Composition of Free and Hydrolyzable Phenolic Acids in the Flours and Hulls of Ten Legume Species

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The phenolic constituents in the defatted flours and hulls of 10 legume species were fractionated into free acid, soluble ester, and residue components, followed by alkaline hydrolysis and quantitation by capillary gas–liquid chromatography. The flours contained only soluble esters, and hydrolyses revealed the presence of *trans*-ferulic, *trans*-*p*-coumaric, and syringic acids in nearly all species. Mung bean, field pea, lentil, fababean, and pigeon pea contained only 2–3 mg of phenolic acids/100 g of flour, but higher levels were obtained in navy bean, lupine, lima bean, chickpea, and, especially, cowpea. The hulls contained *p*-hydroxybenzoic, protocatechuic, syringic, gallic, *trans*-*p*-coumaric, and *trans*-ferulic acids in the soluble ester fraction and, to a lesser extent, in the insoluble residue. Dehulling would reduce the phenolic composition of the flour substantially for pigeon pea, fababean, mung bean, and lentil but would have little effect on the phenolic composition of field pea, navy bean, lima bean, or chickpea products.

Except for oilseed species, there have been few published reports on the phenolic constituents in legume seeds. Immature legume seeds have been analyzed for phenolic acids (Reschke and Herrmann, 1982; Schmidtlein and Herrmann, 1975), and there have been published reports on tannin contents (Khan et al., 1979; Ma and Bliss, 1978; Price et al., 1980) and a recent review on polyphenols (Salunkhe et al., 1982). The objective of the present investigation was to determine the nature of soluble phenolic acids, esters, and glycosides in mature grain legumes and their quantitiative distribution, including the proportion in hulls.

The analyses were conducted by the rapid procedure adopted by Dabrowski and Sosulski (1984) using capillary GLC. Although oilseed legumes were not included in this investigation, the cotyledons were defatted because chickpea and lupine contain significant levels of triglycerides that interfere with the analysis (Krygier et al., 1982a).

The colors of legume flours and those that develop during aqueous food processing would be due to a wide range of flour constituents including flavanoid, quinone, and terpenoid compounds as well as simple phenolic acids and esters. An estimate of the contributions of the latter compounds to flour color was made with the Hunterlab color difference meter and by spectrophotometry of the methanol–acetone–water extracts.

EXPERIMENTAL SECTION

Materials. The samples analyzed for phenolic acid composition included mung bean (*Vigna radiata*), smooth field pea (*Pisum sativum*), yellow lentil (*Lens culinaris*), small fababean (*Vicia faba minor*), pigeon pea (*Cajanus cajan*), navy bean (*Phaseolus vulgaris*), white lupine (*Lupinus albus*), baby lima bean (*Phaseolus lunatus*), chickpea (*Cicer arietinum*), and cowpea (*Vigna unguiculata*).

The seed samples were composites of lots obtained from several commercial sources. Seeds were cracked in a Unimec hammer mill and the hulls removed from cotyledons by aspiration and hand separation. The cotyledons were ground, defatted by refluxing with hexane in a Soxhlet apparatus, reground, and defatted a second time to produce the legume flours.

The hulls obtained in the above process were discarded because of contamination with cotyledonary material. Pure hulls were obtained, and the hull contents were determined by the Deshpande et al. (1982) procedure. Seeds were soaked in distilled water (1:0.5 w/v) for 2 h at 6 °C. Seed coats were removed manually and dried in an air oven at 45 °C for 24 h. The product was weighed and ground for phenolic acid analysis.

Methods. The procedures for extraction, hydrolysis, and quantitation of phenolic compounds by capillary GLC have been described previously (Dabrowski and Sosulski, 1984). The contents of phenolic acids are reported as the mean of duplicate determinations in milligrams per 100 g of flour, fat free, dry basis. Quantities of less than 0.3 mg/100 g of product are reported in the tables as traces (tr). Contents of phenolic acids in the whole seed were calculated from the proportions and compositions of the seed parts but lipid contents were not taken into account.

Color Evaluation. One gram of each flour was extracted 3 times with 25 mL of acetone–methanol–water (7:7:6) in the Polytron and centrifuged, and the combined extracts were made up to 100.0 mL with the above solvent system. The colors of the dry legume flours were evaluated by using the Hunterlab D25D2M digital color difference meter equipped with the M optical head. A white tile (No. C2-5470: $L = 94.7$; $a = -0.9$; $b = 0.5$) was used to standardize the instrument. The color intensities of the above extracts, before concentration, were also determined directly and after alkalization to pH 9.5 with 0.02 N NaOH by spectrophotometric measurements. The wavelength of maximum absorption was found for each extract at both pH values, and then the absorbance was measured by using the Cary 118C spectrophotometer (Varian).

TLC Separation. The above extract of lentil was concentrated about 10 times at room temperature under nitrogen and then loaded on TLC plates coated with silica gel IB2-F containing fluorescent indicator UV-254 (J. T. Baker Chemical Co.) The solvent system of butanol–acetic acid–water (40:7:32) was used for separation as in Dabrowski and Sosulski (1984).

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Table I. Phenolic Acids Liberated from Soluble Esters in Legume Flours by Alkaline Hydrolysis (mg/100 g of Flour)

phenolic acid	mung bean	field pea	lentil	fababean	pigeon pea	navy bean	lupine	lima bean	chickpea	cowpea
p-hydroxybenzoic	tr			tr		tr	1.2			
syringic	tr	1.2		tr	1.8	tr	1.1	tr	5.2	tr
trans-*p*-coumaric	1.8		1.1	1.6	tr	1.3	0.9	4.9		7.4
trans-ferulic	tr	1.4	1.9	1.5	tr	4.2	3.9	3.9	4.3	8.9
total	1.8	2.6	3.0	3.1	1.8	5.5	7.1	8.8	9.5	16.3

Table II. Phenolic Acids Liberated from Soluble Esters and Extracted Residues of Legume Hulls by Alkaline Hydrolysis (mg/100 g of Hulls)

legume species:	mung bean	field pea	lentil	fababean	pigeon pea	navy bean	lupine	lima bean	chickpea	cowpea
% hull:	7.9	6.3	6.7	14.5	13.6	7.2	18.9	6.4	4.3	4.2
Phenolic Acids in Soluble Esters										
p-hydroxybenzoic	1.0	0.4	1.0	0.6	0.7		1.3	0.3	0.8	0.5
protocatechuic	0.9	tr	1.0	3.6	5.6			0.3		0.8
syringic	2.0		3.0		8.4					1.3
gallic	1.2		1.7	9.2	2.6					1.9
trans-*p*-coumaric	1.5	tr	1.1	0.5		0.4	tr	0.7	0.3	3.2
trans-ferulic	0.7	0.7	1.5	1.0		1.0	2.0	0.8		2.2
Phenolic Acids in Residues										
p-hydroxybenzoic	0.5			tr	tr		1.1	tr		
protocatechuic	0.3		0.3	0.3	1.5			tr		0.7
syringic			1.4		1.9					
gallic	0.5		0.3	0.3	1.0			tr		1.5
trans-*p*-coumaric				tr				tr		
trans-ferulic				tr				1.5		
total	8.6	1.1	11.3	15.5	21.7	1.4	4.4	3.6	1.1	12.1
% of whole seed phenolics	29.4	2.8	21.3	46.0	65.4	2.0	12.6	2.7	0.1	3.2

Table III. Measurement of Color Parameters of Legume Flours on Hunterlab Color Difference Meter and Absorbance of Legume Flour Extracts

	color parameters[a]			λ_{max} (pH 7.1),	absorbance at λ_{max}	
	L	*a*	*b*	nm	pH 7.1	pH 9.5
mung bean	87.9	−1.4	10.5	323.0	0.165	0.640
field pea	90.0	−0.0	10.6	323.0	0.076	1.080
lentil	90.1	−2.2	13.7	323.0, 347.0[b]	0.223, 0.199	1.420
fababean	89.1	−1.1	11.0	322.0	0.110	1.260
pigeon pea	88.9	−2.9	16.4	323.0	0.189	1.160
navy bean	90.9	−0.3	6.3	318.5	0.225	1.460
lupine	90.7	−2.3	15.6	327.0	0.777	1.790
lima bean	91.7	−0.8	9.5	319.5	0.319	1.220
chickpea	90.9	−0.4	8.5	318.5	0.107	0.940
cowpea	89.3	−0.4	9.2	318.5	0.431	1.320

[a] L = 100 (white), L = 0 (black); $+a$ = red, $-a$ = green; $+b$ = yellow, $-b$ = blue. [b] Second absorption peak.

RESULTS AND DISCUSSION

Analyses of the tetrahydrofuran extracts of the legume flours and hulls indicated the complete absence of any phenolic acids in the free state. This would be expected, especially in the case of hydroxycinnamic acids, due to their reactive groups and susceptibility to oxidation (Pierpoint, 1969).

Methanol–acetone–water extracts from legume flours yielded, upon alkaline hydrolysis, only four phenolic acids in relatively low concentrations. *trans*-Ferulic, *trans*-*p*-coumaric, and syringic acids were found in the soluble ester fraction of most legume species, while *p*-hydroxybenzoic acid was a significant component in lupine (Table I). Mung bean and pigeon pea contained only 2 mg/100 g of flour of *trans*-*p*-coumaric and syringic acids, respectively. *trans*-Ferulic acid was a major component of the soluble ester fraction in the other eight legume flours. Field pea, lentil, and fababean contained low concentrations of *trans*-ferulic as well as total phenolic acids. Navy bean, lupine, lima bean, chickpea, and cowpea contained 4–9 mg/100 g of flour of *trans*-ferulic and progressively greater quantities of total soluble phenolic acids. Schmidtlein and Herrmann (1975) analyzed immature samples of field pea, common bean, and fababean and found a greater number of hydrolyzed phenolic acids, but the concentrations of individual constituents were of the same order (<0.1–5.2 mg/100 g of seed) as in the present study.

The residues of the flour remaining after extraction of soluble esters failed to yield any phenolic acids on hydrolysis with alkali. Similar results were obtained with rapeseed (Krygier et al., 1982b) and potato (Sosulski et al., 1982), but in the latter study, cereals exhibited 70–300 ppm of residue phenolic acids.

Lupine, fababean, and pigeon pea seeds contained high proportions of hulls (18.9–13.6%), especially in comparison with those of chickpea and cowpea (4.3–4.2%) (Table II). In previous studies (Fan and Sosulski, 1974; Sosulski and Youngs, 1979) in our laboratory, similar yields of hulls from these legumes species were obtained, although the values were not reported.

The phenolic acids released on hydrolysis of the soluble ester fraction from hulls included *p*-hydroxybenzoic, *trans*-*p*-coumaric, and *trans*-ferulic acids in most legume species (Table II). Protocatechuic, syringic, and gallic acids were also present in several legumes. Of interest were the comparatively high levels of gallic acid in fababean and syringic acid in pigeon pea hulls. Total phenolic acids in the soluble fractions varied from 1.1 to 17.3 mg/100 g of hull, which was very similar to the range exhibited by the flours.

Hydrolysis of the insoluble hull residues yielded the same six phenolic acids as the soluble fraction from hulls, but several legume species (field pea, navy bean, chickpea)

were devoid of these residue constituents (Table II). Protocatechuic, syringic, and gallic acids were detected in several legumes, essentially the same species that exhibited these acids in the soluble ester fraction. The presence of gallic acid in the hydrolyzed fractions of hulls was expected, due to the presence of hydrolyzable tannins in the hulls.

Pigeon pea and fababean contained the highest levels of total (esterified plus residue) phenolic acids in the hulls, followed by cowpea and lentil (Table II). In the case of pigeon pea and fababean, the hulls were estimated to contain 65 and 46%, respectively, of the total seed phenolics. In most other legumes, the hulls contributed only a minor fraction of total seed phenolic constituents. Thus, dehulling of legumes would be effective in reducing the phenolic acid contents only in the case of pigeon pea, fababean, and, possibly, lentil and mung bean.

The legume flours showed little variation in brightness with the L values being in the range of 88–92 (Table III). However, distinct yellow-green colors were exhibited by pigeon pea, lupine, and lentil. In contrast, the flour of navy bean was essentially white. Peak absorbance values for the methanol–acetone–water extracts of the legume flours were about 323 nm for mung bean, field pea, lentil, fababean, and pigeon pea while values of 319 nm were obtained for navy bean, lima bean, chickpea, and cowpea. Lupine gave a slightly higher λ_{max} of 327 nm and lentil gave a second peak at 347 nm.

Absorbance at λ_{max} of the legume flour extracts was quite low for field pea, fababean, and chickpea; intermediate values were obtained for mung bean, lentil, pigeon pea, and navy bean (Table III). Lima bean, cowpea, and lupine gave high absorbance values of 0.319, 0.431, and 0.777, respectively. These absorbance values gave nonsignificant correlation coefficients of +0.36 with the b values in Table III and +0.44 with total phenolic acids in the flours in Table I. It was evident that phenolic compounds were not major factors influencing the natural colors of the flours or of aqueous extracts at neutral pH. Under alkaline conditions (pH 9.5), all extracts exhibited significant increases in color intensity. Presumably this was due to oxidative changes in the soluble phenolic esters as well as flavanoid and quinone compounds and their interactions under alkaline conditions. When the alkaline extracts are in contact with the protein components of the legume or oilseed meals, brown and green protein isolates are often obtained (Sosulski and Bakal, 1969).

The extract from lentil was fractionated by TLC to demonstrate the presence of several colored constituents. Only three spots gave blue fluorescence under long-wave UV light, suggesting a phenolic nature. It appeared that a broader study than the present investigation of phenolic compounds would be required for identification of all major color-forming compounds in these legume flours.

Registry No. *trans*-Ferulic acid, 537-98-4; *trans*-*p*-coumaric acid, 501-98-4; syringic acid, 530-57-4; *p*-hydroxybenzoic acid, 99-96-7; protocatechuic acid, 99-50-3; gallic acid, 149-91-7.

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Quantitative Structure–Activity Relationships in the Inhibition of Photosystem II in Chloroplasts by Phenylureas

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Quantitative structure–activity relationships (QSAR) have been formulated for the inhibition of the Hill reaction in spinach chloroplasts by 1,1-dimethyl-3-(X-phenyl)ureas and 1-methyl-1-methoxy-3-(X-phenyl)ureas. These mathematical models are compared with other such QSAR for Hill reaction inhibitors. From these results it is clear that the molecular requirements for inhibitors are an active polarizable sp^2 nitrogen atom attached to a large lipophilic moiety. Although the lipophilic area in chloroplasts is shown to be very large and surprisingly free of steric effects, one significant steric effect caused by branching of substituents near the phenyl ring has been clearly estabished. Evidence is marshaled to show that the optimum lipophilic effect occurs with inhibitors having a log P of 5–6.

A natural route open to organic and physical organic chemists for the study of the interaction of organic compounds with macromolecules has grown out of the extrathermodynamic approach to rate and equilibrium studies pioneered by L. P. Hammett. The great success of Hammett's σ constants inspired Taft to develop the steric parameter E_s, which then was followed by the formulation of a hydrophobic constant (π or log P) for substituents (Hansch and Leo, 1979). With these parameters the

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