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Microwave-Assisted Extraction of Bound Phenolic Acids in Bran and Flour Fractions from Sorghum and Maize Cultivars Varying in Hardness

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ABSTRACT: To release bound phenolic acids, a microwave-assisted extraction procedure was applied to bran and flour fractions obtained from eight sorghum and eight maize cultivars varying in hardness. The procedure was followed by HPLC analysis, and the identities of phenolic acids were confirmed by MS/MS spectra. The extraction of sorghum and maize bound phenolic acids was done for 90 s in 2 M NaOH to release ferulic acid and *p*-coumaric acid from bran and flour. Two diferulic acids, 8-*O*-4′- and 8-5′-benzofuran form, were identified and quantitated in sorghum bran, and only the former was found in maize bran. The contents of ferulic acid and diferulic acids in sorghum bran were 416−827 and 25−179 μg/g, respectively, compared to 2193−4779 and 271−819 μg/g in maize. Phenolic acid levels of sorghum were similar between hard and soft cultivars, whereas those of maize differed significantly ($p < 0.05$) except for ferulic acid in flour. Sorghum phenolic acids were not correlated with grain hardness as measured using a tangential abrasive decortication device. Maize ferulic acid ($r = -0.601$, $p < 0.01$), *p*-coumaric acid ($r = -0.668$, $p < 0.01$), and 8-*O*-4′-diferulic acid ($r = -0.629$, $p < 0.01$) were significantly correlated with hardness.

KEYWORDS: *microwave-assisted extraction, phenolic acids, diferulic acids, sorghum, maize, kernel hardness*

INTRODUCTION

Besides being energy sources, maize and sorghum are increasingly being recognized as sources of phenolic acids with potential health-promoting antioxidant activity. These phenolic compounds are found in both free and bound forms. However, only a small portion of the phenolic acids is in the free form, and the majority are bound to cell walls.[1] In mature plant cell walls, hydroxycinnamic acids, particularly ferulates, are thought to undergo etherification with the earlier esterified phenolic acids,[2] hence forming ester−ether bridges between lignin and polysaccharides. Esterified ferulic acid forms ether linkages with lignin quinone methide intermediates in lignified cell walls. This cross-linking is likely to reinforce cell walls and affect grain mechanical properties such as hardness. Grain hardness is the most important parameter for sorghum and maize dry milling quality.[3] However, studies have not fully established the role of bound phenolic acids on sorghum and maize grain hardness. Hence, there is a need to effectively extract the bound phenolic acids and relate them to grain hardness.

Microwave-assisted extraction (MAE) offers an alternative to the traditional, protracted alkaline refluxing used for releasing ester and ether-bound phenolic acids. The advantages of MAE include rapidity, reduced solvent consumption, and high phenolic yield.[4] Furthermore, the technique combines high temperature and high pressure for optimal release of phenolic acids with the concomitant breakdown of the cell walls. This procedure used previously to release etherified ferulic acid in several plant tissues, including cereal straws,[5] flaxseed, [4] and herbs,[6] resulted in higher quantities of ferulic acid than traditional alkaline hydrolysis. Rose and Inglett[7] applied microwave irradiation to hydrolyze maize bran samples with the aim of releasing feruloylated arabinoxyl-oligosaccharides in which water was used for MAE followed by alkaline hydrolysis. In this study, sorghum and maize cereal grains were hydrolyzed using MAE with the objective of maximizing the release of bound phenolic acids and studying their relationship to grain hardness.

MATERIALS AND METHODS

Materials. A study was conducted on eight sorghum and eight maize cultivars grown in South Africa, representing commercial hybrids varying in grain hardness, from the National Cultivar Trials harvested during the 2008/2009 growing season. Maize cultivars were white dent types grown in Potchefstroom. Sorghum cultivars were red, non-tannin, grown in Platrand. All cultivars were grown in dryland

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Table 1. Physical and Hardness Characteristics of Sorghum Cultivars[a,b]

	TW	TKW	>4.00 mm	>3.35, <4.00	>3.15, <3.35	>2.36, <3.15	TADD
Hard Cultivars							
range	75.9−77.7	25.0−26.2	0.3−3.6	52.5−65.8	11.8−18.8	9.9−13.2	26.7−37.6
mean	77.0a (0.7)	25.6a (0.9)	1.3a (1.46)	60.9a (5.6)	16.5a (3.0)	11.9a (1.4)	33.3a (4.9)
Soft Cultivars							
range	75.2−77.7	19.8−28.0	0.1−2.2	19.4−65.8	12.5−31.2	8.8−38.5	37.6−49.2
mean	76.2a (1.0)	25.0a (3.5)	1.4a (0.9)	50.7b (19.7)	17.9a (8.3)	17.6a (13.0)	42.6a (6.3)

[a]TW, test weight (kg/hL); TKW, thousand kernel weight (g); kernels passing through >2.36 and >4.00 mm (g); TADD, % kernel removed by TADD abrasion. [b]Figures in parentheses are standard deviations. Different letters in the same column denote significant differences ($p < 0.05$) between hard and soft cultivars, respectively.

Table 2. Physical and Hardness Characteristics of Maize Cultivars[a,b]

	TW	SB	KS	TKW	TADD	NIT
Hard Cultivars						
range	79.4−82.0	1.98−2.73	74.5−83.0	397−444	23.4−25.2	91.0−99.6
mean	80.6 (1.4)	2.38 (0.76)	78.2 (6.5)	421 (48)	24.1 (1.9)	95.8 (6.2)
Soft Cultivars						
range	78.6−86.1	1.7−4.11	77.0−83.7	373−422	29.1−31.2	84.1−95.7
mean	81.2 (4.7)	3.35 (1.13)	80.9 (3.5)	403 (33)	30.3 (2.1)	87.8 (6.9)

[a]TW, test weight (kg/hL); SB, % breakage susceptibility by Steiner breakage tester; TKW; thousand kernel weight (g); TADD, % kernel removed by TADD abrasion; KS; % kernel size ≥8 mm; NIT, near-infrared transmittance milling index. [b]Figures in parentheses are standard deviations. Means were not significantly different ($p < 0.05$).

conditions, harvested at <14% moisture, and dried slowly. Their physical and hardness properties are shown in Tables 1 and 2. Phenolic acid standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC grade hexane, ethyl acetate, and methanol were used for phenolic acids extraction. MS grade methanol and acetic acid were used in LC-MS/MS. The solvents were also purchased from Sigma-Aldrich Chemical Co. For phenolic assays, bran and flour portions were separated by decorticating grain to 80% extraction rate with a Tangential Abrasive Dehulling Device (TADD), model 4E- 115 (Venables Machine Works, Saskatoon, SK, Canada). Separation was achieved by optimizing decortication time for each cultivar to abrade 20% of the kernel.

Microwave-Assisted Sample Extraction. A 200 mg portion of the maize or sorghum sample was extracted with 5 mL of 2 M NaOH using a 45 mL PTFE acid digestion bomb vessel (Parr Instrument Co., Moline, IL, USA). The bomb vessel was placed in a 1400 W domestic microwave oven (Diplomat model D811, Danby, Suweon, Korea) set at 100% power to achieve 190 °C. Samples were digested for 45 s. The hydrolysate was adjusted to a pH of 1.5−2.0 using 6 N HCl and extracted three times with 15 mL of hexane to remove lipids.[8] The organic phase was removed with a separator and the aqueous phenolic phase extracted three times with ethyl acetate to obtain the alkali-released phenolics. The organic phase was further dehydrated with 1 g of Na_2SO_4. The combined ethyl acetate extracts were dried and concentrated under vacuum using a rotary evaporator. The dried phenolic extracts were redissolved in 2 mL of 50% methanol and filtered with a 22 μm nylon filter before HPLC and MS/MS analysis for phenolic acids.

Analyses. *HPLC-MS/MS Analysis.* HPLC analysis of phenolic acids was performed on a Waters 2695 HPLC equipped with a Waters 996 photodiode array (PDA) and an autosampler (717 Plus, Waters) to inject 10 μL of sample described by Qiu et al.[8] but with modifications. Instead, a Shimadzu RP analytical column (250 × 4.6 mm, 5 μm) was used and phenolic acid separation was achieved using a 70 min linear solvent gradient at a flow rate of 0.7 mL/min. The gradient mobile phase solvent A was 0.1% acetic acid in high-purity water, and solvent B was 0.1% acetic acid in methanol. The solvent gradient was as follows: 0 min, 4% B; 18 min, 18% B; 35 min, 30% B; 58 min, 42% B; 70 min, 60% B; and 10 min to rinse and equilibrate the column. Phenolic acid quantitation was based on the standard curves of the corresponding phenolic acids at a wavelength of 320 nm, and peak area was used for calculations. Identification of phenolic acids was performed by comparison to the retention time and MS/MS spectra with external standards. MS/MS was conducted using a quadrupole time-of-flight mass spectrometer (Q-TOF MS) (Micromass, Waters Corp., Milford, MA, USA) according to the method of Qiu et al.[8] In this study, full mass spectra were acquired in the negative mode using cone and capillary voltages of 30 V and 1.6 kV, respectively. Desolvation and cone gases (He) were set to flow at 900 and 35 L/h, respectively, whereas the desolvation and source temperatures were 350 and 150 °C, respectively. MS/MS spectra were acquired using collision energy of 25 V in the range m/z 100−1500.

Statistical Analysis. All experiments were done in triplicate. Means were compared by Fisher's least significant differences. Pearson's correlation was performed to determine the relationship between parameters. Calculations were performed using Statgraphics Centurion XV (StatPoint, Herndon, VA, USA).

RESULTS AND DISCUSSION

Optimization of Microwave-Assisted Alkaline Extraction Conditions. Samples were microwaved at 15 s intervals in 2 M NaOH to optimize extraction. Complete solubilization was achieved after 45 s in both sorghum and maize grain samples compared to the conventional alkaline hydrolysis, which takes at least 2 h. In conventional alkaline hydrolysis sample solubilization was incomplete, as shown by visible particles after hydrolysis. The duration of extraction used in this study was shorter than reported previously.[5,7] The differences in extraction conditions could be attributed to different microwave units, power settings, extractants, and sample types. Microwave oven temperature was set at 190 °C, a temperature deemed sufficient to break the ether bonds, which are heat labile at 170 °C.[9]

Identification of Microwave-Extracted Bound Phenolic Acids in Sorghum. Phenolic compounds were identified by comparing their retention times (t_R) and mass spectra with those of external standards. *p*-Coumaric acid and ferulic acid were the only phenolic acids positively identified in

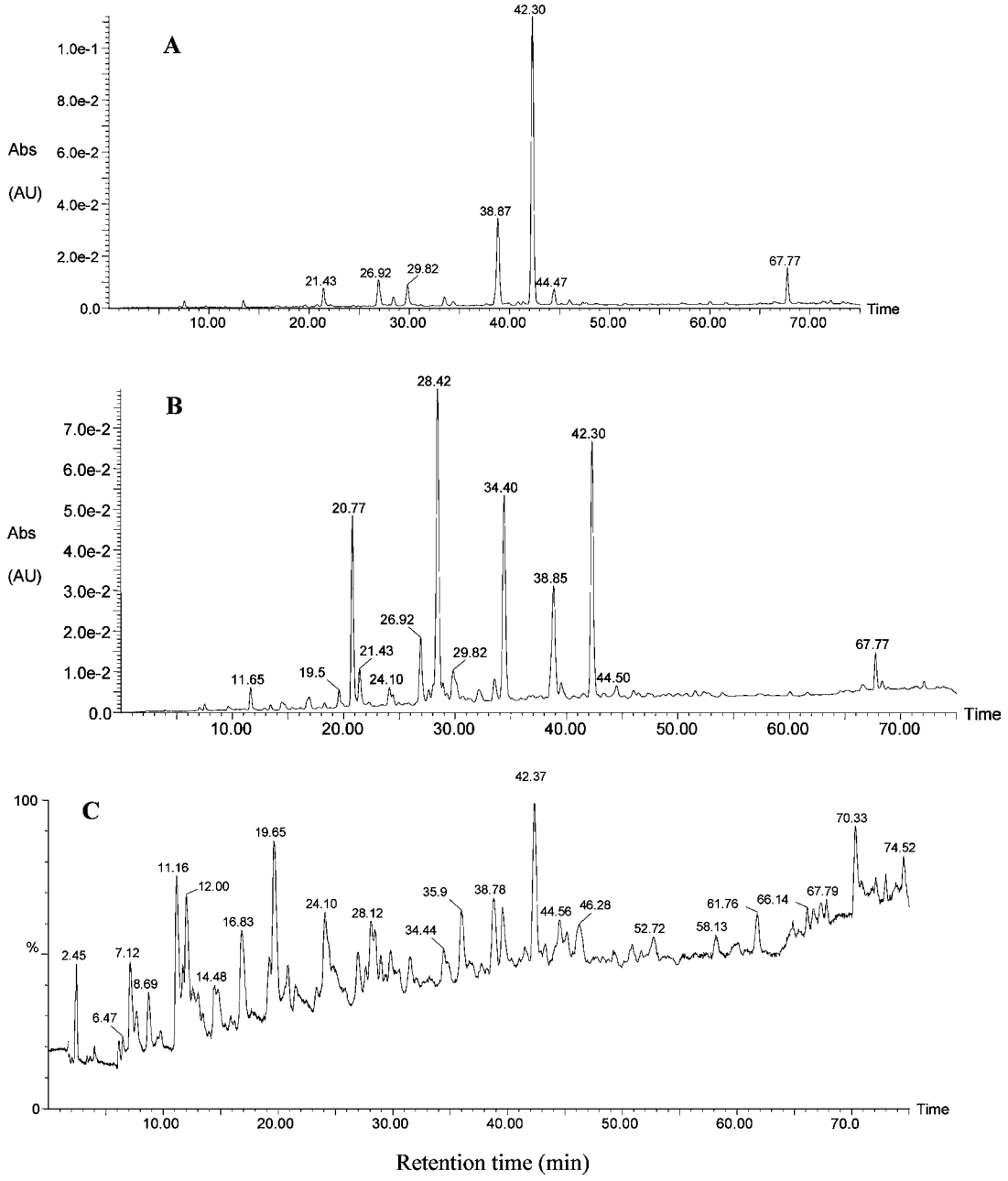

Figure 1. LC chromatogram of phenolic acids in sorghum bran of cultivar PAN 8564 at (A) 320 nm and (B) 280 nm and (C) the total ion chromatogram.

both sorghum and maize. Two diferulic acids were identified in sorghum, and panels A, B, and C of Figure 1 show peaks obtained at 320 and 280 nm and the total ion chromatogram, respectively. There were more compounds detected at 280 nm than at 320 nm, although most of the compounds could not be identified with t_R and masses of the external standards. *p*-Coumaric acid and ferulic acid were the only monomeric phenolic acids identified at t_R 38.9 and 42.3 min, respectively. The identity of the peaks was confirmed with mass spectrometric data acquired in the negative ionization mode based on the molecular ions $[M - H]^-$ and fragmentation patterns (Figure 2). Panels C and D of Figure 3 show the main fragments and mass spectra of ferulic acid and *p*-coumaric acid, respectively. Ferulic acid (m/z 193) produced a fragment at m/z 178 due to the loss of a methyl group (15 Da) from the parent ion. Another fragment was generated at m/z 134 due to the loss of both the methyl group and carbon dioxide. *p*-Coumaric acid (m/z 163) produced fragment ions at m/z 119 corresponding to the loss of carbon dioxide (44 Da). Hossain et al.[10] reported the loss of carbon dioxide in *p*-coumaric acid and ferulic acid from the parent ions characterized in Lamiaceae spices. Besides ferulic acid and *p*-coumaric acid, three peaks at t_R 21.4, 26.9, and 29.8 min were obtained at both 280 and 320 nm. Their identities were not confirmed with external standards and mass spectrometric data but they were assumed to be derivatives of hydroxycinnamic acids, due to their absorption pattern. The rest of the peaks were detected only at 280 nm, and excluded at 320 nm, typical of hydroxybenzoic acid derivatives.

Two diferulic acids with m/z 385 were identified. The diferulic acids were eluted at t_R 66.7 and 44.5 min, and their mass spectra corresponded to 8-*O*-4′- (Figure 2A) and 8-5′-

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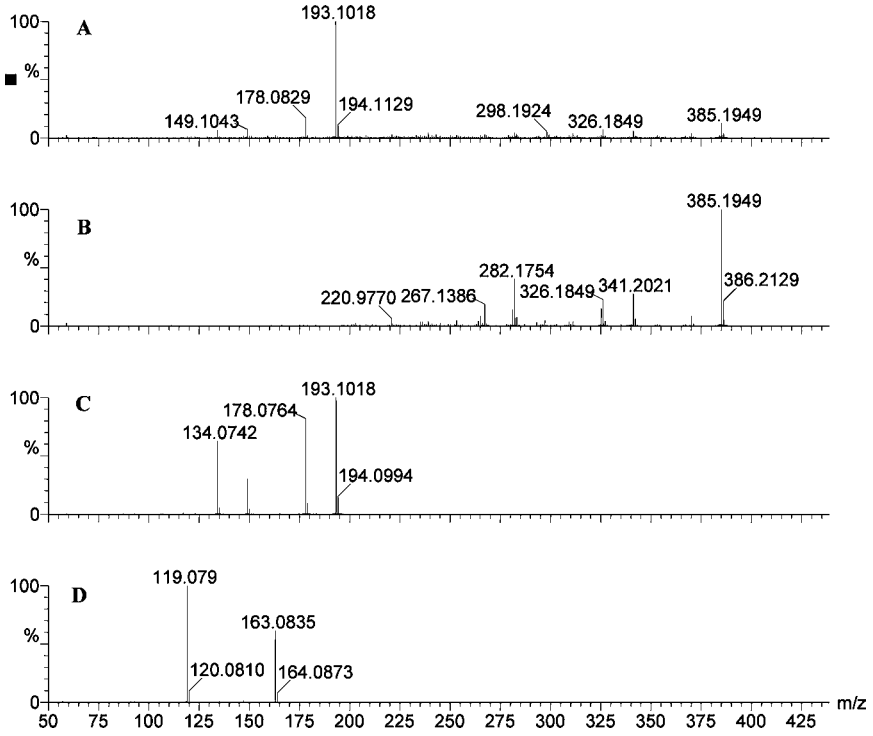

Figure 2. MS/MS spectra of (A) 8-*O*-4′-diferulic acid (66.8 min), (B) 8-5′-benzofuran form diferulic acids (44.5 min), (C) ferulic acid (42.3 min), and (D) *p*-coumaric acid (38.9 min) of sorghum bran cultivar PAN 8564.

benzofuran form, (Figure 2B), respectively. The assignments were in agreement with mass spectra data and fragmentation patterns reported in the literature.[8,11,12] The deprotonated 8-5′-benzofuran form diferulic acid $[M - H]^-$ produced a fragment with *m*/*z* 341 due to the loss of carbon dioxide (44 Da) from the carboxylic acid group. Both diferulic acids produced a fragment with *m*/*z* 326 due to the loss of carbon dioxide and a methyl group (59 Da). The loss of carbon dioxide is typical of phenolic acids with the resultant $[M - H - COO]^-$ anion.[10,13] The diferulic acids were expected and confirmed earlier reports that 8-*O*-4′- and 8-5′-benzofuran form were the most abundant diferulic acid in cereals.[14,15]

Identification of Microwave-Extracted Bound Phenolic Acids in Maize. Several peaks were obtained in maize samples as shown (Figure 3). However, only *p*-coumaric acid and ferulic acid were identified using retention times of external standards. An unknown peak that eluted at t_R 33.7 min at both 280 and 320 nm was assumed to be a hydroxycinnamic derivative. Most of the peaks detected at 280 nm but not detected at 320 nm would be hydroxybenzoic acid derivatives. The unconfirmed peaks eluted at t_R 19.67, 20.8, 28.6, and 34.6 min (Figure 3B) were also observed in sorghum.

MS/MS data were used to confirm the identities of the phenolic acids. The fragmentation pattern of *p*-coumaric acid and ferulic acid followed that of sorghum as described above. The identity of the other compounds could not be confirmed using their MS/MS spectra. One diferulic acid was obtained at t_R 67.8, similar to that identified in sorghum, although two diferulic acids were detected in the latter. The diferulic acid was assigned 8-*O*-4′ due to its fragmentation pattern. 8-*O*-4′-Diferulic acid is among the commonly found diferulic acids in cereal grains.[14]

Quantitation of Microwave-Extracted Bound Phenolic Acids of Sorghum Bran and Flour Fractions. The quantities of sorghum bran and flour phenolic acids are shown in Table 3. Within the hard cultivars, bran of PAN 8488 had the lowest *p*-coumaric acid and ferulic acid contents, whereas PAN 8902 had the highest ferulic acid and diferulic acid contents. Within the bran of soft cultivars, *p*-coumaric acid occurred over a wider range (38−222 μg/g) than hard cultivars (85−228 μg/g). The significant differences in bran *p*-coumaric acid ($p < 0.05$) of soft cultivars could be useful to distinguish among hard and soft grain samples. Bran of PAN 8903 had the lowest phenolic acid content, particularly *p*-coumaric acid and diferulic acids, which were up to 6 and 7 times less than other cultivars, respectively. Overall, there were no significant differences ($p > 0.05$) in bran phenolic acid content between the hard and soft cultivars following MAE.

p-Coumaric acid and ferulic acid were identified and quantitated in the sorghum flours. Within the hard cultivars, the ferulic acid content of PAN 8564 was up to 3 times less than the other cultivars. Overall, *p*-coumaric acid and ferulic acid contents of flours were not significantly different ($p > 0.05$) between hard and soft cultivars. Because most phenolic acids are concentrated in the bran, significant variations in their levels were not expected in the endosperm, the major component of the flours. Diferulic acids content was also not significantly different ($p > 0.05$) between the hard and soft sorghum cultivars. PAN 8903 was an exception as it contained almost 7 times less diferulic acids than both hard and soft cultivars. PAN 8903 was also characterized by lower values of *p*-coumaric acid and ferulic acid within the soft sorghum cultivars. With the exception of PAN 8903, the diferulic acids content of the sorghum cultivars was between 4 and 6 times less than ferulic acid content due to the lack of lignified cell walls in the endosperm.

Quantitation of Microwave-Extracted Bound Phenolic Acids of Maize Bran and Flour Fractions. The contents of

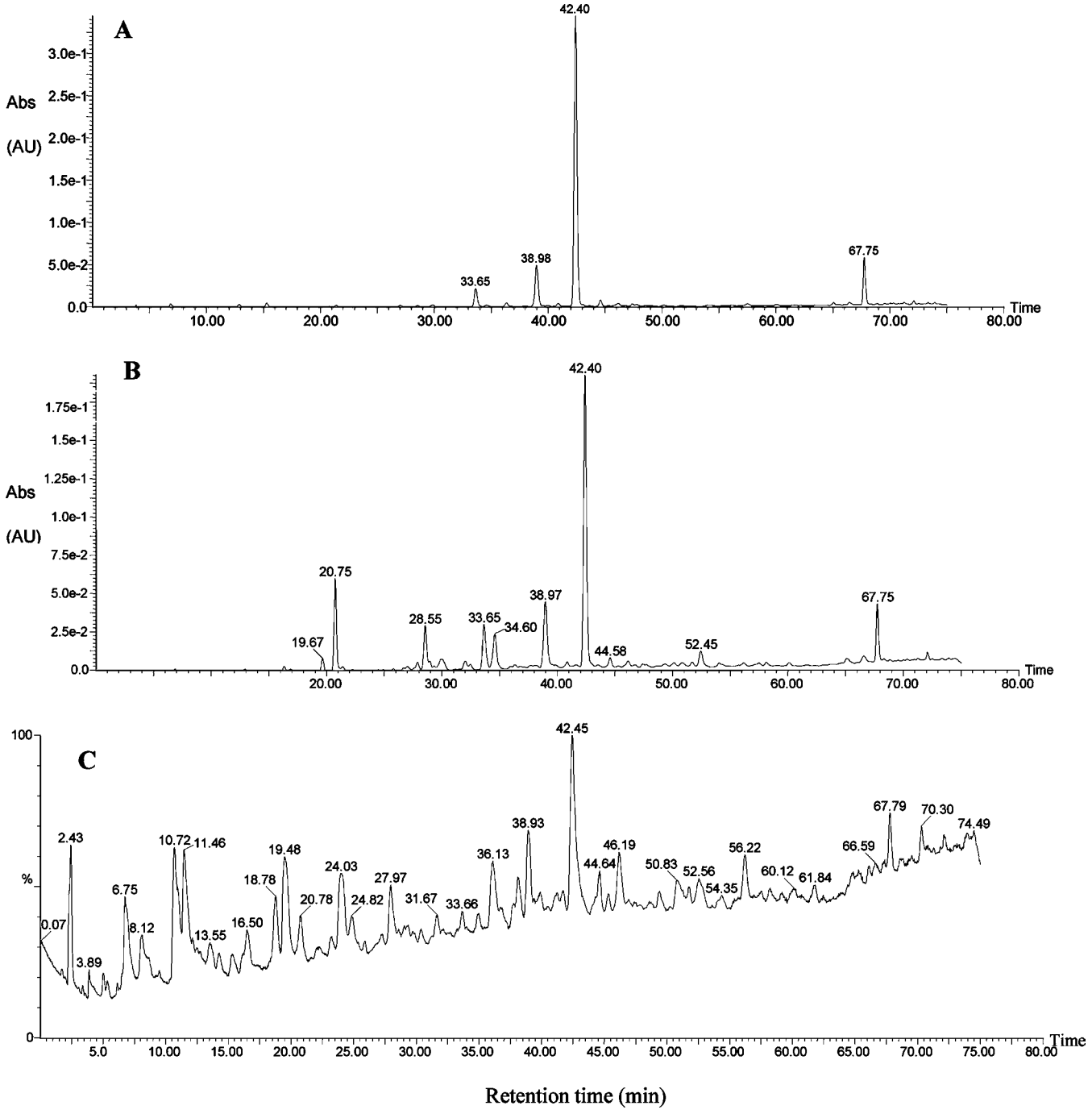

Figure 3. LC chromatogram of phenolic acids in maize bran of cultivar AFG 4555 at (A) 320 nm and (B) 280 nm and (C) the total ion chromatogram.

bran phenolic acids varied among the maize cultivars. There were significant differences ($p < 0.05$) in bran phenolic acid content between hard and soft samples (Table 4). Ferulic acid was the predominant phenolic acid in the bran as previously reported,[14] followed by *p*-coumaric acid. On average, there was a larger variation in FA (range 1189 μg/g) than *p*-coumaric acid (range 179 μg/g) between hard and soft cultivars. AFG 4555 had the highest amount of ferulic acid (4779 μg/g) with at least 25% more ferulic acid than the other hard cultivars. PAN 6223 B had up to 38% more ferulic acid than bran from other soft cultivars. Diferulic acids were significantly different ($p < 0.05$) between the hard and soft cultivars. Although AFG 4555 had the highest ferulic acid, its diferulic acids content was the lowest within the hard maize cultivars. This trend was not observed for soft cultivars as high ferulic acid content corresponded with high diferulic acids content and vice versa.

Similarly, ferulic acid and *p*-coumaric acid were the only phenolic acids identified and quantitated in maize flours. *p*-Coumaric acid was almost 4 times higher in hard cultivars than in soft cultivars. However, within the hard cultivars there was a large variation in *p*-coumaric acid (25−159 μg/g). There were no significant differences ($p > 0.05$) in the *p*-coumaric acid of soft cultivar flours. Ferulic acid was similar between hard and soft cultivars, although cultivars AFG 4555 and AFG 4473 had

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Table 3. Bound Phenolic Acids of Sorghum Bran and Flour Fractions Released by Microwave-Assisted Alkaline Extraction (Micrograms per Gram)[a]

	bran			flour	
	PCA	FA	DFA	PCA	FA
Hard Cultivars					
PAN 8902	108bcB (8)	827aA (10)	179aA (4)	24.5dC (1.8)	98abB (4)
PAN 8905	228aA (11)	445dC (18)	108cC (4)	32.2cB (1.8)	110aA (2)
PAN 8564	106bcB (5)	643bcB (14)	131bB (6)	40.1bA (1.5)	37eC (2)
PAN 8488	85dC (5)	416dC (6)	112cC (6)	41.6abA (3.1)	111aA (8)
mean	132α (60)	583α (178)	133α (30)	34.6α (7.5)	89α (33)
Soft Cultivars					
PAN 8901	222aA (10)	680bA (8)	170aA (9)	16.7fC (1.8)	108aA (4)
PAN 8903	38eD (3)	445dC (7)	25dC (1)	22.5eB (2.0)	65dC (3)
PAN 8906	90cC (3)	619cB (6)	110cB (2)	14.8fC (0.9)	84bcB (3)
PAN 8904	122bB (5)	613cB (12)	172aA (5)	48.1aA (0.7)	78cdB (1)
mean	118α (72)	589α (94)	119α (64)	25.6α (14.3)	84α (17)

[a]Figures in parentheses are standard deviations. Different lower case, upper case, and Greek letters in the same column denote significant differences ($p < 0.05$) among all cultivars, within the hard and soft and between hard and soft cultivars, respectively. PCA, *p*-coumaric acid; FA, ferulic acid; DFA, diferulic acid.

exceptionally high ferulic acid content in the hard and soft groups, respectively.

The high temperature used for extraction likely degraded some monomeric phenolic acids. However, the stable ferulic acid and *p*-coumaric acid were retained. In contrast, high temperature was expected to break down ether bonds in cell walls and increase the release and diffusion of oligomeric phenolic acids into the alkali. However, only two diferulic acids were identified in sorghum and one in maize. The diferulic acids released by MAE were also less than the quantities reported for maize dietary fiber,[16] probably owing to sample composition and preparation before extraction. The latter extracted the diferulic acids from isolated cell walls. Although the microwave conditions were set such that the temperature was sufficient to cause ether bond breakage, generally sorghum diferulic acids content was low. Moreover, total solubilization of the sample was expected to increase phenolic acid release by means of cell wall breakage. There were large differences between the sorghum and maize phenolic acid contents despite the two cereal grains being similar in structure, chemical composition, and basis for grain hardness.[17] The differences in the diferulic acids content and composition of sorghum and maize cultivars could be due to variations in their biosynthesis and cross-linking, hence affecting the degree of alkaline hydrolysis and breakage of ether bonds.[18] The time for extraction could have been insufficient to break all of the sorghum ether bonds and release the diferulic acids despite the likelihood that the temperature generated would enhance mass transfer by increasing solid solubility and diffusion of solubilized phenolic acids into the alkali and reduce extraction time.[19] Similar extraction conditions were applied to both sorghum and maize as this was a comparative study, but it seems that optimal conditions for phenolic extraction vary between the two cereal grains and, in particular, for release of diferulic acids in sorghum.

Effect of Microwave-Assisted Extraction on Sorghum and Maize Phenolic Acids. Several phenolic acids have been identified and confirmed in sorghum and maize grains. However, in this study, only two phenolic acids were positively identified, which shows that microwaving had an effect on phenolic acid composition. The stability of phenolic acids varies with treatment conditions. With conventional alkaline hydrolysis, the pH is known to affect phenolic acid stability. Among the phenolic acids studied, caffeic acid was reported to be pH sensitive and might undergo irreversible chemical transformation with increasing pH.[20] Transformations were thought to be due to oxidative degradation or formation of unstable quinone intermediates, which would form diketo derivatives and degradation products.[20] This was attributed to the structure of caffeic acid, which has two phenolic hydroxyl groups in the ortho position capable of forming quinones. Although similar to

Table 4. Bound Phenolic Acids of Maize Bran and Flour Fractions Released by Microwave-Assisted Alkaline Extraction (Micrograms per Gram)[a]

	bran			flour	
	PCA	FA	DFA	PCA	FA
Hard Cultivars					
IMP 52-11	255deD (21)	3592cC (119)	622cC (12)	151aA (5)	345bB (10)
DKC 77-61 B	345cC (38)	3564cC (112)	819aA (2)	159aA (7)	260cdC (8)
AFG 4555	547aA (15)	4779aA (220)	599cdC (12)	37bB (2)	470aA (13)
LS 8521 B	463bB (31)	3944bB (163)	746bB (25)	25cC (2)	287cC (11)
mean	403α (121)	3970α (538)	696α (97)	93α (66)	341α (87)
Soft Cultivars					
PAN 6223 B	319cdA (15)	3517cA (100)	573dA (37)	24cA (1)	235dC (8)
PAN 4P-313 B	185efC (8)	2862dB (65)	507eB (35)	25cA (2)	281cB (16)
AFG 4473	221efB (15)	2193fD (39)	271fC (15)	24cA (2)	435aA (13)
AFG 4517	171fC (11)	2551eC (17)	536deAB (23)	23cA (2)	273cB (12)
mean	224β (63)	2781β (522)	472β (130)	24β (2)	306α (82)

[a]Figures in parentheses are standard deviations. Different lower case, uppercase, and Greek letters in the same column denote significant differences ($p < 0.05$) among all cultivars, within the hard and soft cultivars, and between hard and soft cultivars, respectively. PCA, *p*-coumaric acid; FA, ferulic acid; DFA, diferulic acid.

Table 5. Pearson Correlation Coefficients between Sorghum Physical and Hardness Characteristics and Phenolic Acids of Bran Fractions[a,b]

	TW	TKW	KS 4.00	KS 3.35	KS 3.15	KS 2.36	TADD	PCA	FA
PCA	0.0291	0.126	−0.074	0.015	−0.053	−0.008	0.078		
FA	0.233	0.005	0.502*	0.092	−0.141	0.106	−0.468	0.111	
DFA	−0.058	−0.305	0.128	−0.235	0.194	0.384	−0.453	0.471	0.733**

[a]TW, test weight (kg/hL); TKW, thousand kernel weight (g); KS 4.00, kernel size >4.00 mm; KS 3.35, kernel size <4.00 and >3.35 mm, KS 3.15, kernel size <3.35 and >3.15 mm; KS 2.36, kernel size <3.15 and >2.36; TADD, % kernel abraded by a Tangential Abrasive Dehulling Device; PCA, *p*-coumaric acid; FA, ferulic acid; DFA, diferulic acids. [b]Significance at $p < 0.05$ denoted by *.

Table 6. Pearson Correlation Coefficients between Maize Physical and Hardness Characteristics and Phenolic Acids of Bran and Flour Fractions[a,b]

	TW	SB	SC	SCI	TKW	TADD	KS	NIT	PCA	FA
PCA	0.434	0.371	−0.267	−0.123	−0.029	−0.601**	0.173	0.188		
FA	0.313	0.363	−0.368	−0.239	−0.179	−0.668**	0.293	0.169	0.891***	
DFA	−0.140	0.0527	−0.315	−0.241	−0.373	−0.629**	0.306	0.397	0.504*	0.642**

[a]TW, test weight (kg/hL); SB, % breakage susceptibility by Stein breakage tester; TKW, thousand kernel weight (g); TADD, % kernel abraded by a Tangential Abrasive Dehulling Device; KS; % kernel size ≥8 mm; NIT, NIT milling index, PCA, *p*-coumaric; FA, ferulic acid; DFA, diferulic acid. [b]Significance at $p < 0.01$ and 0.001 denoted by ** and ***, respectively.

caffeic acid, ferulic acid has a methylated phenolic hydroxyl group, which is not capable of forming quinones, and the methyl group may confer better resonance stability and, hence, stability to both alkaline and acidic pH changes. *p*-Coumaric acid is known to confer the greatest stability,[21] which is attributed to the small number of substituents in the aromatic ring.[22] Hence, the identification and quantitation of ferulic acid and *p*-coumaric acid in most cereals could be attributed to their chemical stability. Liazid[22] studied the stability of phenolic acids including hydroxycinnamic and hydroxybenzoic acid derivatives during MAE. The hydroxycinnamic and hydroxybenzoic acid derivatives were stable up to 150 °C, and their contents were reduced thereafter, attributed to their degradation at high temperatures. The extraction temperature of 190 °C could partly explain why few phenolic acids were identified. As described above, the chemical structure of the phenolic acids plays a role in their stability. Phenolic acids with a few substituent groups in the aromatic ring were found to be more stable during MAE.[22] Moreover, among phenolic acids with the same number of substituents, those that had more methyl groups than hydroxyl groups were less susceptible to degradation, as confirmed previously.[21] This would be true for ferulic acid and *p*-coumaric acid in the sorghum and maize samples studied. Compared to other phenolic acids, the stability of the phenolic acids was influenced by the methyl group and the small substituent groups in ferulic acid and *p*-coumaric acid, respectively.

Rose and Inglett[7] used MAE to release esterified ferulic acid from maize bran. The highest amounts of ferulic acid (1.94 and 2.13 g/100 g) were obtained after the samples had been extracted for 5 and 10 min, respectively, at 200 °C, a temperature higher than the 190 °C currently used. However, lower amounts of ferulic acid were obtained under conditions optimal for the release of arabinoxylo-oligosaccharides and would imply an inverse relationship between the release of oligosaccharides and ferulic acid. A combination of MAE and extract purification and preconcentration using solid phase extraction were found ideal for phenolic acid evaluation.[23] Solid phase extraction reduced interference by pigmented compounds and coelution of substances during HPLC analysis. However, in this study, the purification step was not included, which could have been useful in sorghum to avoid elution of interfering pigmented compounds because the sorghums evaluated were red types.

Relationship between Phenolic Acids of Sorghum and Maize with Hardness Parameters. Microwave-extracted sorghum bran phenolic acids were not correlated with grain hardness except the significant correlation between kernel size >4.00 mm and ferulic acid ($r = 0.502$, $p < 0.05$) (Table 5). However, this was not the case with maize as TADD hardness was moderately correlated with *p*-coumaric acid ($r = -0.601$), ferulic acid ($r = -0.668$), and diferulic acids ($r = -0.629$) at $p < 0.01$ (Table 6). The result is of interest as it shows that diferulic acids influence maize grain hardness and the higher the content, the harder the maize. Also, the results imply that microwave extraction was able to release most of the bound phenolic acids in maize, particularly diferulic acids.

The positive correlations between maize ferulic acid and diferulic acids with TADD hardness confirm the influence of these phenolic acids on grain hardness. However, microwaving sorghum at similar conditions with maize does not yield the same results. Because this study is the first to report on microwave-extracted sorghum phenolic acids, it is evident that mechanisms of phenolic extraction between the two cereal grains are different. More work is required to optimize microwave conditions for extracting bound sorghum phenolic compounds and ascertain the identity of the several unknown compounds, which were unidentified using the external standards and their masses.

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Notes

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