JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Accelerated Solvent Extraction of Alkylresorcinols in Food Products Containing Uncooked and Cooked Wheat

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ABSTRACT: This research focuses on the overall extraction process of alkylresorcinols (ARs) from uncooked grains and baked products that have been processed with wheat, corn, rice, and white flour. Previously established extraction methods developed by Ross and colleagues, as well as a semiautomated method involving accelerated solvent extraction (ASE), were applied to extract ARs within freshly ground samples. For extraction of alkylresorcinols, nonpolar solvents such as ethyl acetate have been recommended for the extraction of uncooked foods, and polar solvents such as 1-propanol:water (3:1 v/v) have been recommended for the extraction of baked foods that contain rye, wheat, or other starch-rich grains. A comparison of AR extraction methods has been investigated with the application of gas chromatography and a flame ionization detector (GC-FID) to quantify the AR content. The goal of this research was to compare the rapid accelerated solvent extraction of the alkylresorcinols (ASE-AR) method to the previous manual AR extraction methods. Results for this study as well as the investigation of the overall efficiency of ASE-AR extraction with the use of a spiking study indicated that it can be comparable to current extraction methods but with less time required. Furthermore, the extraction time for ASE (approximately 40 min) is much more convenient and less tedious and time-consuming than previously established methods, which range from 5 h for processed foods to 24 h for raw grains.

KEYWORDS: *accelerated solvent extraction, alkylresorcinols, ARs, ASE, lipids, wheat*

INTRODUCTION

Alkylresorcinols (ARs) are 1,3-dihydroxy-alkylbenzene phenolic lipids that are found within a variety of plants, fungi, and bacteria.[1,2] With regard to wheat (*Triticum aestivum*), ARs have been reported to be one of the major groups of phenolic compounds located within the outer bran layer (i.e., pericarp, testa, and aleurone), with 5-*n*-alkyl-derivatives with odd alkyl chain lengths being most common.[3] Common alkyl tail lengths of ARs (Figure 1) can range from 17 to 25 carbons, and the chain is usually saturated.[4] The overall structure of ARs exhibits amphiphilic properties due to having a polar "head" (the dihydroxybenzene group) and a nonpolar "tail" (the alkyl chain).

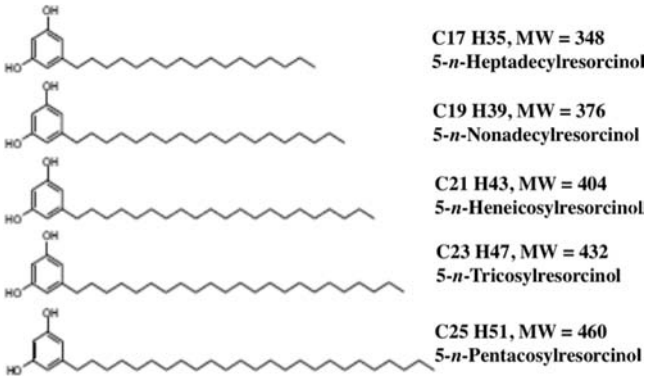

Figure 1. Major 5-*n*-ARs found in cereal grains. The common homologues have odd alkyl chains in the range of C17:0−C25:0.

Modern methods of extraction, preconcentration, and clean up of analytes include liquid−liquid extraction and solid-phase extraction.[5,6] Their application in the process of analyte dissolution as well as the removal of many interfering compounds make these methods very suitable as alternatives in extraction overall.[5] However, with regard to AR extraction, Ross et al. have currently developed methods with the use of both polar and nonpolar solvents. A recent report[3] examined the baking process of wheat-based products and its effect on ARs and observed that nonpolar solvents were unable to achieve sufficient extraction, thereby suggesting that ARs were denatured and degraded due to the high temperatures of the baking process.[7,8] However, the use of hot 1-propanol:water (3:1 v/v) was able to achieve 90−100% extraction of ARs, suggesting that they are bound in flour once it is wetted and heated. This is due to starch−lipid complexes, making extraction difficult for nonpolar solvents.[9]

Currently, accelerated solvent extraction (ASE) is a semi-automated technique currently accepted by the U.S. Environmental Protection Agency for solid and semisolid extraction.[10] This type of extraction utilizes higher temperatures and pressures during the extraction process. Elevated pressures

Received: January 12, 2012
Revised: April 17, 2012
Accepted: April 24, 2012
Published: April 24, 2012

Table 1. Comparison of Total AR Content and AR Homologues (C17:0−C25:0) for Wheat Bran and Processed Food Samples under Ross Extraction and ASE Methods[a]

sample	solvent	C17	C19	C21	C23	C25	AR total
wheat bran	ethyl acetate						
Ross extraction		58.4 ± 1.3	447.8 ± 4.3	719.1 ± 6.5	132.3 ± 6.2	6.9 ± 0.98	1364.5 ± 11.8
ASE extraction		59.1 ± 1.1	450.3 ± 8.6	716.7 ± 7.3	130.7 ± 6.4	8.7 ± 1.30	1365.4 ± 14.2
Pepperidge Farm 100% Wheat Bread	1-propanol−water						
Ross extraction		7.3 ± 0.1	39.9 ± 1.0	44.1 ± 1.6	8.0 ± 0.2	5.0 ± 0.9	104.3 ± 3.3
ASE extraction		6.0 ± 0.3	39.2 ± 1.01	45.8 ± 1.5	6.8 ± 0.1	4.7 ± 1.0	102.6 ± 2.3
white bread	1-propanol−water						
Ross extraction		1.0 ± 0.1	2.2 ± 0.1	2.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	7.2 ± 0.2
ASE extraction		1.2 ± 0.1	1.4 ± 0.2	1.1 ± 0.3	0.9 ± 1.0	0.9 ± 0.3	5.5 ± 0.7
Triscuits	1-propanol−water						
Ross extraction		12.9 ± 0.4*	67.4 ± 3.4	101.2 ± 0.8	18.2 ± 0.4	5.7 ± 0.1	205.3 ± 3.8
ASE extraction		9.8 ± 1.0*	68.9 ± 1.7	102.5 ± 3.6	14.9 ± 0.8	4.6 ± 0.4	200.8 ± 2.0
saltines	1-propanol−water						
Ross extraction		2.1 ± 0.3	2.5 ± 0.1	2.8 ± 0.2	2.2 ± 0.2	1.3 ± 0.8	10.9 ± 1.5
ASE extraction		2.5 ± 0.1	0.8 ± 0.7	1.7 ± 1.2	1.6 ± 0.8	1.1 ± 0.6	7.8 ± 1.8
Hodgson Mill Whole Wheat Pasta	1-propanol−water						
Ross extraction		6.6 ± 0.2	18.3 ± 1.7	66.8 ± 2.0	16.1 ± 0.4	8.4 ± 2.0	116.3 ± 1.4
ASE extraction		6.3 ± 0.8	18.6 ± 0.8	73.1 ± 0.2	20.8 ± 1.0	8.1 ± 0.6	126.9 ± 2.0
Pasta La Bella White Pasta	1-propanol−water						
Ross extraction		1.2 ± 0.1	2.8 ± 0.1	6.3 ± 1.2	2.9 ± 0.1	2.1 ± 0.2	15.3 ± 1.0
ASE extraction		1.9 ± 0.4	2.9 ± 0.6	5.6 ± 3.6	2.3 ± 0.2	2.3 ± 0.8	15.1 ± 5.2
Wheaties	1-propanol−water						
Ross extraction		9.3 ± 2.3	63.6 ± 1.7	79.1 ± 1.4	12.3 ± 1.3	5.9 ± 0.8	170.3 ± 3.5
ASE extraction		11.7 ± 0.6	60.6 ± 1.6	74.8 ± 2.7	12.0 ± 0.5	6.8 ± 0.5	165.9 ± 4.8
Fiber One	1-propanol−water						
Ross extraction		11.2 ± 0.3	74.3 ± 2.0	94.2 ± 1.4*	16.2 ± 0.2	5.4 ± 0.5	201.3 ± 3.2*
ASE extraction		11.3 ± 0.3	80.7 ± 0.4	105.8 ± 2.0*	18.6 ± 0.2	6.4 ± 0.7	222.8 ± 2.2*
Rice Krispies	1-propanol−water						
Ross extraction		1.3 ± 0.6	ND	3.1 ± 0.1	1.1 ± 0.4	2.4 ± 1.0	7.8 ± 2.9
ASE extraction		1.3 ± 0.4	ND	2.2 ± 1.1	0.7 ± 0.5	0.9 ± 0.5	5.1 ± 0.5
Corn Flakes	1-propanol−water						
Ross extraction		1.1 ± 0.4	ND	2.7 ± 0.3	ND	2.3 ± 0.2	6.1 ± 0.6
ASE extraction		2.6 ± 0.7	ND	0.9 ± 0.1	ND	1.2 ± 0.7	4.7 ± 1.5
King Arthur 100% Whole Wheat Flour	1-propanol−water						
Ross extraction		9.9 ± 0.1	66.1 ± 1.9*	101.7 ± 0.3	15.6 ± 1.2	3.9 ± 0.5	197.1 ± 3.1
ASE extraction		11.7 ± 1.6	75.3 ± 3.8*	98.0 ± 5.1	18.9 ± 0.4	2.0 ± 0.2	205.9 ± 10.3

[a]The units presented are μg AR/g fresh weight ± standard deviation. For each sample and homologue marked with *, the two values are significantly ($p < 0.05$) different based on the Tukey multiple comparison method.

(>1000 psi) allow for solvents to be heated at temperatures higher than their normal boiling point, resulting in fast, efficient extractions. Automation of the system also reduces analyst time. Because of its automation and convenience to time, this current study was undertaken to compare the efficiency of ASE-AR extraction to current methods developed by Ross and colleagues[3] to see if it can be utilized as an alternative for AR extraction of food products composed of wheat.

MATERIALS AND METHODS

Sample Material and Preparation. Wheat bran (Bob's Red Mill, Milwaukie, OR) and processed food samples that included whole wheat bread (Pepperidge Farm 100% Whole Wheat Bread), refined grain bread (white bread), whole grain crackers (Triscuits), refined grain crackers (saltine crackers), whole wheat pasta (Hodgson Mill Whole Wheat Penne Pasta), refined grain pasta (Pasta LaBella Spaghetti), ready-to-eat whole wheat breakfast cereal (General Mills Wheaties), ready-to-eat breakfast cereal containing whole wheat (General Mills Fiber One), nonwheat ready-to-eat breakfast cereals (Rice Krispies and Kellogg's Corn Flakes), and whole wheat flour (King Arthur 100% Whole Wheat Flour) were purchased at local markets and stored at 4 °C. All samples were ground to 20 mesh using a Wiley mill (Thomas Scientific) prior to extraction. The moisture content for all samples was determined by oven drying each sample at 55 °C for 2 h. Oven-dried samples for the breads and whole wheat flour were used for AR extraction due to their convenience in milling. The AR contents for the breads and flour were then extrapolated from dried weight and presented as μg AR/g fresh weight. To investigate if oven drying had an effect on AR content, samples were also lyophilized overnight to remove moisture using a Flexi-Dry Freeze-Dryer (FTS Systems, Stone Ridge, New York). Afterward, AR extraction was then performed under extraction methods developed by Ross and colleagues.[3] The lyophilization AR content was then compared to the oven-drying AR content.

Extraction. All samples were extracted by methods developed by Ross and colleagues[3] and by ASE. ASE was performed using a Dionex ASE 200 (Sunnyvale, CA) using 11 cc extraction vessels. Nonpolar solvent (ethyl acetate) was used for wheat bran, whereas polar solvent [1-propanol:water (3:1 v/v)] was used for all processed food samples. Methyl behenate (C22:0, fatty acid methyl ester) dissolved in ethyl acetate was used as the internal standard (0.5 mg/mL). After extraction and drying, ethyl acetate (1 mL) was added to redissolve

each sample before AR content analysis. All reagents and solvents were of reagent or chromatography grade.

AR Extraction of Wheat Bran as Reported by Ross.[3] Milled wheat bran (1 g) with 1 mL of internal standard (0.5 mg/mL) was extracted with 40 mL of ethyl acetate for 24 h at room temperature with continuous shaking using a wrist-action shaker (Burrell Corp.). Extracts were decanted and dried under a gentle stream of nitrogen gas at 55 °C.

AR Extraction of Processed Foods as Reported by Ross.[3] Milled sample (1 g) with 1 mL of internal standard (0.5 mg/mL) was extracted with 10 mL of 1-propanol/water (3:1 v/v) in a boiling water bath. This was performed three times (2 × 2 h and 1 × 1 h) with fresh solvent each time for the same sample. Extracts were pooled and dried under a gentle stream of nitrogen at 70 °C.

AR Extraction Using ASE (ASE200). A filter on the outlet was fitted for each extraction vessel. The milled sample (1 g) with 1 mL of internal standard (0.5 mg/mL) was placed in the extraction vessel. Ottawa sand (20−30 mesh; Fisher, P/N 523-3) was added to fill the remaining dead volume. The Ottowa sand in this procedure was used as a dispersing agent to allow increased permeation of the sample and solvent. Each prepared cell was inserted into the top carousel, corresponding to each collection vial in the bottom carousel. Nonpolar solvent (ethyl acetate) was used for wheat bran and polar solvents [1-propanol:water (3:1)] were used for processed foods. The conditions for extraction were as follows: pressure, 1000 psi; temperature, 100 °C; heating period of 5 min; extraction (static) time of 10 min, with three static cycles per sample; flush volume, 100%; and purge volume, 60 s. The recovered solvent was dried under a gentle stream of nitrogen gas at either 55 or 70 °C, depending on the type of solvent used.

Spiking Study of ASE with Synthetic C20:0 AR Standard. To determine the overall efficiency of ASE-AR extraction, a spiking study was conducted. A nonwhole wheat sample expected to have little to no amount of AR content and a synthetic AR standard were used. In this case, saltine crackers with a synthetic C20:0 AR standard were applied to assess the efficiency of extraction. Two known concentrations of C20:0 AR standard (0.25 and 0.5 mg/mL) in ethyl acetate were added to milled samples of saltine crackers (1 g each) with 1 mL of internal standard (0.5 mg/mL) in the extraction vessels prior to extraction. The standard protocol for extraction was performed under the same procedure as the protocol for ARs of ASE.

Gas Chromatography−Flame Ionization Detector (GC-FID) Analysis of AR Content. The AR content analysis was performed using a Hewlett-Packard HP 6890 Series GC System that was coupled to an HP 6890 Series Mass Analyzer (MS) for peak identification and a FID for quantification. The column used was a HP-5MS 5% Phenyl Methyl Siloxane, 30 m × 250 μm × 0.25 μm nominal. The flow rate was 1.0 mL/min. For analysis using the FID, gases used were as follows: hydrogen (30 mL/min), oxygen (300 mL/min), and nitrogen carrier gas (28 mL/min). All gases were of chromatography grade. The method of Ross et al.[11] was employed. Separation was performed under the following temperature program: 120 (0 min), 200 (5 min), 320 (20 min), and 320 °C (40 min). Using methyl behenate (0.5 mg/mL) as the internal standard, ARs (C17:0−C25:0) were quantified (Table 1). The relative response factors of methyl behenate and heneicosylresorcinol (C21:0) were established as 1:0.9. All ARs were assumed to have the same response factors as heneicosylresorcinol.[12] AR extraction performed for both methods (Ross and ASE) as well as the spiking study was conducted in triplicate for each sample.

Statistical Analysis. Analysis of variance was conducted to calculate pooled variances across the samples and methods. Pair wise comparisons were tested for statistical significance ($p < 0.05$) by the Tukey multiple comparison technique.[13] Significantly different values were identified for each sample using the two extraction methods. This technique was also used to identify significance differences between oven drying and lyophilization.

RESULTS AND DISCUSSION

A spiking study was conducted using a synthetic C20:0 AR standard to test the quantitative recovery of ARs in the new ASE extraction method. AR standard at two known concentrations (0.25 and 0.5 mg/mL) was added to samples with low levels of total AR content (saltines), and the spiked samples were extracted under the ASE protocol. The levels of recovered ARs were then quantified via GC-FID (Figure 2) and compared to a standard curve of synthetic C20:0 AR standard. It was found that 99.1% of the C20:0 (0.25 mg/mL) was recovered and 98.4% of the C20:0 (0.5 mg/mL) was recovered, which provided good evidence in support of this new extraction method.

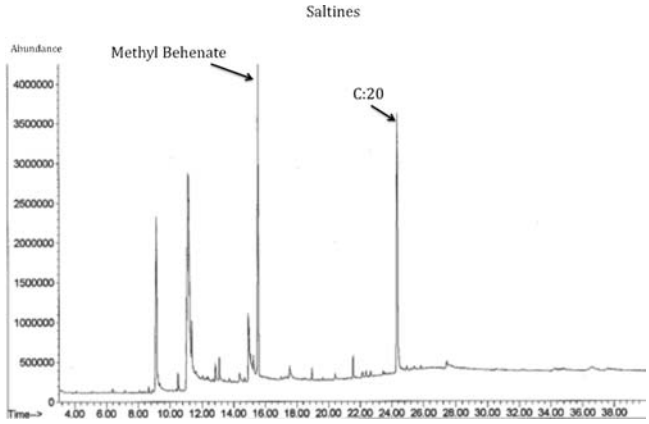

Figure 2. Spiking study example of GC-FID chromatogram of saltine crackers with methyl behenate (0.5 mg/mL) and a synthetic AR standard (C20:0, 0.5 mg/mL) identified.

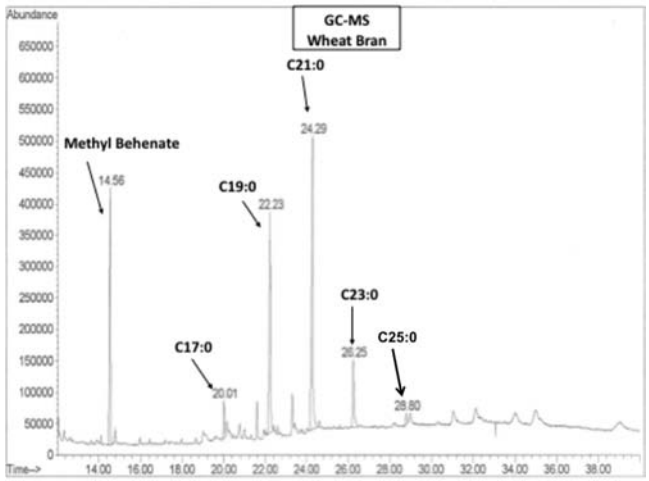

Figure 3. Example of GC-MS chromatogram of wheat bran with methyl behenate and AR homologues identified.

When comparing the AR homologues and total AR content between methods (Table 1), ASE results were comparable to the current extraction methods by Ross (only four pairs of data points out of a total of 72 pairs were statistically different).[3] With regard to processing time, the Ross extraction times can vary from 5 to 24 h, whereas the ASE extraction time is only 40 min, thereby making it much more time efficient and less tedious to the analyst. This reduction in time and the semiautomation of the technique result in quicker sample turnaround time, improved productivity, and are more convenient for the analyst.

The moisture content for all samples was determined by oven drying at 55 °C for 2 h. Excluding the whole wheat and white breads, the moisture content ranged from 0.46 to 10.12%.

dx.doi.org/10.1021/jf3001422 | J. Agric. Food Chem. 2012, 60, 4799−4802

Table 2. Comparison of AR Homologues and Total AR Content between Oven-Dried and Lyophilized Sample[a]

sample	drying method	C17	C19	C21	C23	C25	AR total
white bread	oven	1.2 ± 0.1	1.4 ± 0.2	1.1 ± 0.3	0.9 ± 1. 0	0.9 ± 0.3	5.5 ± 0.7*
	lyophilizer	1.5 ± 0.1	2.9 ± 0.6	2.9 ± 0.4	1.6 ± 0.14	1.7 ± 0.6	10.5 ± 0.8*
Pepperidge Farm 100% Whole Wheat Bread	oven	6.0 ± 0.3*	39.2 ± 1.0	45.8 ± 1.5	6.8 ± 0.1*	4.7 ± 1.0*	102.6 ± 2.3
	lyophilizer	7.9 ± 0.1*	37.0 ± 0.9	43.7 ± 1.9	10.3 ± 1.9*	2.4 ± 0.1*	101.3 ± 1.0
King Arthur 100% Whole Wheat Flour	oven	9.9 ± 0.1*	66.1 ± 1.9*	101.7 ± 0.3*	15.6 ± 1.2*	3.9 ± 0.5*	197.1 ± 3.1
	lyophilizer	14.2 ± 0.8*	77.2 ± 2.0*	89.5 ± 0.9*	10.8 ± 1.5*	1.5 ± 0.3*	193.1 ± 1.5

[a]The units presented are μg AR/g fresh weight ± standard deviation. For each sample and homologue marked with *, the two values are significantly ($p < 0.05$) different based on the Tukey multiple comparison method.

The moisture content for the bread samples ranged from 30.14 to 37.54%.

With regard to the total AR content for all samples (Table 1), wheat bran (Figure3) gave the highest total AR amount of ~1365 μg/g for both methods. With respect to the processed foods, Triscuits and Fiber One cereal had the highest amount of ARs, 200.8 and 222.8 μg/g, respectively, in this study. With white flour, the bran layer is removed during processing, which is where the ARs are located;[14] therefore, as expected, the foods that do not contain whole wheat or wheat bran (e.g., white flour, corn, and rice-based cereals) contained little to no ARs.

It should be noted that when comparing the levels of individual and total ARs with the two extraction methods, values were closest in samples with high levels of total AR contents (wheat bran, Triscuits, Wheaties, Fiber One, and King Arthur's 100% Whole Wheat Flour) and were more deviating in samples with low levels of total AR contents (white bread, saltines, Rice Krispies, and Kellogg's Corn Flakes). We believe that the GC-FID method used in this analysis mainly accounts for this difference, which is more accurate at higher levels of ARs, but is subjected to a high degree of background "noise" at lower levels of ARs.

It has been previously reported[3] that moist samples such as breads need to be lyophilized overnight to remove moisture before grinding and extraction. To investigate if oven drying could be used as a more rapid drying process, bread samples were lyophilized to remove the moisture content, and AR extraction was performed using the Ross methods. As shown (Table 2), the results indicate that oven drying and lyophilization produce similar, but not identical, results (unfortunately, nine pairs of data points out of a total of 18 pairs were statistically different, so more studies will be required to compare lyophilization and oven drying).

This current study revealed that ASE extraction of ARs can be comparable to current extraction methods. Spiking studies with a synthetic AR standard have shown that ASE-AR extraction is around 99%. The extraction time for ASE is 40 min, which is much more convenient and less tedious and time-consuming than methods developed by Ross and colleagues,[3] which are 5 h for processed foods and 24 h for raw grains. The AR contents between oven drying and lyophilzation were similar but not identical, suggesting that more studies need to be performed. Overall, this study demonstrated that ASE-AR extraction provides results comparable to, and is a much faster alternative for, AR extraction.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. John Phillips for valuable assistance with statistical analyses.

ABBREVIATIONS USED

ARs, alkylresorcinols; ASE, accelerated solvent extraction; GC-FID, gas chromatography−flame ionization detector; GC-MS, gas chromatography−mass spectrometry

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