Phenolic Constituents in Sunflower Flour

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Eight of the ten phenolic compounds in the aqueous methanolic extracts from three varieties of sunflower were tentatively identified and quantitated by their spectrophotometric and chromatographic characteristics. Chlorogenic acid, one of its isomers, and caffeic acid constituted 70% of the total phenolic compounds in the flour of each variety. Compounds related to *p*-coumaric, isoferulic, and sinapic acids and a hydroxycinnamic acid–sugar ester were also detected by tlc and glc analyses. The sinapic acid like compound represented 15% of the total phenolic compounds in the three flours. Two minor components with long retention times were also present in the neutral fraction.

Sunflower ranks as second in importance among world sources of vegetable oils and there is considerable interest in utilizing the light-colored defatted flour for food purposes. The utilization of the high protein by-product from oilseed crushing in foods has been limited by the presence of phenolic constituents which are readily oxidized into dark green and brown compounds. A number of extraction procedures have been devised to remove the phenolic compounds from sunflower kernels and flours (Gheyasuddin *et al.*, 1970; Sosulski *et al.*, 1972) but the economic feasibility of these processes is unknown.

Chlorogenic acid has been identified as the major phenolic compound in sunflower kernels with minor constituents being caffeic acid, 3,5-dicaffeoylquinic acid, and a disubstituted cinnamic acid (Mikolajczak *et al.*, 1970; Milic *et al.*, 1968). The present study was undertaken to identify additional phenolic constituents in sunflower flour. The study was conducted on the three major biotypes of sunflower grown in North America—Commander, the large-seeded confectionery type; Majak, a high-oil Russian variety; and Valley, a Canadian hybrid. The techniques used to separate, identify, and quantitate the phenolic compounds included thin-layer chromatography (tlc), gas–liquid chromatography (glc), and spectrophotometry.

EXPERIMENTAL SECTION

Sample Preparation. The phenolic compounds in the defatted flours from Commander, Majak, and Valley sunflowers were extracted by refluxing with 80% aqueous methanol for 5 hr at a flour to solvent ratio of 1:100 (Mikolajczak *et al.*, 1970). The methanol extracts were evaporated to dryness at 40° under vacuum. The dried material was dissolved in HCl (pH 2) and the phenolic compounds were isolated by continuous extraction with ether for 19 hr (Ribereau-Gayon, 1972). The ether was evaporated under vacuum at room temperature and the residue dissolved in absolute methanol for tlc and glc analyses.

The phenolic constituents were separated into neutral and acidic fractions by dissolving an aliquot of the methanol solution in 5% sodium bicarbonate (pH 8.5) and continuously extracting with ether for 19 hr (Ribereau-Gayon, 1972). The neutral components were isolated by evaporation of the ether extract, while the aqueous phase was adjusted to pH 2 before extraction for acidic components with ether, evaporation, and redissolution in absolute methanol for glc analyses.

Tlc Procedure. The phenolic compounds in methanol were fractionated on silica gel G plates along with several authentic hydroxycinnamic acids including chlorogenic acid. Three solvent systems were employed: BzDA, benzene–dioxane–acetic acid (90:25:4); BzMA, benzene–methanol–acetic acid (90:16:8); and BAW, butanol–acetic acid–water (40:7:32). The phenolic constituents were detected by fluorescence under short- and long-ultraviolet (uv) light, the colors recorded, and the R_f values measured in duplicate by the procedure of Steck (1967). Each compound was eluted with absolute methanol for the determination of absorption maxima and the spectral shift after addition of aqueous 0.5 *N* NaOH (1 drop/ml).

Glc Technique. Aliquots of the phenolic compounds in methanol were combined with kaempferol as an internal standard (IS) before evaporation to dryness under nitrogen at 40°. Traces of water were removed with benzene (Davison and Young, 1969) and the phenolic compounds were silylated with *N,O*-bis(trimethylsilyl)acetamide (BSA) by incubation overnight at room temperature (Morita, 1972). The TMS derivatives of the phenolic compounds were chromatographed on a F&M Model 402 gas chromatograph equipped with a hydrogen flame detector and peak area integrator. The 6-ft glass column, 0.25 in. o.d., was packed with 1.5% SE-30 on 80–100 mesh Chromosorb W (HP), conditioned with silyl-8 (Pierce) at 100°. The flow rate for the carrier gas, helium, was 30 cm^3/min. The injection and detector temperatures were 300°. Four minutes after sample injection, the oven temperature was raised from 100 to 260° at 5°/min. After identification, the TMS derivatives of the phenolic compounds in sunflower flour were quantitated from the integrated peak areas and weight ratios of TMS derivatives of authentic phenolic compounds and the internal standard, kaempferol (Mason and Slover, 1971).

RESULTS AND DISCUSSION

The fluorescence colors and R_f values from tlc and absorption maxima of the most common hydroxycinnamic acids and the relative retention times (T_{RR}) on glc of their TMS derivatives were determined. The characteristics of the phenolic compounds which corresponded to those found in sunflower flour are summarized in Table I.

The phenolic compounds extracted from the three varieties of sunflower were separated into seven constituents by tlc (Table II). These compounds were detected on the chromatograms by their fluorescence colors under short and long uv light in the presence and absence of ammonia vapors. CG_1 exhibited R_f values characteristic of chlorogenic acid under the three solvent systems and gave a bathochromic shift of 47 nm after the addition of alkali. Steck (1967) also reported that chlorogenic acid (3-caffeoylquinic acid) had an absorption maximum of 376 nm under alkaline conditions. Based on its fluorescence and absorption maxima in methanol and alkaline methanol, the compound, CG_3, appeared to be similar to chlorogenic acid. However, the greater mobility in BzDA and BzMA and the lower R_f value in BAW suggested it may be a less polar isomer of chlorogenic acid. (CG_2) demonstrated a

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Table I. Fluorescence Colors, R_f Values and Absorption Maxima of Some Cinnamic Acids, and the Relative Retention Times (T_{RR}) of Their TMS Derivatives

Compd	Fluorescence colors[a]				R_f values in solvent			Absorption maxima, nm		
	254 nm	+NH_3	366 nm	+NH_3	BzDA	BzMA	BAW	CH_3-OH	+Na-OH	T_{RR}
Chlorogenic acid	BL-Vl	GN	BL	GN	0.00	0.00	0.39	328	375	1.05
Caffeic acid	BL-Vl	BL	BL	BL-GN	0.22	0.32	0.72	320	345	0.60
p-Coumaric acid	dk Vl	Vl	f	Vl	0.37	0.49	0.74	310	330	0.49
Isoferulic acid	BL-Vl	GN	BL-Vl	GN	0.38	0.49	0.70	315	340	0.56
Ferulic acid	BL-Vl	b BL-Vl	BL	b BL	0.44	0.54	0.74	315	340	0.57
Sinapic acid	BL-Vl	BL	BL	BL-GN	0.38	0.50	0.70	318	340	0.64
trans-Cinnamic acid										0.22

[a] BL, blue; Vl, violet; GN, green; dk, dark; f, faint; b, bright.

Table II. Fluorescence Colors, R_f Values, Absorption Maxima and the Identification of Phenolic Compounds from Commander, Majak, and Valley Sunflower Flour by Tlc Technique

Compd	Fluorescence colors				R_f values in solvent			Absorption maxima, nm		Tentative ident.
	254 nm	+NH_3	366 nm	+NH_3	BzDA	Bz-MA	BAW	CH_3-OH	+Na-OH	
CG_1	BL-Vl	GN	BL	GN	0.00	0.00	0.39	328	375	Chlorogenic acid
(CG_2)	BL	GN	BL	GN	0.05	0.04	0.20	328	382	Hydroxycinnamic acid–sugar ester
CG_3	BL	GN	BL	GN	0.12	0.13	0.05	328	375	Chlorogenic acid isomer
CA	BL-Vl	BL	BL	BL-GN	0.22	0.32	0.72	320	345	Caffeic acid
(IF-SN)	BL-Vl	b BL-Vl	BL-Vl	GN	0.39	0.50	0.73	315	340	Isoferulic + sinapic acid like
(PC)	dk Vl	Vl	f	Vl	0.42	0.56	0.75	310	330	*p*-Coumaric acid like
UK	Vl	Vl	f	Vl	0.80	0.89	0.94	275	275	Unknown

spectral shift of 54 nm, which corresponded to the changes in absorption maxima of 1-feruloylglucose and 1-*p*-coumaroylglucose (Steck, 1967) which are sugar esters of hydroxycinnamic acids.

The presence of caffeic acid has been widely reported by other investigators (Mikolajczak *et al.*, 1970; Milic *et al.*, 1968) and compound CA obtained in the present study (Table II) was identical with authentic caffeic acid according to tlc and absorption characteristics (Table I). The fluorescent color changes and spectral shift of compound (IF-SN) (Table II) were similar to those of isoferulic and sinapic acids but the R_f values suggested that the unknown compound may be an unresolved mixture of isoferulic and sinapic acids. (PC) was similar to *p*-coumaric acid although the R_f values in BzDA and BzMA tended to be high. The low intensity of fluorescence, high R_f values, and lack of spectral shift indicated that the unknown, UK, compound might not be a cinnamic acid derivative.

An excellent resolution of silylated phenolic constituents in sunflower flour was obtained by glc. The TMS derivatives were fractionated into ten peaks in each sunflower variety. A typical chromatogram of the phenolic compounds in Majak is illustrated in Figure 1. A relative retention time of 1.00 was assigned to the internal standard (kaempferol) and the T_{RR} values of the silylated phenolic constituents in the three varieties are tabulated (Table III).

To further define their characteristics, the methanol extract was fractionated into acidic and neutral compounds. A silylated acidic compound with short retention time on the glc column was positively identified as *trans*-cinnamic acid by cochromatography with the authentic acid (Table III). *trans*-Cinnamic acid is not a phenolic compound and did not fluoresce under uv light in the tlc analyses. The concentrations of *trans*-cinnamic acid in the three varieties were very low.

The TMS derivatives of (PC) and (IF) were similar in T_{RR} values and uv fluorescence (Tables II and III) to

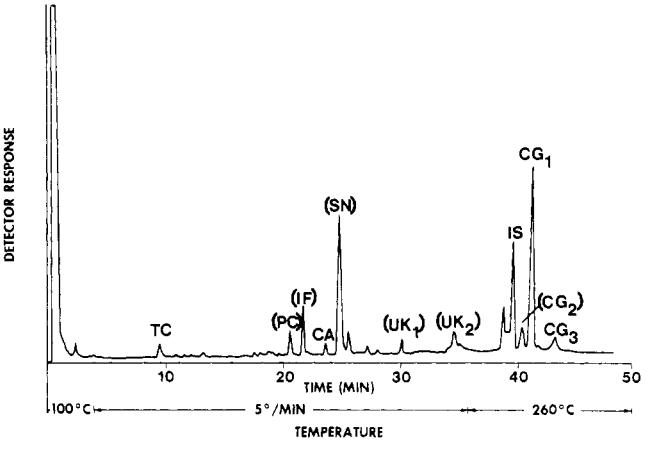

Figure 1. Chromatogram of TMS derivatives of the phenolic compounds from Majak sunflower flour.

those of *p*-coumaric and isoferulic acids (Table I). Since both unknown compounds were found in the neutral fraction, they were assumed to occur in the ester or reduced alcohol forms in the sunflower flours. The caffeic acid peak, CA, appeared in the acidic fraction at about the same concentration as reported by Mikolajczak *et al.* (1970) in the Russian variety, Armavirec.

The glc peak, (SN), had a retention time which approached the value for the TMS derivative of authentic sinapic acid. As a neutral component, (SN) may be sinapyl alcohol, ester, or aldehyde. (SN) constituted a major phenolic constituent, averaging about 0.5 g/100 g of flour in the three varieties.

Two unknown components, (UK_1) and (UK_2), with relatively long retention times of their TMS derivatives were detected in the neutral fraction (Table III). Their concentrations were too low for accurate quantitation by the present procedures.

Table III. Tentative Identification, T_{RR} Values, and Quantitative Analysis by Glc of the TMS Derivatives of Phenolic Sunflower Extracts

Compd	T_{RR} values			Ident.	Composition, g/100 g of flour		
	Commander	Majak	Valley		Commander	Majak	Valley
				Bicarbonate Soluble Fraction (Acid)			
TC	0.22	0.22	0.22	*trans*-Cinnamic acid	0.06	0.07	0.05
CA	0.60	0.60	0.61	Caffeic acid	0.18	0.16	0.17
CG_1	1.05	1.05	1.05	Chlorogenic acid	1.97	1.94	2.08
CG_3	1.09	1.09	1.09	Chlorogenic acid isomer	0.17	0.13	0.12
				Bicarbonate Insoluble Fraction (Neutral, Phenol)			
(PC)	0.51	0.51	0.51	*p*-Coumaric acid like	0.09	0.11	0.10
(IF)	0.56	0.57	0.56	Isoferulic acid like	0.17	0.16	0.14
(SN)	0.62	0.62	0.62	Sinapic acid like	0.48	0.57	0.48
(UK_1)	0.75	0.76	0.77	Unknown	Trace	Trace	Trace
(UK_2)	0.88	0.87	0.88	Unknown	Trace	Trace	Trace
(CG_2)	1.02	1.02	1.02	Hydroxycinnamic acid–sugar ester	0.15	0.18	0.20

On the basis of T_{RR} values and cochromatography with pure chlorogenic acid, the major phenolic constituent in the flours, CG_1, was positively identified as chlorogenic acid (Table III). The three sunflower flours contained 1.9–2.1% of chlorogenic acid which corresponds to the 2.0% obtained by Mikolajczak *et al.* (1970). Other investigators (Milic *et al.*, 1968; Gheyasuddin, 1970; Sosulski *et al.*, 1973) reported a wider range of values but only peak absorptions at 324 or 328 nm were used as the measure of chlorogenic acid. A minor component in the neutral fraction, (CG_2), had a similar retention time to that of chlorogenic acid (Table III) and may be the glucose ester of hydroxycinnamic acid which was obtained by tlc (Table II). The glucose esters like 1-feruloylglucose and 1-*p*-coumaroylglucose would have mildly acidic characteristics. The acidic constituent, CG_3, was only slightly higher in the T_{RR} value than chlorogenic acid and was assumed to be an isomer of chlorogenic acid.

Coarse *et al.* (1965) demonstrated that isochlorogenic acid was a mixture of three dicaffeoylquinic acids and the 3′-methyl ether of 3,5-dicaffeoylquinic acid. In the present study, authentic isochlorogenic acid was fractionated into four spots of R_f values 0.39, 0.49, 0.63, and 0.77 by tlc analyses using the BAW solvent system. The phenolic compound in the spot of the lowest R_f value (0.39) was identical with chlorogenic acid in R_f value (Table I). Therefore, one of the dicaffeoylquinic acids could probably be eluted with chlorogenic acid in the tlc analyses of the phenolic compound from sunflower flour. Mikolajczak *et al.* (1970) have identified 3,5-dicaffeoylquinic acid in the aqueous methanolic extracts from Armavirec sunflower meal. In the present study, dicaffeoylquinic acid was not detected in the aqueous methanolic extracts from sunflower flour by glc analyses because the dicaffeoylquinic acids were either disintegrated on the column or failed to elute. The disintegration of dicaffeoylquinic acids was indicated by the appearance of a caffeic acid peak when the TMS derivative of isochlorogenic acid was chromatographed.

Chlorogenic acid and related compounds constituted about 70% of the total phenolic compounds in each variety. Caffeic acid was a minor phenolic acid in sunflower but neutral compounds related to isoferulic, sinapic, and *p*-coumaric acids were also detected by tlc and glc techniques.

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