Composition of Free and Hydrolyzable Phenolic Acids in Defatted Flours of Ten Oilseeds

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The phenolic compounds in 10 oilseed flours were separated into free, esterified, and bound residue phenolic acids, and the liberated phenolic acids were quantitated by capillary gas–liquid chromatography. Phenolic acids were not present in the free form; soluble esters yielded the major proportion of phenolic compounds, but most flour residues also contained small, hydrolyzable quantities of the same phenolic compounds. *p*-Hydroxybenzoic, *trans*-*p*-coumaric, *trans*-ferulic, and *trans*-caffeic acids in the ester form occurred in all flours, with syringic acid also being a common constituent. Coconut, cottonseed, and sesame contained low concentrations of total phenolic acid esters. Soybean, flax, safflower, and peanut were intermediate in total phenolics, the principal acids obtained on hydrolysis of esters being syringic, *trans*-ferulic, *trans*-sinapic, and *trans*-*p*-coumaric acids, respectively. *p*-Hydroxybenzoic acid occurred in high concentrations in mustard, *trans*-sinapic acid in rapeseed and mustard, and chlorogenic acid in sunflower flour.

Oilseed flours may contain considerable amounts of certain phenolic constituents. Sinapine, the major phenolic component of rapeseed (Clandinin, 1961; Austin and Wolff, 1968), has been found to vary from 1.2 to 2.5% of defatted meal depending on location and cultivar (Mueller et al., 1978). Similarly, a wide range of sinapine values (0.4–1.8% of dry matter) was reported for several cultivars of Cruciferae crops including the *Brassica* species (Kerber and Buchloh, 1980). Qualitiative data and relative concentrations of other phenolic acids in rapeseed have been reported (Durkee and Thivierge, 1975; Fenton et al., 1980; Kozlowska et al., 1975, 1983; Krygier et al., 1982; Sosulski et al., 1980).

Chlorogenic acid has been reported as the major phenolic compound in sunflower kernels with much smaller concentrations of caffeic acid being present (Mikolajczak et al., 1970; Milic et al., 1968). Eight phenolic compounds in aqueous (unhydrolyzed) extracts of sunflower flours were tentatively identified and quantified (Sabir et al., 1974). Recently, Leung et al. (1981) evaluated acid and base hydrolysates of phenolic compounds in seven cultivars of sunflower and reported the presence of caffeic acid as the predominant phenolic acid, along with *p*-hydroxybenzoic, *p*-coumaric, cinnamic, *m*-hydroxybenzoic, vanillic, and syringic acids. Chlorogenic acid in sunflower flours is of particular importance because of the dark green and brown colors that develop under alkaline conditions or during aqueous processing. Color problems are the major factor prohibiting the widespread use of sunflower flours in food products.

Ethanolic extracts of soybean contained seven phenolic acids (Arai et al., 1966; How and Morr, 1982). Maga and Lorenz (1974) found a wide distribution of free phenolic acids in soybean, peanut, and cottonseed flours with syringic, ferulic, and vanillic acids being the major components.

The objectives of the present investigation were to apply the Sosulski et al. (1980) procedure for the fractionation of phenolic constituents into free, esterified, and residual components to oilseed flours using capillary gas–liquid chromatography for quantitation of the hydrolyzed phenolic acids (Dabrowski and Sosulski, 1984). The study encompassed 10 oilseed species and was restricted to the dehulled, fat-free flours that are, or have the potential of being, primary food products.

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EXPERIMENTAL SECTION

Materials. The samples analyzed for phenolic acid composition included coconut (*Cocos nucifer* L.), glanded and glandless cottonseed (*Gossypium hirsutum* L.), sesame (*Sesamum indicum* L.), soybean (*Glycine max* L.), Virginia-type peanut (*Arachis hypogaea* L.), safflower (*Carthamus tinctorius* L.), flax (*Linum usitatissimum* L.), yellow mustard (*Brassica hirta* L.), rapeseed (*Brassica campestris* L.), and sunflower (*Helianthus annuus* L.). Seed were obtained in bulk from commerical wholesalers except that cottonseed was supplied by Dr. E. Lusas, Food Protein Research and Development Center, Texas A&M University. The seed samples were dehulled, ground, and defatted by refluxing with hexane in a Soxhlet apparatus, reground, and extracted a second time.

Methods. The procedure for phenolic compound fractionation, purification and quantitation by capillary GLC has been described previously (Dabrowski and Sosulski, 1984). Due to hydrolysis of chlorogenic acid and loss of the constituent aglycon, caffeic acid [Figure 3 and Table III in Dabrowski and Sosulski (1984)], chlorogenic acid in sunflower flour was determined spectrophotometrically by the method of Dorrell (1976). The contents of phenolic acids are expressed as the means of duplicate determinations in milligrams per 100 g of defatted flour, dry basis.

RESULTS AND DISCUSSION

Extraction of flours with tetrahydrofuran demonstrated that no free phenolic acids were present in the oilseed samples in measureable quantities. Similar results were obtained in an investigation of phenolic constituents in 10 legume flours where all phenolic compounds were bound to other constituents in soluble forms or in the residue (Sosulski and Dabrowski, 1984).

Alkaline hydrolysis of the soluble phenolic compounds in the oilseed flours released a wide range of phenolic acids (Table I). Phenolic acids which were present in all oilseed flours were *p*-hydroxybenzoic, *trans*-*p*-coumaric, *trans*-ferulic, and *trans*-caffeic acids. Syringic acid was identified in seven oilseed species while vanillic and *trans*-sinapic

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Table I. Phenolic Acids Liberated from Soluble Esters of Oilseed Flours (mg/100 g of Flour)

phenolic acid	coconut	cottonseed		sesame	soybean	flax	safflower	peanut	mustard	rapeseed	sunflower
		glanded	glandless								
p-hydroxybenzoic	1.4	1.1	1.0	trace	13.0	2.6	25.9	2.0	269.4	5.6	6.0
vanillic						trace	trace		trace	0.7	0.8
protocatechuic									trace	trace	
syringic	trace				26.4	0.4	trace		3.0	1.1	2.2
trans-*p*-coumaric	5.4	3.9	3.9	5.7	9.4	4.9	20.9	134.7	2.2	trace	5.6
trans-ferulic	1.7	4.2	5.0	2.6	14.5	33.3	24.4	14.0	8.3	15.0	5.8
trans-caffeic	1.0	0.5	1.8	8.8	5.2	3.6	26.3	1.6	2.5	trace	960.9
cis-sinapic									29.7	33.0	
trans-sinapic						29.1	34.6	8.1	702.6	1110.7	
total	9.5	9.7	11.7	17.1	68.5	73.9	132.1	160.4	1017.7	1166.1	981.3

Table II. Phenolic Acids Liberated from Insoluble Residue of Oilseed Flours (mg/100 g of Flour)

phenolic acid	coconut	cottonseed		sesame	soybean	safflower	peanut	flax	mustard	rapeseed	sunflower
		glanded	glandless								
p-hydroxybenzoic	trace			trace	0.9	2.4		trace	1.2		1.4
syringic					2.2	1.2					1.4
trans-*p*-coumaric	trace	0.4	0.2	1.5		trace	11.7	1.2	trace		trace
trans-ferulic	trace	0.3	0.5	3.1	1.2	0.9	2.2	4.3	1.7		1.4
trans-caffeic				1.0	0.8	trace	1.2	1.7	1.1		18.2
total	trace	0.7	0.7	5.6	5.1	4.5	15.1	7.2	4.0	0.0	22.4

Table III. Total Phenolic Acids in Flours (mg/100 g of Flour)

phenolic acid	coconut	cottonseed		sesame	soybean	flax	safflower	peanut	mustard	rapeseed	sunflower
		glanded	glandless								
p-hydroxybenzoic	1.4	1.1	1.0	trace	13.9	2.6	28.3	2.0	270.6	5.6	7.4
vanillic						trace	trace		trace	0.7	0.8
protocatechuic									trace	trace	
syringic	trace				28.6	0.4	1.2		3.0	1.1	3.6
trans-*p*-coumaric	5.4	4.3	4.1	7.2	9.4	6.1	20.9	146.4	2.2	trace	5.6
trans-ferulic	1.7	4.5	5.5	5.7	15.7	37.6	25.3	16.2	10.0	15.0	7.2
trans-caffeic	1.0	0.5	1.8	9.8	6.0	5.3	26.3	2.8	3.6	trace	979.1
cis-sinapic									29.7	33.0	
trans-sinapic						29.1	34.6	8.1	702.6	1110.7	
chlorogenic acid[a]											(2850.0)
total	9.5	10.4	12.4	22.7	73.6	81.1	136.6	175.5	1021.7	1166.1	1003.7
fraction											
soluble ester	100.0	93.3	94.3	75.3	93.1	88.8	96.7	91.4	99.6	100.0	97.8
residue	0.0	6.7	5.7	24.7	6.9	11.2	3.3	8.6	0.4	0.0	2.2
major acid	coumaric	ferulic	ferulic	caffeic	syringic	ferulic	coumaric	coumaric	sinapic	sinapic	caffeic (chlorogenic)

[a] Determined spectrophotometrically on an 80% aqueous-ethanolic extract without hydrolysis.

acids occurred in only five species. The cis isomer of sinapic acid in the two *Brassica* species is reported to arise from the natural trans form during hydrolytic procedures, exposure to daylight, and air (Fenton et al., 1980; Sosulski et al., 1980). In the present study, isomerism in the extracts was suppressed by exclusion of light, maintenance of a nitrogen atmosphere in the flasks and rapid analyses.

The total phenolic acid contents of the soluble ester fractions of coconut, glanded and glandless cottonseed, and sesame were only 10–17 mg/100 g of flour (Table I). Each oilseed fraction contained small quantities of *p*-hydroxybenzoic, *trans*-*p*-coumaric, *trans*-ferulic, and *trans*-caffeic acids. There were no differences in phenolic acid composition between glanded and glandless cottonseed, as gossypol was not quantitated by the present techniques.

Soybean, flax, safflower, and peanut flours were next in order of magnitude, having 68–160 mg of total phenolic acids/100 g of flour (Table I). In these oilseeds the predominant phenolic acids were syringic in soybean, *trans*-ferulic in flax, *trans*-sinapic in safflower, and *trans*-*p*-coumaric in peanut flour.

The total phenolic acids released from the soluble ester fraction of mustard, rapeseed, and sunflower flours averaged 1.0 g/100 g of flour (Table I). A wide range of phenolic acids was identified in each flour but the sinapic acids predominated in mustard and rapeseed while *trans*-caffeic acid was the major component of sunflower esters. The high content of *p*-hydroxybenzoic acid in yellow mustard has been noted previously (Kozlowska et al., 1983) and likely arose from the alkaline degradation of (*p*-hydroxybenzyl)glucosinolate, kaempferol glycosides (Schulz and Herrmann, 1981), and 4-hydroxybenzolcholine (Clausen et al., 1982). The *trans*- and *cis*-sinapic acids in mustard and rapeseed arose primarily from sinapine, the choline ester of sinapic acid, and, to a lesser extent, from glucose esters of sinapic acid (Durkee and Thivierge, 1975; Fenton et al., 1980). *Brassica* species also contain methyl sinapate, an antithiamine factor, in low concentration (Bhattacharya and Chaudhuri, 1973).

Sunflower contained nearly 1% of caffeic acid which was known to be largely lost during alkaline hydrolysis (Table I). Spectrophotometric analysis of the soluble ester fraction indicated the presence of 2.85% of chlorogenic acid in the flour. Thus, the recovery of caffeic acid was about 66%, which was substantially better than in samples with low concentrations of caffeic acid (Dabrowski and Sosulski, 1984). The chromotograms also yielded about 35 mg of quinic acid/100 g of flour but the solvents employed in the extractions do not recover the sugars quantitatively. The compositions of chlorogenic acid in sunflower reported by other investigators were 1.6% of the kernel by Milic et al. (1968) and, on flour basis, 2.0% by Mikolajczak et al. (1970), 3.1% by Sosulski et al. (1973), and 1.9–2.1% by Sabir et al. (1974). Other phenolic acids in sunflower flour were *p*-hydroxybenzoic, vanillic, syringic, *trans*-*p*-coumaric, and *trans*-ferulic, most of which were also identified in sunflower flour by Leung et al. (1981).

Hydrolysis of the residues from the methanol–acetone–water (7:7:6) extractions of the flours released *p*-hydroxybenzoic acid, syringic, trans-*p*-coumaric, *trans*-ferulic, and *trans*-caffeic acids but the quantities were quite low (Table II). Rapeseed, coconut, and cottonseed were devoid or very low in bound phenolic acids. Mustard, safflower, sesame, soybean, and flax had only 4.0–7.2 mg/100 g of flour. Peanut contained somewhat more *trans*-*p*-coumaric and sunflower was high in *trans*-caffeic acid, both of which occurred in high concentration in the soluble ester fraction. The caffeic acid level of 18.2 mg/100 g of sunflower corresponded to 35.8 mg of chlorogenic acid/100 g of flour. Sosulski et al. (1973) estimated that a reduction of chlorogenic acid to 0.3% of the flour, or more specifically the protein concentrate, would provide a product with essentially white color characteristics. Therefore, it can be assumed that these extracted residues would be relatively free of color or flavor problems arising from interactions with phenolic acids.

The total phenolic acid contents of the oilseed meals are shown in Table III. The proportions of the total phenolic acids in the soluble ester fraction varied from 91.4% (peanut) to 100.0% (coconut and rapeseed) except for sesame in which 24.7% was bound to residues. *p*-Hydroxybenzoic acid constituted a high proportion of mustard phenolics. Syringic predominated in soybean and *trans*-*p*-coumaric in peanut while *trans*-ferulic occurred widely in all oilseed flours. Caffeic acid was the major acid in sunflower, primarily as a component of chlorogenic acid, and sinapic acid was the major acid in rapeseed and mustard, arising primarily from sinapine.

Registry No. *p*-Hydroxybenzoic acid, 99-96-7; vanillic acid, 121-34-6; protocatechuic acid, 99-50-3; syringic acid, 530-57-4; *trans*-*p*-coumaric acid, 501-98-4; *trans*-ferulic acid, 537-98-4; *trans*-caffeic acid, 501-16-6; *cis*-sinapic acid, 7361-90-2; *trans*-sinapic acid, 7362-37-0; chlorogenic acid, 327-97-9.

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