checking for wheat contamination in products. On the basis of these results, it could be estimated that a person eating 100−200 g of refined wheat-based food would eat 2−4 mg of AR/day. This would still be far lower than people who habitually eat wholegrain cereals (18−31 mg/day) (*32*).

The concentration of AR in the cereal products analyzed (**Table 4**) is similar to that in previous studies using GC-FID analysis (*1*, *8*, *23*, *33*) and is somewhat lower than the results found using HPLC-UV (*20*). A possible reason for this difference is coeluting peaks increasing the apparent area of AR peaks in

**Table 3.** AR Content in Different Cereal Flours from China (CN), Hungary (H), India (IN), Italy (IT), the Philippines (PN), Sweden (SE), Switzerland (CH), and the United Kingdom (UK)

| | % of each AR homologue | | | | | |
|---|---|---|---|---|---|---|
| | C17:0 | C19:0 | C21:0 | C23:0 | C25:0 | total[a] |
| white wheat/spelt flour (CH) | 6 | 24 | 39 | 15 | 17 | 31.8 |
| organic white wheat flour (CH) | 3 | 25 | 36 | 20 | 16 | 26.3 |
| white wheat flour 1 (CH) | 7 | 26 | 35 | 17 | 15 | 34.7 |
| white wheat flour 2 (CH) | 7 | 23 | 34 | 19 | 17 | 29.9 |
| white wheat flour 1 (SE) | 12 | 22 | 38 | 11 | 17 | 13.5 |
| white wheat flour 2 (SE) | 7 | 11 | 50 | 17 | 15 | 27.6 |
| wheat semolina (CH) | 1 | 10 | 49 | 22 | 18 | 46.6 |
| half-white wheat flour 1 (CH) | 6 | 27 | 40 | 14 | 14 | 42.6 |
| half-white wheat flour 2 (CH) | 8 | 26 | 39 | 15 | 13 | 44.0 |
| "rustic" wheat/rye flour (CH) | 15 | 28 | 29 | 14 | 13 | 85.3 |
| brown wheat flour 1 (CH) | 4 | 30 | 45 | 12 | 8 | 117.0 |
| brown wheat flour 2 (CH) | 4 | 30 | 49 | 11 | 6 | 215.6 |
| brown wheat flour 3 (CH) | 4 | 30 | 51 | 11 | 4 | 162.9 |
| brown wheat flour 4 (CH) | 4 | 30 | 51 | 10 | 4 | 134.2 |
| brown wheat flour 5 (CH) | 4 | 28 | 52 | 11 | 5 | 98.1 |
| brown wheat flour 6 (CH) | 3 | 29 | 52 | 11 | 5 | 195.5 |
| wholegrain wheat flour 1 (CH) | 4 | 33 | 48 | 11 | 4 | 660.3 |
| wholegrain wheat flour 2 (CH) | 4 | 32 | 48 | 12 | 4 | 600.7 |
| wholegrain wheat flour 3 (CH) | 4 | 31 | 47 | 13 | 5 | 285.4 |
| wholegrain wheat flour 4 (CH) | 5 | 32 | 48 | 11 | 4 | 575.8 |
| organic wholegrain wheat flour (CH) | 4 | 32 | 50 | 11 | 4 | 488.9 |
| wholegrain wheat flour 1 (IN) | 5 | 29 | 48 | 11 | 7 | 138.4 |
| wholegrain wheat flour 2 (IN) | 5 | 30 | 49 | 11 | 6 | 202.6 |
| wholegrain wheat flour 3 (IN) | 4 | 30 | 51 | 11 | 4 | 345.5 |
| wholegrain white wheat (PN) | 5 | 30 | 47 | 13 | 5 | 419.8 |
| wholegrain white wheat (CH) | 5 | 32 | 45 | 13 | 5 | 555.5 |
| barley 1 (H) | 1 | 6 | 17 | 15 | 62 | 76.8 |
| barley 2 (CH) | 1 | 2 | 19 | 17 | 60 | 51.0 |
| spelt grains (CH) | 4 | 32 | 49 | 11 | 4 | 455.3 |
| spelt flour (SE) | 4 | 32 | 49 | 11 | 4 | 507.3 |
| wholegrain rye flakes 1 (UK) | 22 | 25 | 24 | 15 | 13 | 703.4 |
| wholegrain rye flakes 2 (UK) | 23 | 26 | 24 | 15 | 13 | 683.9 |
| rye bran (SE) | 28 | 28 | 21 | 13 | 11 | 1266.1 |
| maize semolina (CH) | nd[b] | nd | nd | nd | nd | nd |
| brown rice (UK) | nd | nd | nd | nd | nd | nd |
| brown rice (IT) | nd | nd | nd | nd | nd | nd |
| rolled oats (UK) | nd | nd | nd | nd | nd | nd |
| rolled oats (CH) | nd | nd | nd | nd | nd | nd |
| buckwheat (CN) | nd | nd | nd | nd | nd | nd |

[a] Total concentration, $\mu$g/g of DM, means of triplicates (CV <10%). [b] Not detected. Detection limit: 1 ng injected.

HPLC-UV analysis. In the first analysis of wholegrain white wheat samples for AR content, they did not differ from other varieties of wheat (**Table 3**). Analysis of unprocessed oats, maize semolina, and buckwheat did not find any AR in these cereals or pseudocereals (**Table 3**), which was confirmed by GC-MS analysis.

Initially, AR were also detected using a diode array detector (DAD), set between 250 and 350 nm, with quantification at 280 nm. The sensitivity and resolution with the 14 min run time was found to be sufficient, but required that samples be more concentrated (e.g., for white flour and barley samples, 4 mL of extract evaporated, and reconstituted in 100 $\mu$L of methanol) and large injection volumes be used (50 $\mu$L). The DAD was useful for discriminating between possible AR peaks (two spectra maxima at 275 and 280 nm) and non-AR eluting at a similar time, but also highlighted the problem of interfering peaks due to many compounds with absorbance at 280 nm. Results using DAD and CA detection were similar (data not shown), but CA was used due to the greater specificity, lower injection volume, and lower sample concentration required.

**Table 4.** AR Content in Different Cereal Products[a]

| | % of each AR homologue | | | | | |
|---|---|---|---|---|---|---|
| | C17:0 | C19:0 | C21:0 | C23:0 | C25:0 | total[b] |
| refined couscous (IS) | 4 | 31 | 47 | 10 | 9 | 15.1 |
| wholegrain couscous 1 (IS) | 5 | 32 | 47 | 9 | 7 | 76.6 |
| wholegrain couscous 2 (SE) | 0 | 11 | 53 | 26 | 10 | 368.8 |
| soba noodles (CN) | 3 | 26 | 50 | 12 | 10 | 27.7 |
| wholegrain instant noodles (IN) | 4 | 31 | 45 | 12 | 8 | 50.2 |
| refined wheat pasta (IT) | 0 | 13 | 56 | 20 | 11 | 32.1 |
| wholegrain wheat pasta (CH) | 0 | 8 | 56 | 27 | 9 | 40.8 |
| wholegrain wheat pasta 1 (IT) | 1 | 14 | 57 | 22 | 6 | 340.5 |
| wholegrain wheat pasta 2 (IT) | 0 | 12 | 55 | 25 | 9 | 313.7 |
| wholegrain wheat pasta 1 (UK) | 1 | 11 | 59 | 23 | 7 | 171.7 |
| wholegrain wheat pasta 2 (UK) | 1 | 13 | 52 | 25 | 9 | 435.5 |
| white wheat bread 1 (CH) | 8 | 29 | 50 | 16 | 5 | 25.9 |
| white wheat bread 2 (CH) | 10 | 30 | 41 | 14 | 6 | 47.4 |
| mixed wheat/rye white bread (CH) | 22 | 28 | 29 | 11 | 10 | 94.5 |
| brown wheat bread (CH) | 7 | 30 | 45 | 13 | 5 | 109.3 |
| wholegrain wheat bread 1 (UK) | 4 | 32 | 49 | 11 | 4 | 608.5 |
| wholegrain wheat bread 2 (UK) | 4 | 35 | 50 | 9 | 2 | 422.8 |
| wholegrain wheat bread 3 (UK) | 4 | 36 | 48 | 9 | 2 | 437.7 |
| wholegrain wheat bread 4 (UK) | 5 | 36 | 49 | 8 | 2 | 481.6 |
| wholegrain wheat/rye bread (SE) | 18 | 29 | 29 | 15 | 10 | 323.7 |
| wholegrain rye bread (SE) | 25 | 27 | 24 | 14 | 10 | 514.0 |
| wholegrain rye bread (DK) | 25 | 26 | 21 | 16 | 12 | 519.5 |
| wholegrain gruel powder (SE) | 14 | 31 | 33 | 12 | 9 | 56.4 |
| breakfast cereal (maize, 0% wholegrain; CH) | nd | nd | nd | nd | nd | nd |
| breakfast cereal (rice, 0% wholegrain; CH) | nd | nd | nd | nd | nd | nd |
| breakfast cereal (0% wholegrain; CH) | 8 | 30 | 41 | 14 | 7 | 7.0 |
| breakfast cereal (27% wholegrain; CH) | 6 | 31 | 50 | 9 | 4 | 133.1 |
| breakfast cereal (57% wholegrain; UK) | 6 | 37 | 48 | 7 | 2 | 369.2 |
| breakfast cereal (65% wholegrain; CH) | 4 | 32 | 50 | 10 | 40 | 403.1 |
| breakfast cereal (67% wholegrain; UK) | 5 | 29 | 49 | 13 | 5 | 374.6 |
| breakfast cereal (74% wholegrain; UK) | 4 | 29 | 46 | 15 | 7 | 53.0 |
| breakfast cereal (94% wholegrain; UK) | 5 | 30 | 48 | 13 | 5 | 480.0 |
| breakfast cereal (100% wholegrain; UK) 1 | 6 | 30 | 50 | 11 | 3 | 389.2 |
| breakfast cereal (100% wholegrain; UK) 2 | 4 | 32 | 50 | 11 | 3 | 606.4 |
| breakfast cereal (100% wholegrain; UK) 3 | 5 | 29 | 48 | 13 | 5 | 499.4 |
| breakfast cereal (100% wholegrain; UK) 4 | 6 | 30 | 47 | 12 | 5 | 420.4 |
| wholegrain crisps (UK) | 6 | 36 | 43 | 6 | 9 | 87.4 |
| wholegrain wheat crackers 1 (IT) | 4 | 35 | 49 | 9 | 3 | 166.5 |
| wholegrain wheat crackers 2 (IT) | 4 | 36 | 49 | 8 | 3 | 204.3 |
| wholegrain wheat crackers 3 (IT) | 6 | 32 | 44 | 13 | 5 | 161.0 |
| wholegrain wheat crackers 4 (IT) | 6 | 32 | 43 | 13 | 6 | 164.2 |
| multigrain crackers (IS) | 9 | 30 | 41 | 14 | 7 | 183.6 |

[a] Country of origin is indicated in parentheses after the sample name: China (CN), Denmark (DK), India (IN), Israel (IS), Italy (IT), Sweden (SE), Switzerland (CH), and the United Kingdom (UK). [b] Total concentration, $\mu$g/g of DM, means of triplicates (CV <10%).

The use of HPLC-CA was tested on one plasma sample. It was possible to detect AR in a purified plasma sample of high concentration (405 nmol/L determined by GC-MS) with HPLC-CA, but only C19:0 and C21:0 were easily detectable (**Figure 3**). The unpurified plasma sample contained a large peak that coeluted with C23:0, as well as a peak that eluted at the same time as C17:0. On the basis of 5 nmol/L for an individual homologue in a plasma sample with a low AR concentration (*8*), then 200 $\mu$L of plasma would contain approximately 0.35 ng, which is just below the LOD for the CA detector, assuming that all extract is injected onto the HPLC. It is conceivable that a limited analysis of plasma samples using this method could be performed, based on the quantification of C19:0 and C21:0 and whether C17:0 is detectable or not. For quantification, this HPLC-CA method is not an improvement on current GC-MS

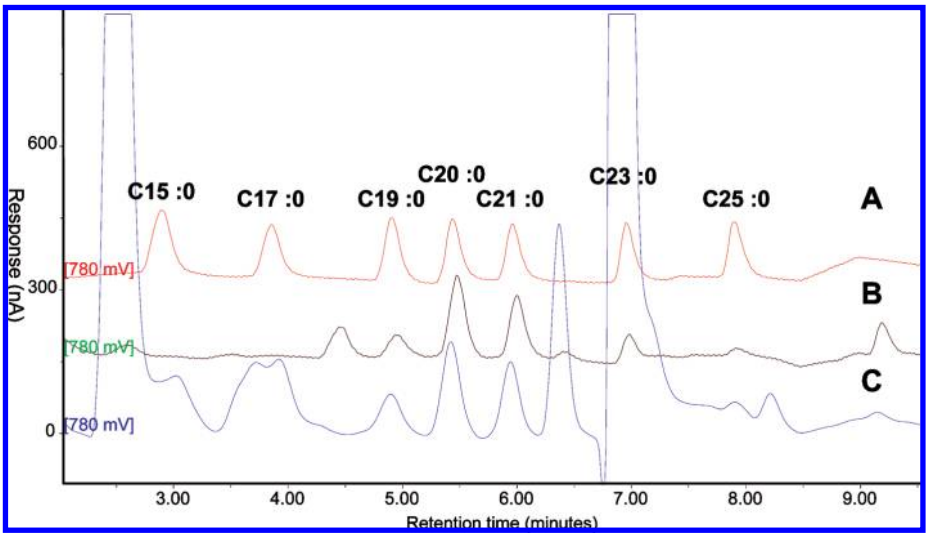


**Figure 3.** Plasma alkylresorcinols analyzed by HPLC-CA detection: **A**, mix of synthetic standard alkylresorcinols; **B**, purified plasma extract; **C**, unpurified plasma extract. The large peak at 7 min in the unpurified extract is cholesterol. The alkylresorcinol concentration of the plasma sample is 405 nmol/L.

methods used to analyze AR in plasma, due to the greater sensitivity and specificity of MS detectors.

This HPLC method for AR in cereals and cereal products is faster than previously reported HPLC methods, and the use of a CA detector allows more sensitive analysis than do GC-FID or HPLC-UV. The finding of low concentrations of AR in all refined wheat flours analyzed may explain the presence of low concentrations of AR in people who do not habitually eat wholegrain wheat or rye.

## ABBREVIATIONS USED

AR, alkylresorcinols; CA, coularray; CV, coefficient of variation; DM, dry matter; FID, flame ionization detector; GC, gas chromatography; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MSTFA + 1% TMCS, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane; UV, ultraviolet.

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