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High-Performance Liquid Chromatography Determination of Phenolic Constituents in 17 Varieties of Cowpeas

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Seventeen varieties of cowpeas grown in Arkansas were analyzed for their phenolic constituents using high-performance liquid chromatography (HPLC). Protocatechuic acid was identified as the major phenolic acid present in esterified forms. The amount of protocatechuic acid increased from trace−3.6 to 9.3−92.7 mg/100 g of flour in the 17 varieties of cowpeas after hydrolysis. Six other phenolic acids, including, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid, were also identified. These phenolic acids were evenly distributed mainly in free acid forms at <7 mg/100 g of flour. Total phenolic contents determined using Folin−Ciocalteu's reagent were largely different among the 17 varieties, ranging from 34.6 to 376.6 mg/100 g of flour. A comparison of the HPLC chromatograms of the 17 cowpea phenolics before and after alkali hydrolysis indicated the conversion of a pattern with evenly distributed peaks to one with a single major peak for protocatechuic acid, suggesting that the chromatograms before hydrolysis better represent the identities of the cowpea varieties.

KEYWORDS: Cowpea; HPLC; phenolics; protocatechuic acid

INTRODUCTION

Cowpeas (*Vigna unguiculata*) or southern peas have been recognized as a beneficial source of proteins and other nutrients such as vitamin B for the diets of people around the world, especially in developing countries (*1*, *2*). Although cowpeas have been part of a supplemental diet in many parts of the world, information on the phytochemicals in cowpeas is limited, yet these phytochemicals may bring nutraceutical and functional benefits to food systems.

One of the most important phytochemical groups is phenolics. Plant phenolics are antioxidants in many food systems (*3*, *4*). Through the scavenging of free radicals or the quenching of radical reactions responsible for lipid rancidity, phenolics prevent food deterioration (*5*). Many phenolics show both antioxidant activity and antimutagenicity and are functional food components possessing health benefits or being able to prevent disease in human beings (*6*). Catechins in tea and anthocyanins in many vegetables and fruits, for example, are believed to have health functions in preventing carcinogenic disease *(6*, *7*). Phenolics in wine, on the other hand, are important in furnishing the color of wine, producing the astringent taste, and offering a reservoir for oxygen reduction (*8*). Plant phenolics also provide metabolic functions during the seedling and growing stages of plants and prevent secretion against insect and disease damage in injured plants (*5*, *9*).

Plant phenolics are present in free, ester, and insoluble bound forms (*5*, *10*). Free and esterified phenolics are soluble in methanol and found to be the predominant phenolics in some plants (*10*). Hydrolysis will convert the esterified phenolics to simple phenolic acids.

Unlike phenolics from herbs (*11*) and soybeans (*12*), which have been extensively investigated, phenolics from cowpeas have received limited attention. In a study on the composition of free and hydrolyzable phenolic acids in flours and hulls of 10 legumes using alkali hydrolysis and gas chromatographic determination, Sosulski and Dabrowski (*13*) reported that field peas had a phenolic content of 2−3 mg/100 g of flour. Although field pea hulls contained only 0.4 mg of *p*-hydroxybenzoic acid/100 g of flour, trace amounts of protocatechuic acid and *p*-coumaric acid (<0.3 mg/100 g of flour), and 0.7 mg of ferulic acid/100 g of flour, field pea flour contained 1.2 mg of syringic acid/100 g of flour and 1.4 mg of ferulic acid/100 g of flour. Kahkonen et al. (*4*) reported a total phenolic content of 1.6 mg/g methanol extract for peas (*Pisum savitum*) using a colorimetric method, which was 56.6 mg/100 g of flour. There were also some reports about a heat-stable antioxidant of phenolic origins carried by the superoxide dismutase isolated from dried peas (*14*). No information is readily available for cowpea phenolics. Our objective was to determine the phenolic constituents in 17 varieties of cowpeas in order to provide information regarding the levels of phytochemicals in cowpeas. This paper reports our findings on the phenolic constituents of 17 varieties of cowpeas determined using high-performance liquid chromatography

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(HPLC). Chromatogram patterns of phenolics in methanolic extracts from 17 cowpeas before and after alkali hydrolysis were compared.

MATERIALS AND METHODS

Sources of Materials. Standard phenolic acids, including gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, gentisic acid, (±)-catechin, vanillic acid, caffeic acid, syringic acid, (−)-epicatchin, *p*-coumaric acid, sinapic acid, benzoic acid, 2,4-dimethoxybenzoic acid, *o*-coumaric acid, and cinnamic acid, were purchased from Sigma Chemical Co. (St. Louis, MO). Ferulic acid was purchased from Aldrich Chemical Co. (Milwaukee, WI). Seventeen varieties of cowpeas were provided by the Department of Agriculture, University of Arkansas at Pine Bluff. The methanol, acetonitrile, and water used for HPLC analysis were of HPLC grade. All other chemicals, unless stated otherwise, were of verified ACS grade and purchased from VWR Scientific Products Inc. (Fayetteville, AR).

Methanol Extraction of Phenolics from Cowpea Flours. The cowpea flours were prepared by grinding the seed in a coffee grinder model KSM 2B (Gillette Canada, Mississauga, ON, Canada) for 1 min. The moisture contents of 17 varieties of cowpea flours were determined to be between 9 and 11%. Cowpea phenolics were extracted according to the method described by Cai and Arntfield (*15*) with some modifications. Five hundred milligrams of cowpea flours was weighed into a 250-mL distilling vessel connected with a distilling/condensing system. Twenty milliliters of methanol was then added to the flours, and the mixture was refluxed for 2 h in a water bath of 95 °C while stirring using a magnetic stirring bar. After extraction, the mixture was cooled to ambient temperature. The mixture was then filtered through a Whatman no. 4 filter paper. The solid layer was discarded. The liquid phase was then evaporated to dryness under a stream of nitrogen at room temperature. The dried extract was then redispersed into 1 mL of methanol. A portion (200 μL) of the methanol solution was filtered through a 0.2-μm filter with a 1-mL syringe. The filtered liquid was used for HPLC analysis. The rest (800 μL) was used for hydrolysis.

Hydrolysis of Cowpea Phenolics. The methanol extracts were hydrolyzed to convert the phenolics from esterified forms to free phenolic acids. Eight hundred milliliters of the methanol redispersed solution was evaporated to dryness in a 5-mL test tube under a stream of nitrogen. One milliliter of 5 N NaOH was then added to the tube, and the mixture was stirred with a stream of nitrogen for 4 h. After hydrolysis, the pH of the mixture was adjusted to 2.0 with 5 N HCl to precipitate any proteinaceous materials (*16*). The final volume of the mixture was readjusted with deionized water to 1.0 mL to compensate any vapor loss during alkaline hydrolysis. Then, to the mixture was added 1.0 mL of methanol, and the mixture was vortexed for 5 s. The mixture was then centrifuged at 10000 rpm with an Eppendorf centrifuge model 5415C (Brinkman Instruments Inc., Westbury, NY) for 5 min and passed through a 0.2-μm filter before analysis by HPLC.

HPLC Analysis. Chromatographic equipment consisted of a Hewlett-Packard (Avondale, PA) liquid chromatograph model 1090 equipped with a diode array ultraviolet (UV) detector. A TSK-GEL Super-ODS (Supelco, Bellefonte, PA) column was used. The absorbance of the effluent was monitored at 254 and 238 nm. The mobile phase consisted of solvents A−C using three pumps equipped with the chromatograph. Solvent A was 0.1% trifluoroacetic acid in acetonitrile, solvent B, 0.1% trifluoroacetic acid in HPLC grade water, and solvent C 100% methanol. Flow rate was set at 1.0 mL/min, and column temperature was maintained at 37 °C throughout the test. The initial solvent condition was 100% solvent B. A linear gradient was used to increase solvent A from 0 to 10% within 7 min. This solvent composition was maintained at an isocratic flow for 3 min. Solvent A was then increased from 10 to 40% using a 20-min linear gradient. This composition was then maintained for 2 min and returned to the initial condition in 3 min. Solvent C was used for column washings between and after runs. Sample sizes of 4 μL for the intact phenolics and 12 μL for hydrolyzed phenolics were injected during HPLC analysis. The use of different sample sizes was due to the different phenolic concentrations in intact and hydrolyzed samples. The concentrations of phenolic acids in cowpea flours were calculated from standard curves calibrated using the 16 phenolic standards. The phenolic contents were expressed as milligrams per hundred grams of cowpea flours.

Standard Calibration. Stock solutions (2 mg/mL) for phenolic acids were prepared by accurately weighing 5 mg of each substance into 2.5 mL of methanol. Dilution of the above stock solutions gave a set of standard solutions of 200, 100, 50, and 25 μg/mL for each phenolic acid, respectively. A 4-μL mixture of phenolic acid solutions was injected into the HPLC column using a 25-μL automatic sample loop. Calibration curves were obtained for each phenolic acid, respectively, by plotting concentrations versus peak areas. Regression equations were obtained from the calibration curves for each individual phenolic compound. Identification of the phenolic compounds was done by comparing the retention time of the unknown with those of authentic phenolics at two wavelengths (254 and 238 nm). The identities were then confirmed by spiking the unknown samples with authentic compounds.

Calculation of Phenolic Acid Contents. Phenolic acid contents of the individual cowpea flours were calculated using the equation

$$\text{PA (mg/100 g)} = \frac{(aA + b)V_c}{V_s V_t W} \times 100$$

where PA = the phenolic acid content, a and b = slope and y-intercept of the standard curves for individual phenolic acids, respectively, A = peak area, V_c = injection volume for calibration (μL), V_s = injection volume for sample (μL), V_t = volume of solvent added to sample (1 mL), and W = weight of the flour (g).

Total Phenolic Contents. Total phenolic contents of the cowpea flours were determined using Folin−Ciocalteu's reagent according to the method of Swain and Hillis (*17*) and Schanderl (*18*). Fifty milligrams of cowpea flour was extracted with 10 mL of 80% ethanol in capped test tubes at 95 °C for 20 min with occasional shaking. An aliquot of the extracted solution (0.5 mL) was diluted to 7 mL with deionized water and vortexed for 5 s. Then, 0.5 mL of Folin−Ciocalteu's reagent was added. The mixture was vortexed for another 5 s and allowed to stand for 3 min. To the mixture was then added 1.0 mL of saturated sodium carbonate, and the mixture was made up to 10 mL with water and vortexed for another 5 s. After 1 h, absorbance was determined at 725 nm. Results were expressed as protocatechuic acid equivalents. Protocatechuic acid was used to calibrate the concentration as a function of absorbance.

Statistical Analysis. All values are reported as means of three determinations. Analysis of variance (ANOVA) was conducted using the Statistical Analysis System (SAS 8.1, SAS Institute Inc., Cary, NC, 2000). Duncan's multiple-range test was performed using the SAS program for pair comparison. Significance of difference was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Phenolic Acids. The contents of phenolic acids of 17 varieties of cowpeas determined by HPLC and the total phenolic contents determined using Folin−Ciocalteu's reagent are given in **Table 1**. Overall, phenolic acid contents ranged from trace to 6.2 mg/100 g of flour. Seven phenolic acids have been identified as protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid. The amount of protocatechuic acid ranged from trace in cowpea variety AR 91-245 to 3.6 mg/100 g of flour in variety Louisiana Purplehull. The amount of *p*-hydroxybenzoic acid ranged from trace in varieties AR 91-245 and AR 91-333 to 3.5 mg/100 g of flour in variety Louisiana Purplehull. Caffeic acid was lower than 1.0 mg/100 g of flour in all 17 cowpea flours. *p*-Coumaric acid and ferulic acid were 4.2 and 6.2 mg/100 g of flour, respectively, in variety Louisiana Purplehull, the highest compared to other cowpea varieties. 2,4-Dimethoxybenzoic acid was also found to be highest in variety Louisiana Purplehull, with a content of 2.5 mg/100 g of flour. Cinnamic acid was found to be highest in Texas Pinkeye, with a content

Table 1. Phenolic Acid Contents of 17 Varieties of Cowpeas before Hydrolysis (Milligrams per 100 g of Flour)[a]

order	variety name	PCA	HBA	CA	CMA	FA	DMBA	CNA	total phenolics[b]
1	Ms Silver	0.8	0.4	0.3	1.0	1.3	tr	tr	143.6 ± 20.6 de
2	AR 91-135	0.7	0.7	tr	0.4	0.7	0.3	tr	78.4 ± 17.3 ghij
3	AR 91-245	tr	tr	0.6	tr	tr	tr	tr	34.6 ± 4.3 k
4	AR 95-105	1.0	1.5	0.3	1.2	1.6	tr	tr	42.7 ± 13.9 jk
5	AR 95-104	0.2	2.8	0.6	0.9	1.2	tr	tr	326.2 ± 28.6 bc
6	AR 92-552	1.3	2.2	0.4	1.3	2.5	tr	tr	68.4 ± 6.6 hijk
7	CT Pinkeye	2.5	3.1	0.6	0.9	2.4	1.6	0.6	38.1 ± 7.3 k
8	Early Scarlet	2.0	2.3	0.5	2.8	2.4	1.9	tr	109.1 ± 18.1 efg
9	Arkansas Blackeye	tr	1.4	tr	0.3	0.6	0.3	tr	306.8 ± 26.3 c
10	AR 91-333	1.0	tr	0.4	0.8	1.3	0.4	tr	61.8 ± 5.1 ijk
11	Excel	0.6	2.7	0.4	0.7	1.1	2.1	tr	103.5 ± 19.6 fgh
12	AR 91-285	0.4	2.2	0.4	0.9	1.8	0.4	0.3	151.9 ± 16.6 d
13	AR 92-574	2.8	3.1	1.0	3.4	2.5	1.1	0.3	92.8 ± 6.1 fghi
14	Early Acre	0.4	3.0	0.6	0.9	1.8	0.3	tr	59.9 ± 5.2 ijk
15	Texas Pinkeye	2.9	2.2	0.1	2.4	2.1	1.3	1.2	131.2 ± 11.2 def
16	Black Crowder	1.0	0.6	0.6	1.1	tr	tr	tr	376.6 ± 28.9 a
17	Louisiana Purplehull	3.6	3.5	0.8	4.2	6.2	2.5	1.0	347.7 ± 16.9 ab

[a] All values are dry basis. Values are means of three determinations. PCA, protocatechuic acid; HBA, *p*-hydroxybenzoic acid; CA, caffeic acid; CMA, *p*-coumaric acid; FA, ferulic acid; DMBA, 2,4-dimethoxybenzoic acid; CNA, cinnamic acid; tr, trace (<0.3 mg/100 g). [b] Column values with the same letters were not significantly different ($p > 0.05$).

Table 2. Phenolic Acid Contents (Free and Hydrolyzed) of 17 Varieties of Cowpeas after Hydrolysis (Milligrams per 100 g of Flour)[a]

order	variety name	PCA[b]	HBA	CA	CMA	FA	DMBA	CNA
1	Ms Silver	9.3 ± 1.3 e	0.4	0.7	1.3	2.2	0.6	0.3
2	AR 91-135	11.0 ± 0.1 kl	tr	0.4	1.0	0.4	tr	tr
3	AR 91-245	30.0 ± 2.2 h	tr	0.5	1.0	0.5	tr	tr
4	AR 95-105	16.3 ± 0.2 j	0.8	1.2	2.1	6.4	0.4	0.4
5	AR 95-104	35.9 ± 2.3 g	2.3	2.4	3.4	5.1	0.3	0.6
6	AR 92-552	38.4 ± 1.8 g	1.7	1.8	2.5	4.2	0.6	0.9
7	CT Pinkeye	13.6 ± 0.4 jk	2.0	0.5	3.4	6.9	1.1	tr
8	Early Scarlet	54.4 ± 2.5e	0.3	0.5	1.5	0.9	0.3	0.6
9	Arkansas Blackeye	57.6 ± 3.1 de	1.4	0.9	2.7	3.2	0.9	0.9
10	AR 91-333	23.5 ± 3.3 i	1.3	0.9	1.6	1.8	0.19	tr
11	Excel	30.3 ± 2.6 h	2.2	2.2	2.0	8.8	0.9	0.6
12	AR 91-285	82.9 ± 2.3 b	3.1	2.4	3.4	2.7	0.4	0.4
13	AR 92-574	65.2 ± 0.7 c	2.0	1.1	1.8	7.0	tr	tr
14	Early Acre	61.4 ± 2.2 cd	2.4	1.0	1.8	8.3	0.6	0.4
15	Texas Pinkeye	24.5 ± 0.2 l	1.6	0.4	2.1	3.7	tr	tr
16	Black Crowder	43.5 ± 1.5 f	0.6	0.9	2.5	4.7	tr	tr
17	Louisiana Purplehull	92.7 ± 0.8 a	3.9	0.57	5.6	12.4	1.6	0.8

[a] All values are dry basis. Values are means of three determinations. PCA, protocatechuic acid; HBA, *p*-hydroxybenzoic acid; CA, caffeic acid; CMA, *p*-coumaric acid; FA, ferulic acid; DMBA, 2,4-dimethoxybenzoic acid; CNA, cinnamic acid; tr, trace (<0.3 mg/100 g). [b] Column values with the same letters were not significantly different ($p > 0.05$).

of 1.2 mg/100 g of flour. In comparison, gentisic, syringic, *o*-coumaric, salicylic, caffeic, *p*-coumaric, and ferulic acid were previously reported to be present in soybeans (*19*).

The total phenolic acid contents varied largely among different varieties. Variety Black Crowder had the highest total phenolic content (376.6 mg/100 g), followed by varieties Louisiana Purplehull and AR 95-104 (347.7 and 326.2 mg/100 g of flour, respectively). Lower total phenolic contents were found for varieties AR 91-245 and CT Pinkeye (25.8 and 35.9 mg/100 g of flour, respectively). In comparison, soybean has ~23.4 mg/100 g of flour (*20*).

Phenolics after Hydrolysis. Hydrolysis converted phenolic esters to simple phenolic acids. Therefore, HPLC determination of the hydrolyzed phenolic acid reflected the sum of free phenolic acid and phenolic acids released after hydrolysis from esters. The contents of hydrolyzed phenolic acids are given in **Table 2**. All phenolic acids identified before hydrolysis were also observed after hydrolysis. However, only protocatechuic acid had a remarkable increase in the amount after hydrolysis. The amount of protocatechuic acid ranged from 9.3 mg/100 g in variety Ms Silver to 92.7 mg/100 g of flour in variety Louisiana Purplehull. Protocatechuic acid was found to be the major phenolic acid in the methanol extract after hydrolysis in all varieties. Cowpea variety Louisiana Purplehull contained the highest amount of protocatechuic acid (92.7 mg/100 g of flour), followed by AR 91-285 and AR 92-574, which had protocatechuic acid contents of 82.9 and 65.2 mg/100 g, respectively. A comparison of the content of protocatechuic acid before and after hydrolysis (**Tables 1** and **2**) indicated that protocatechuic acid was present mainly in esterified forms. The esterified protocatechuic acids were converted to free protocatechuic acid after hydrolysis.

Although there were changes in amounts for other phenolic acids after hydrolysis, these changes were much smaller than those observed for protocatechuic acid. The amounts of *p*-hydroxybenzoic acid ranged from trace in varieties AR 91-135 and AR 91-245 to 3.9 in variety Louisiana Purplehull. Caffeic acid ranged from 0.4 mg/100 g in variety AR 91-135 to 2.4 mg/100 g in AR 95-104 and AR 91-285. *p*-Coumaric acid ranged from 1.0 mg/100 g in varieties AR 91-135 and AR 91-245 to 5.6 mg/100 g in variety Louisiana Purplehull. Ferulic acid had a relatively large increase after hydrolysis, ranging from

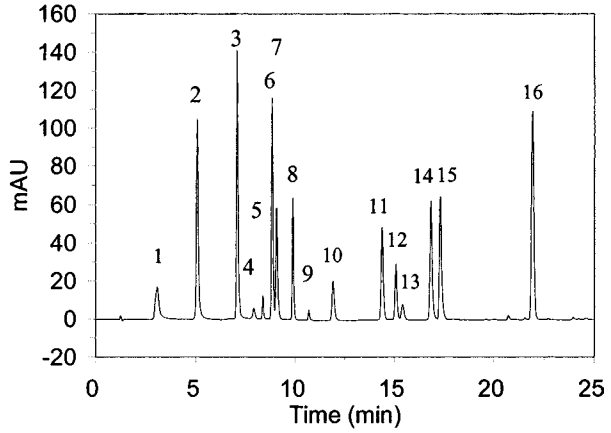

Figure 1. HPLC chromatogram of standard phenolic acids. Peaks: 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, gentisic acid; 5, (±)-catechin; 6, vallinic acid; 7, caffeic acid; 8, syringic acid; 9, (–)-epicatchin; 10, *p*-coumaric acid; 11, ferulic acid; 12, sinapic acid; 13, benzoic acid; 14, 2,4-dimethoxybenzoic acid; 15, *o*-coumaric acid; 16, cinnamic acid. Detector was set at 254 nm.

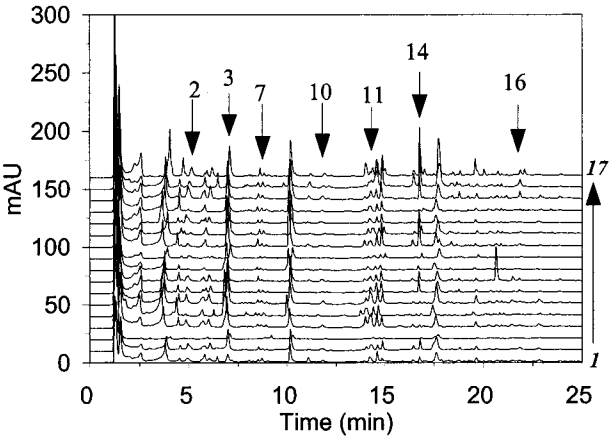

Figure 2. HPLC chromatograms of free phenolic acids from 17 varieties (1–17) of cowpeas. Phenolic compounds correspond to peaks 2, 3, 7, 10, 11, 14, and 16 as in **Figure 1**. Labels are as in **Figure 1**. Other peaks were not identified. Variety orders 1–17 and names are as in **Table 1**. Detector was set at 254 nm.

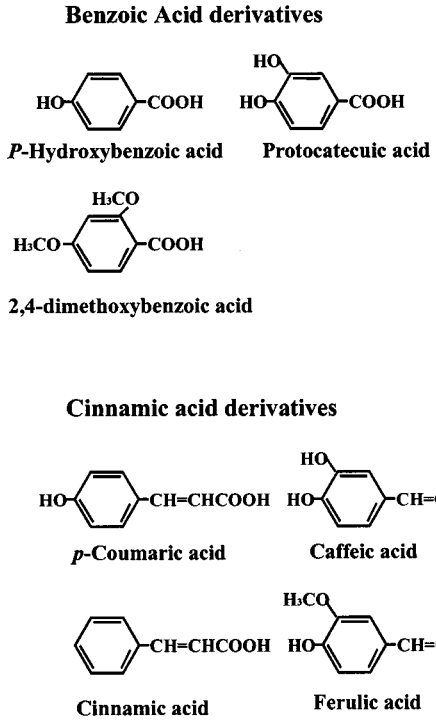

Figure 3. Structures of the phenolic compounds identified in **Figure 2**.

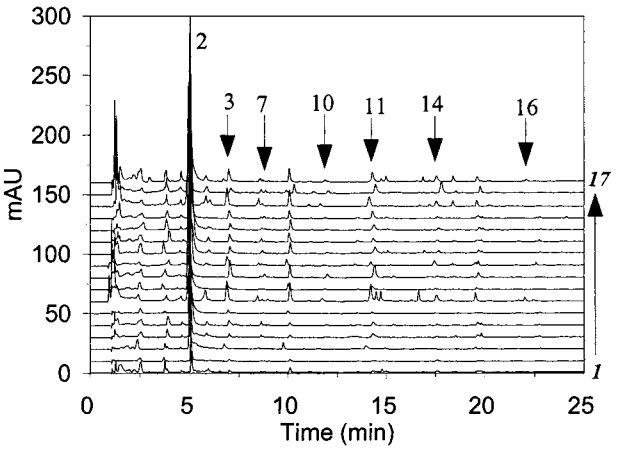

Figure 4. HPLC chromatograms of hydrolyzed phenolic acids from 17 varieties (1–17) of cowpeas. Phenolic compounds correspond to peaks 2, 3, 7, 10, 11, 14, and 16 as in **Figure 1**. Labels are as in **Figure 1**. Other peaks were not identified. Variety orders 1–17 and names are as in **Table 1**. Detector was set at 254 nm.

0.4 mg/100 g in variety AR 91-135 to 12.4 mg/100 g in variety Louisiana Purplehull, compared to 6.2 mg/100 g of flour before hydrolysis. 2,4-Dimethoxybenzoic acid and cinnamic acid were present in small quantities, mostly <1.6 mg/100 g of flour. Unlike protocatechuic acid, which was present in high amount in cowpea flour, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid were present at low quantity even in esterified forms.

HPLC Chromatograms of Methanol Extracts from 17 Cowpea Flours. The HPLC chromatogram of 16 standard phenolic acids is given in **Figure 1**. Standard phenolic acids were chosen from those commonly reported in plant species. HPLC chromatograms of free phenolic acids in methanol extracts before hydrolysis are given in **Figure 2**. Seven phenolic acids were identified as protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, 2,4-dimethoxybenzoic acid, and cinnamic acid. These compounds belong to the groups of benzoic acid derivatives and cinnamic acid derivatives. The structures of these phenolic acids are given in **Figure 3**. Other peaks were not identified. HPLC chromatograms of phenolic acids after hydrolysis from methanol extracts are given in **Figure 4**. Because of hydrolysis, these phenolic acids should include free phenolic acids and phenolic acids released after hydrolysis. A large peak representing protocatechuic acid was evident in all 17 varieties of cowpeas, representing both free protocatechuic acid and that released after hydrolysis. Compared with the chromatograms before hydrolysis, showing only the free protocatechuic acid, there was a large increase in the content of protocatechuic acid. This indicated that protocatechuic acid was the predominant phenolic acid in cowpeas, mainly present in esterified forms. Structures of the esterified protocatechuic acids need further investigations.

Chromatogram patterns of phenolic extracts have been used as a means of variety identification for canola and other crops (*21*). The chromatogram of the intact phenolic constituents of cowpea flour showed a large difference in the number of peaks and peak distribution at retention times. Peaks that have been identified correspond to compounds in **Tables 1** and **2**. On the other hand, chromatograms of hydrolyzed phenolic acids showed

a rather similar pattern for all 17 cowpea varieties with protocatechuic acid as the major peak. This indicates that chromatograms before hydrolysis better represent the identities of the individual varieties.

Conclusions. Phenolic constituents of 17 cowpea varieties were found to be dominated by protocatechuic acid in esterified forms, which upon hydrolysis released the simple protocatechuic acid, ranging from 9.3 to 92.7 mg/100 g of flour. Six other phenolic acids were also identified to be present in small quantities. Total phenolic contents ranged from 34.6 to 376.6 mg/100 g of flour. The number of peaks and the diversified peak distribution on chromatograms of 17 cowpea phenolics decreased and the chromatograms simplified with a predominant peak for protocatechuic acid after hydrolysis, suggesting that chromatograms of intact phenolics better describe the identities of cowpea varieties. The levels of protocatechuic acid in cowpeas are much higher compared to reported data for other legumes (*13*). The predominant amount of protocatechuic acid present in hydrolyzed cowpea phenolics suggests the major role the compound may play in the nutraceutical and functional properties of cowpeas in the diets.

LITERATURE CITED

(1) Phillips, R.; McWatters, K. Contribution of cowpeas to nutrition and health. *Food Technol.* **1991**, *45* (9), 127−130.

(2) Uzogara, S.; Ofuya, Z. Processing and utilization of cowpeas in developing countries: a review. *J. Food Process. Preserv.* **1992**, *16* (2), 105−147.

(3) Mau, J. L.; Chao, G. R.; Wu, K. T. Antioxidant properties of methanolic extracts from several ear mushrooms. *J. Agric. Food Chem.* **2001**, *49*, 5461−5467.

(4) Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J. P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem*. **1999**, *47*, 3954−3962.

(5) Shahidi, F.; Naczk, M. *Food Phenolics: Sources, Chemistry, Effects and Applications*; Technomic Publishing: Lancaster, PA, 1995; pp 1−20.

(6) Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.

(7) Kalt, W. Health functional phytochemicals of fruit. *Hortic. Rev.* **2001**, *27*, 269−315.

(8) Leo, F.; Massari, S. Phenolic component and antioxidant activity evaluation in red wines produced either through traditional fermentation on carbonic maceration. *J. Commodity Sci.* **2001**, *40* (4), 189−204.

(9) Bouchereau, A.; Hamelin, J.; Renard, M.; Larher, F. Structural changes in sinapic acid conjugates during seedling development of rape. *Plant Physiol. Biochem*. **1992**, *30*, 467−475.

(10) Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified and insoluble phenolic acids. II. Composition of phenolic acids in rapeseed flour and hulls. *J. Agric. Food Chem.* **1982**, *30*, 334−336.

(11) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165−5170.

(12) Pandjaitan, N.; Hettiarachchy, N.; Ju, Z. Y.; Crandall, P.; Sneller, C.; Dombek, D. Evaluation of genistin and genistein contents in soybean varieties and soy protein concentrate prepared with 3 basic methods. *J. Food Sci.* **2000**, *65* (3), 399−402.

(13) Shahidi, F.; Naczk, M. An overview of phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *J. Am. Oil chem. Soc.* **1992**, *69*, 917−924.

(14) Nice, D. J.; Robinson, D. S.; Holden, M. A. Characterization of a heat-stable antioxidant co-purified with the superoxide dismutase activity from dried peas. *Food Chem.* **1995**, *52* (4), 393−397.

(15) Cai, R.; Arntfield, S. D. A rapid high performance liquid chromatographic method for the determination of sinapine and sinapic acid in canola seed and meal. *J. Am. Oil Chem. Soc*. **2001**, *78*, 903−910.

(16) Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified and insoluble phenolic acids. I. Extraction and purification procedure. *J. Agric. Food Chem.* **1982**, *30*, 330−334.

(17) Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus domestica* I. The qualitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63−68.

(18) Schanderl, S. H. Tannins and related phenolics. In *Methods in Food Analysis, Physical Chemical and Instrumental Methods of Analysis*; Joslyn, M. A., Ed.; Academic Press: New York, 1970; pp 701−725.

(19) How, J. S. L.; Morr, C. V. Removal of phenolic compounds from soy protein extracts using activated carbon. *J. Food Sci.* **1982**, *47*, 933−940.

(20) Sosulski, W. F.; Dabrowski, K. J. Composition of free and hydrolyzable phenolic acids in the flours and hulls of 10 legume species. *J. Agric. Food Chem.* **1984**, *32*, 131−133.

(21) Mailer, R. J.; Daun, J.; Scorth, R. Cultivar identification in *Brassica napus* L. by reverse-phase high-performance liquid chromatography of ethanol extracts. *J. Am. Oil Chem. Soc.* **1993**, *70*, 863−866.

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