of alfalfa (Gerloff et al., 1965; Smith, 1966). Those amino acids present in somewhat greater amounts in the celery protein were lysine, aspartic acid, threonine, and serine. Protein content of leaf protein concentrates can vary from as much as 32 to 84% (Gerloff et al., 1965). The higher percentage of protein in these present preparations (85%) was obtained by washing the precipitated protein fraction three times with water and three times with ethanol, thus extracting extraneous material such as carbohydrates and phenolic material, presumably chlorogenic acid, whose presence has also been shown in alfalfa protein concentrate (Free and Satterlee, 1975). The formation of acid-stable products from the reaction between amines and phenolics or carbohydrates is well documented (Van Sumere et al., 1975; Reynolds, 1965) and could lead to some reduction in the amino acid content of the protein. The LJS besides containing 13.5–14.0% crude protein also contained about 50% of carbohydrate-like material as well as 30% of mineral oxides or ash. During the amino acid analysis of the LJS only about 50% of the Kjeldahl nitrogen present in the sample before hydrolysis was recovered in the form of amino acid nitrogen, including ammonia. Most of the losses occurred presumably with the relatively large amounts of humin filtered from these hydrolysates. The results on the LJS fraction are therefore considered preliminary. However, interesting in this analysis of the total amino acids is the high content of aspartic and glutamic acids and the apparent oxidation of methionine and of cysteine to cysteic acid. Analysis for tryptophan was not carried out.

ACKNOWLEDGMENT

The author is grateful to W. M. Johnson, Mines and Petroleum Resources, Victoria, B.C., for the spectrographic analyses of the minerals, and to N. Bluman, Health Protection Branch, Vancouver, for the pesticide residue analysis. Amino acid analyses were carried out by Analytical Chemistry Services, C.B.R.I. Ottawa, Ontario and the animal feeding studies by Warf Institute Inc., Madison, Wisconsin. Thanks are also due to J. A. Kitson, S. C. MacDonald, and J. F. Conroy for support, interest, and encouragement in this project.

LITERATURE CITED

Association of Vitamin Chemists Inc., "Methods of Vitamin Assay", 3rd ed, Interscience, New York, N.Y., 1966 pp 123–195.
Bickoff, E. M., Kohler, G. O., U.S. Patent 3 823 128 (1974); *Food Sci. Technol. Abstr.* **6**, 48 (1974).
deFremery, D., Miller, R. E., Edwards, R. H., Knuckles, B. E., Bickoff, E. M., Kohler, G. O., *J. Agric. Food Chem.* **21**, 886 (1973).
Free, B. L., Satterlee, L. D., *J. Food Sci.* **40** 85 (1975).
Gerloff, E. D., Lima, I. H., Stahmann, M. A., *J. Agric. Food Chem.* **13**, 139 (1965).
Huang, K. H., Tao, M. C., Boulet, M., Riel, R. R., Julien, J. P., Brisson, G. J., *Can. Inst. Food Technol. J.* **4**, 85 (1971).
McLeod, H. A., Ritcey, W. R., Graham, R. A., Bluman, N., Osadchuk, M., McCulley, K. A., in "Analytical Methods for Pesticide Residues in Foods", The Department of National Health and Welfare, Canada, 1973.
Pirie, N. W., in "Leaf Protein: Its Agronomy, Preparation and Use", Pirie, N. W., Ed., Blackwell Scientific Publications, Oxford and Edinburgh, 1971.
Reynolds, T. M., *Adv. Food Res.* **14**, 167 (1965).
Schierbeck, J., personal communication, Celery Debris Project, British Columbia Department Agriculture, 1973.
Smith, R. H., *Adv. Chem. Ser.* **No. 57**, 133 (1966).
Van Sumere, C. F., Albrecht, J., Dedonder, A., De Pooter, H., Pe, I., in "The Chemistry and Biochemistry of Plant Proteins", Harborne, J. B., Van Sumere, C. F., Ed., Academic Press, New York N.Y., 1975 pp 211–264.
Watt, B. K., Merrill, A. L., in "Composition of Foods", Agriculture Handbook No. 8, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C., 1963.

Received for review November 28, 1977. Accepted March 15, 1978. Contribution No. 466, Agriculture Canada Research Station, Summerland, B.C.

Quantitative Determination by GLC of Phenolic Acids as Ethyl Derivatives in Cereal Straws

Ann-Christine Salomonsson, Olof Theander,* and Per Åman

A new method was used to analyze barley, oat, wheat, rye, and rice straws for phenolic acids. The acids were extracted with aqueous alkali. The phenolic acid were recovered from this extract and alkylated with ethyl iodide and identified and quantified by GLC and GLC–MS. Eight phenolic acids, *p*-hydroxybenzoic, vanillic, *cis*-*p*-coumaric, syringic, *trans*-*p*-coumaric, *cis*-ferulic, *trans*-ferulic, and *trans*-sinapic acids, were identified in all straws. *trans*-*p*-Coumaric acid and *trans*-ferulic acid were the dominant acids in the investigated straws.

Benzoic acids and cinnamic acids are widely distributed in plants (Ribéreau-Gayon, 1972). The cinnamic acids are found in various combined forms, for example, as glycosides, sugar esters (Ribéreau-Gayon, 1972), and as esters linked to carbohydrates in the cell walls (Hartley, 1973; Hartley et al., 1973; Harris and Hartley, 1976). Phenolic acids have been analyzed in 80% ethanol extracts and in the extraction residues from mature oats, wheat, sorghum, and corn residues by paper chromatography (Guenzi and McCalla, 1966) and in rice straw by GLC (Kuwatsuka and Shindo, 1973). Diferulic acids together with monomeric phenolic acids have been reported from cell walls of *Lolium multiflorum* (Hartley and Jones, 1976).

trans-Cinnamic acids are known to be partially convertable to their cis analogues by UV light, the conversion being maximized between pH 5.0 and 7.0 (Neish, 1961;

Swedish University of Agricultural Sciences, Department of Chemistry, S-750 07 Uppsala 7, Sweden.

0021-8561/78/1426-0830$01.00/0

Table I. Content of Combined Phenolic Acids in Some Cultivars of Cereal Straw[a]

Phenolic acid	Barley					Oat,	Wheat			Rye,	
	Wing	Särla	Cilla	Ingrid	Senat	Titus	Starke II	Holme	Drabant	Petkus	Rice
p-Hydroxybenzoic	92	26	37	28	170	290	26	42	91	62	Tr
Vanillic	110	93	13	20	120	79	13	24	180	33	17
cis-*p*-Coumaric	390	130	79	180	390	400	240	240	210	530	120
trans-*p*-Coumaric	2800	510	880	1700	2800	3100	920	1500	1700	2900	2600
cis-Ferulic	450	89	170	210	420	540	240	300	270	560	140
trans-Ferulic	2100	440	1000	790	3100	2400	620	1400	1400	2700	1200
Σ identified phenolic acids, %	0.59	0.13	0.22	0.29	0.70	0.68	0.21	0.35	0.39	0.68	0.41
Total ethyl acetate extract, %	3.8	2.3	2.8	3.3	3.9	2.5	2.1	3.0	3.1	3.2	1.3

[a] The results are given as μg/g of the dry weight of the straw. The Σ of the identified phenolic acids and the total ethyl acetate extracts are given as percent of the dry weight of the straw.

Kahnt, 1967). It has, therefore, been suggested that extraction, derivatization, and other manipulations of cinnamic acids should be carried out in the absence of UV and daylight to avoid cis–trans isomerization (Hartley and Jones, 1975).

Since phenolic acids are nonvolatile compounds, they must be converted to volatile derivatives before they can be analyzed by GLC. The most used derivatives are trimethylsilyl ethers (e.g., Kuwatsuka and Shindo, 1973; Hartley and Jones, 1975) and methyl ethers (Erickson et al., 1973). Trimethylsilyl ethers of phenolic acids are relatively unstable compounds because they are easily hydrolyzed by water and methylation gives identical derivatives of several phenolic acids which contain hydroxyl and methoxyl groups. In both methods the derivatives of the phenolic acids are easily identified by GLC–MS.

Our interest in these compounds arises from recent work on alkaline treatment of cereal straw, intended to increase digestibility (e.g., Beckmann, 1921; Jayasuriya and Owen, 1975), where phenolic acids are liberated in the alkaline liquor. When a closed circulating system is used, where only straw, water, and alkali are added and only treated straw is removed (Wehtje, 1977), phenolic acids are accumulated in the alkaline liquor (Theander and Åman, 1977).

In the present work, the phenolic acids in the straw of some Swedish cereals and rice are analyzed quantitatively by GLC after alkylation with ethyl iodide by a procedure previously developed at this department in connection with studies on the herbicide linuron and its soil metabolites (Glad et al., 1977). This procedure was based on that described earlier for the alkylation of some urea herbicides (Lawrence and Laver, 1975).

EXPERIMENTAL SECTION

Reference Phenolic Acids. Benzoic acid, *p*-chlorobenzoic acid, salicylic acid, *p*-hydroxybenzoic acid, veratric acid, vanillic acid, gentistic acid, protocatechuic acid, β-resorcylic acid, syringic acid, *trans*-*p*-coumaric acid, gallic acid, *trans*-ferulic acid, *trans*-caffeic acid, and *trans*-sinapic acid were obtained commercially and *cis*-*p*-coumaric acid, α,β-dihydro-*p*-coumaric acid, and α,β-dihydroferulic acid were obtained from the authors' laboratory. All reference phenolic acids were chromatographically pure (TLC and GLC).

Plant Material. Barley (*Hordeum distichum* L.) straw of the cultivars Wing, Särla, Cilla, Ingrid, and Senat, oat (*Avena sativa* L.) straw of the cultivar Titus, wheat (*Triticum aestivum* L.) straw of the cultivar Starke II, Holme, and Drabant, and rye (*Secale cereale*, L.) straw of the cultivar Petkus were obtained from the Department of Plant Husbandry of this university, air-dried, and milled. Rice (*Oryza sativa*) straw was obtained from Dr. G. O. Kohler, U.S. Department of Agriculture, Berkeley, Calif.

General Methods. All results are calculated on a dry weight basis. Evaporations were performed in vacuo below 40 °C in a Büchi Rotavapor-R apparatus.

Extraction and Fractionation. A suspension of the milled straw (5 g) was refluxed for 3 × 30 min in 80% aqueous ethanol (3 × 100 mL). After each extraction the residue was filtered and washed with 80% aqueous ethanol (50 mL). The residue was air-dried overnight and further extracted with 1.0 M aqueous NaOH at room temperature (200 mL for 16 h). The suspension was centrifuged and the residue was washed with 2 × 50 mL of 1.0 M aqueous NaOH in the centrifuge tubes. The combined supernatants were neutralized with 2.0 M HCl and concentrated to ca. 250 mL. The solution was acidified to about pH 1 with 1.0 M HCl and extracted with 3 × 300 mL of distilled ethyl acetate. When the ethyl acetate phase was not clear it was centrifuged. The combined ethyl acetate phases were dried with anhydrous Na_2SO_4, filtered, evaporated to dryness, and weighed after drying in a dessicator (Table I).

Ethylation of the Phenolic Acids. The ethyl acetate extracted phenolic acids were dissolved in 10.00 mL of methanol and a solution (10 mL) containing 10.00 mg of *p*-chlorobenzoic acid (Kuwatsuka and Shindu, 1973) was added as internal standard. Part of the solution (2.00 mL) was evaporated to dryness, dissolved in 0.5 mL of dimethyl sulfoxide, and transferred to a 20-mL screw-cap tube. Ethyl iodide (0.5 mL) and further NaH (20 mg) was added. The suspension was treated by ultrasonics for 1 min and then submerged for 20 min at 50 °C in a water bath. After cooling to room temperature, *n*-hexane (1.5 mL) was added and the capped test tube gently shaken. Water (10 mL) was carefully added drop by drop to the reaction mixture to decompose the excess of NaH and then the capped tube was vigorously shaken for 0.5 min (Glad et al., 1977). The layers were allowed to separate, and 1.0 μL of the ethylated phenolic acids in the hexane phase was injected in the gas chromatograph. The reference phenolic acids (2.00 mg of each) were ethylated as described above.

Gas–Liquid Chromatography (GLC) and GLC–Mass Spectrometry (MS). GLC with packed columns was carried out with a Varian Model 2700 instrument fitted with glass columns (180 × 0.2 cm i.d.) containing 3% OV-1 on Varaport 30 and flame ionization detectors. The oven was temperature programmed to 100–250 °C at 6 °C/min and the flow rate of nitrogen carrier gas was 25 mL/min. The GLC with a capillary column was carried out with a modified Packard Model 427 instrument fitted with a glass

Table II. Relative Retention Times and Relative Response Factors of Ethylated Phenolic Acids

Phenolic acid	No. (Figures 1–3 and Table III)	Packed OV-1[a]		Capillary OV-101[a]
		Retention time	Response factor	Retention time
Benzoic	1	0.56	0.76	0.53
p-Chlorobenzoic	2	1.00	1.00	1.00
Salicylic	3	1.46	0.78	1.59
p-Hydroxybenzoic	4	1.59	0.76	1.83
Veratric	5	1.98	1.23	2.54
α,β-Dihydro-*p*-coumaric	6	2.01	0.86	2.68
Vanillic	7	2.12	0.97	2.88
Gentisic	8	2.18	0.81	3.07
cis-*p*-Coumaric	9	2.19	0.87	3.04
Protocatechuic	10	2.23	0.79	3.20
β-Resorcylic	11	2.32	0.91	3.42
Syringic	12	2.42	1.34	3.72
trans-*p*-Coumaric	13	2.43	0.84	3.81
α,β-Dihydroferulic	14	2.46	1.05	3.78
cis-Ferulic	15	2.54	*b*	4.11
Gallic	16	2.69	0.86	4.41
trans-Ferulic	17	2.90	1.00	5.11
trans-Caffeic	18	3.01	0.91	5.45
trans-Sinapic	19	3.20	1.20	6.07

[a] For chromatographic conditions, see Experimental Section. [b] Not determined. The relative response factor of *trans*-ferulic acid is used for quantitative determination.

column (2500 cm × 0.05 cm i.d.) containing OV-101 and a flame ionization detector. The oven was temperature programmed to 180–280 °C at 6 °C/min and the flow rate of helium carrier gas was 2 mL/min. An Autolab minigrator was used to determine peak areas.

The mass spectral data were obtained at 70 eV with a Varian MAT CH7 mass spectrometer, equipped with the Spectro System 100 N 101/81 MS and a Varian 1740 gas chromatograph. This was fitted with the 3% OV-1 column and operated as above but using a helium flow rate of 25 mL/min.

Identification of the ethylated phenolic acids was made by comparison of their relative retention times (Table II) and their mass spectra (Table III) with the reference compounds.

Quantitation. Methanol solutions of the reference phenolic acids corresponding to 1, 2, 4, 6 mg and 2 mg of the internal standard (*p*-chlorobenzoic acid) were ethylated and analyzed by GLC as described above. Dry weight ratios (acid/internal standard) were plotted against integrator count ratios (acid/internal standard) (Mason and Slover, 1971). A linear relationship was found in each case, and the relative response factors listed in Table II were calculated from the graphs. The procedures were performed in the absence of UV and sunlight to avoid cis–trans isomerization (Hartley and Jones, 1975). No isomerization was observed when pure *cis*- or *trans*-*p*-coumaric acid, *trans*-ferulic acid, *trans*-caffeic acid, and *trans*-sinapic acid were analyzed.

In order to obtain correction factors for the alkaline treatment of straw and the subsequent working up and GLC procedure, 4-hydroxybenzoic, vanillic, *trans*-*p*-coumaric, and *trans*-ferulic acids (25.00 mg of each) and the alkali stable internal standard (*p*-chlorobenzoic acid, 25.00 mg) were treated with alkali, extracted with ethyl acetate after acidification, and analyzed by GLC as described above. The correction factors obtained, related to the internal standard, were: *p*-hydroxybenzoic acid = 0.86, vanillic acid = 0.78, *trans*-*p*-coumaric acid = 0.86, and *trans*-ferulic acid = 0.71. The integrator count should be divided with the correction factors obtained. The correction factors of the two trans acids were also used for calculation of the content of the corresponding cis acids.

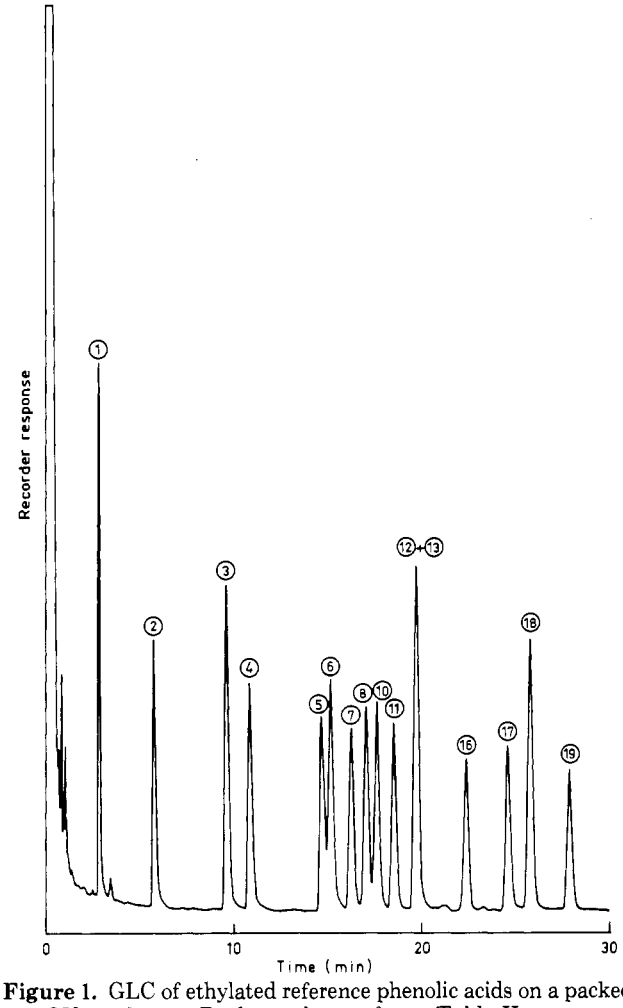

Figure 1. GLC of ethylated reference phenolic acids on a packed 3% OV-1 column. Peak numbers refer to Table II.

RESULTS AND DISCUSSION

Extraction of Phenolic Acids. Dried and milled straw of barley, oat, wheat, rye, and rice were extracted with 80% ethanol in order to remove low molecular weight sugars and other water–ethanol soluble substances. Five–ten percent of the material was soluble in the 80% ethanol and trace amounts of free phenolic acids, mainly *trans*-*p*-coumaric, syringic, and *trans*-ferulic acids were identified in these extracts. After drying, the combined phenolic acids in the residues were extracted with ethyl acetate from alkaline extracts which had been acidified with hydrochloric acid. The extracts accounted for 1.3–3.9% of the straws (Table I).

Analysis of Reference Phenolic Acids. Varying amounts of reference phenolic acids and *p*-chlorobenzoic acid, as internal standard, were alkylated with ethyl iodide and analyzed by GLC and GLC–MS. A typical gas chromatogram on a packed column is shown in Figure 1 and on a capillary column in Figure 2. On the packed column it was not possible to get baseline separation between veratric and α,β-dihydro-*p*-coumaric acids and between gentisic and protocatechuic acids. Syringic and *trans*-*p*-coumaric acids were not separated on this column. These separation problems could be solved by using the capillary column. An analysis on the capillary column (15

Table III. Mass Spectra of Ethylated Phenolic Acids Obtained as Described in the Experimental Section [(M) Indicates the Molecular Ion]

Benzoic acid	*m/e*	150 (M)	122	105	77	51					
	I %	15	29	100	50	22					
p-Chlorobenzoic acid	*m/e*	184 (M)	156	141	139	111	75				
	I %	15	34	34	100	34	27				
Salicyclic acid	*m/e*	194 (M)	166	149	138	122	121	93	65	39	32
	I %	40	15	31	51	18	100	23	33	21	16
	m/e	29									
	I %	32									
p-Hydroxybenzoic acid	*m/e*	194 (M)	166	149	138	121	93	65			
	I %	27	17	45	26	100	15	20			
Veratric acid	*m/e*	210 (M)	182	165	137	79	77	51			
	I %	60	26	100	18	18	18	17			
α,β-Dihydro-*p*-coumaric acid	*m/e*	222 (M)	148	135	123	120	107	91	77		
	I %	30	43	100	18	34	99	16	23		
Vanillic acid	*m/e*	224 (M)	196	179	168	151	123				
	I %	43	25	17	39	100	17				
Gentisic acid	*m/e*	238 (M)	165	164	137	136	135				
	I %	29	16	100	18	70	18				
cis-*p*-Coumaric acid	*m/e*	220 (M)	175	164	148	147	120	119	118	91	65
	I %	62	47	16	35	100	47	37	15	30	21
	m/e	39									
	I %	17									
Protocatechuic acid	*m/e*	238 (M)	210	193	182	181	165	154	138	137	109
	I %	51	25	16	36	17	20	62	17	100	20
β-Resorcylic acid	*m/e*	238 (M)	193	191	177	165	164	149	138	137	136
	I %	46	77	80	27	36	56	21	19	80	100
	m/e	121	108	81	80	39					
	I %	18	46	25	16	19					
Syringic acid	*m/e*	254 (M)	226	225	211	209	198	182	181	154	
	I %	74	100	21	20	19	47	19	97	21	
trans-*p*-Coumaric acid	*m/e*	220 (M)	175	164	148	147	120	119	91	65	
	I %	72	56	17	36	100	46	38	27	20	
α,β-Dihydroferulic acid	*m/e*	252 (M)	165	150	149	137					
	I %	56	49	46	28	100					
cis-Ferulic acid	*m/e*	250 (M)	222	205	194	177	150	149	148	147	145
	I %	89	56	29	31	76	100	21	22	30	72
	m/e	133	117	91	89	78	77	51	42	39	
	I %	28	31	22	37	21	41	41	27	27	
Gallic acid	*m/e*	282 (M)	254	237	226	225	209	198	197	183	170
	I %	88	58	20	51	19	16	73	100	18	50
	m/e	154	153	141	125	113	67	39			
	I %	18	66	24	30	18	16	29			
trans-Ferulic acid	*m/e*	250 (M)	222	205	194	177	150	149	148	147	145
	I %	100	52	29	28	70	85	18	18	25	59
	m/e	133	117	91	89	77	51				
	I %	18	24	17	31	30	25				
Caffeic acid	*m/e*	264 (M)	236	219	208	207	180	179	164	163	162
	I %	100	36	23	29	30	34	32	22	49	16
	m/e	145	136	135	134	133	89	77	51		
	I %	19	37	22	24	26	21	21	17		
Sinapic acid	*m/e*	280 (M)	252	251	235	224	223	207	180	177	175
	I %	97	100	63	23	20	16	31	62	28	29
	m/e	163	147	135	119	91	77	65			
	I %	29	18	18	22	18	17	17			

min) was also much faster than on the ordinary column (30 min). A general advantage of using ethylation instead of methylation is to enable identification and analysis of naturally methoxy compounds and distinguish between related non- and partially methylated compounds, for instance, between salicylic and gallic acids.

The relative retention times are presented in Table II. When the weight ratios (acid/internal standard) were plotted against the integrator count ratios, linear relationships in the investigated intervals were obtained. The relative response factors presented in Table II are calculated from these graphs.

Correction factors for the identified phenolic acids (including alkaline treatment, working up procedure, and GLC analysis) were calculated from analysis of reference compounds. Ester-linked di- and trihydroxy phenolic acids are not expected to survive to any significant extent during the alkaline hydrolysis conditions (compare Forsskåhl et al., 1976). Caffeic acid, for example, could not be detected after the alkaline treatment conditions used in the present investigation or by using a tenth of the alkaline conditions used in the present investigation. If such alkali-labile ester-linked acids are going to be analyzed, enzymatic hydrolysis, if available, could be the best method. When a milder type of alkaline hydrolysis (methanol/0.1 M sodium hydroxide, 7:3) according to Kuwatsuka and Shindo (1973) of barley straw was used, no new peaks appeared on the gas chromatogram, but the yield of the individual acids was lower than under the standard conditions of the present investigation. Very low yield of phenolic acids has previously been reported by Guenzi and McCalla (1966) using acid hydrolysis of straw.

The mass spectral data for the ethylated phenolic acids are given in Table III. In the table, ions are listed only if their relative abundance is >15% of the base peaks. All phenolic acids investigated had molecular ions (M) with *m/e* values agreeing with the calculated molecular weight of the fully ethylated form of each acid, i.e., all phenolic

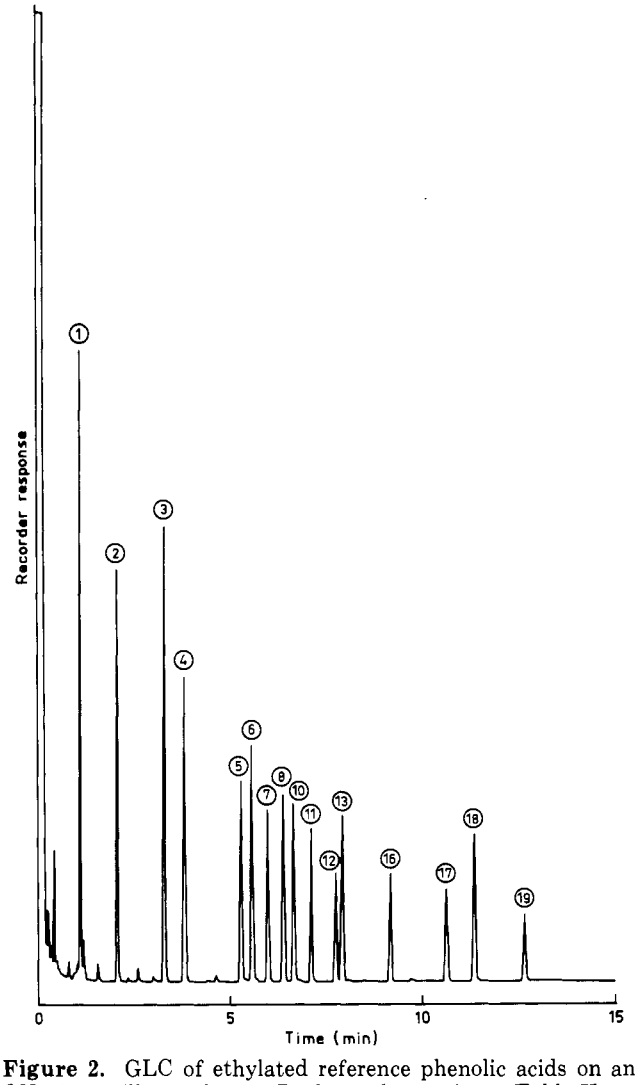

Figure 2. GLC of ethylated reference phenolic acids on an OV-101 capillary column. Peak numbers refer to Table II.

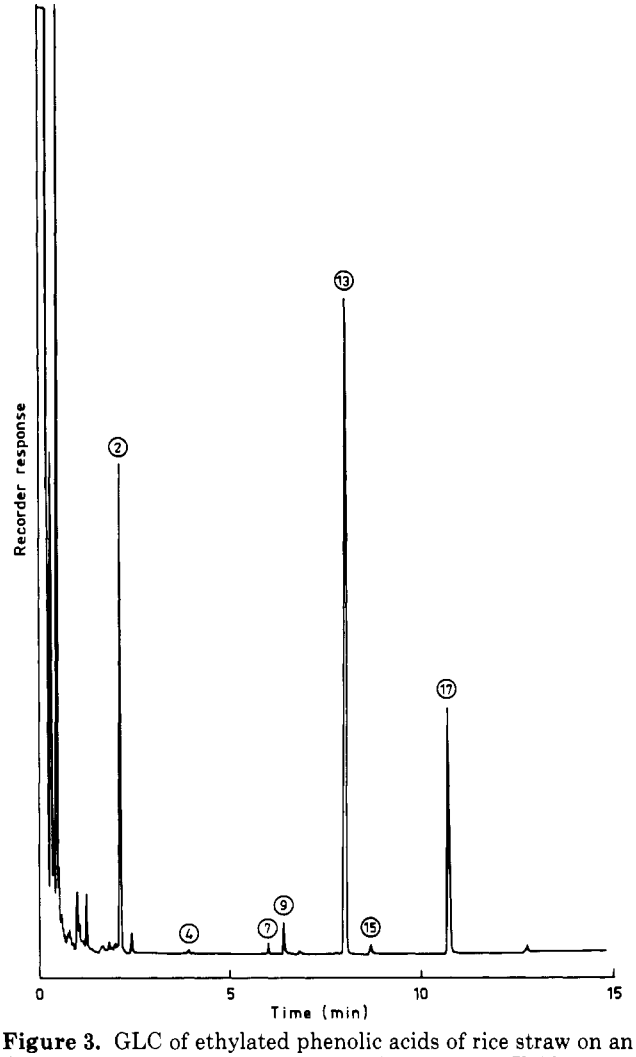

Figure 3. GLC of ethylated phenolic acids of rice straw on an OV-101 capillary column. Peak numbers refer to Table II.

hydroxyl and carboxyl groups had been ethylated. Most of the large ions are due to the loss of one or several mass units 28 (CH_2=CH_2) and/or 45 (CH_3CH_2O·). The saturated cinnamic acids also lose the characteristic mass units of 87 (EtOC(=O)CH_2·) from β cleavage. When there were several substituents on the aromatic ring, the fragmentation patterns were more complex.

Analysis of the Phenolic Acids in the Straw. The phenolic acids in the ethyl acetate extracts were ethylated and analyzed by GLC and GLC–MS. Eight phenolic acids, *p*-hydroxybenzoic, vanillic, *cis*-*p*-coumaric, syringic, *trans*-*p*-coumaric, *cis*-ferulic, *trans*-ferulic, and *trans*-sinapic acids, were identified in the straws. Sinapic acid was not identified in the rice straw sample. The relative retention times and the mass spectra were identical with the reference acids. A typical gas–liquid chromatogram of the phenolic acids in rice straw is shown in Figure 3. As shown in Table I, *trans*-*p*-coumaric acid and *trans*-ferulic acid were the dominant acids in the straw (510–3100 and 440–3100 μg/g of the straw, respectively). *cis*-*p*-Coumaric and *cis*-ferulic acids contents of 130–530 and 100–560 μg/g, respectively, of the straw and small amounts of *p*-hydroxybenzoic acid and vanillic acid were also quantified. The sum of the identified phenolic acids accounted for 0.1–0.7% of the dry weight of the straw. The variation between the five investigated barley cultivars was in the same range as the variation between the different cereals. The pattern of acids for the rice straw is similar to that reported by Kuwatsuka and Shindo (1973), except that no salicylic acid was detected in the present investigation.

ACKNOWLEDGMENT

The skilled technical assistance of Lena Widnersson is acknowledged. The authors are indebted to the Swedish Board for Technical Development for financial support.

LITERATURE CITED

Beckmann, E. *Festschr. Kaiser Wilhelm Ges. Founderung. Wiss Zehnjahr. Jubiläum*, 18 (1921).
Erickson, M., Larsson, S., Miksche, G. M., *Acta Chem. Scand.* **27**, 127 (1973).
Forsskåhl, I., Popoff, T., Theander, O., *Carbohydr. Res.* **48**, 13 (1976).
Glad, G., Popoff, T., Theander, O., *Swed. Weed Conf., Rep.* **18**, 11 (1977).
Glad, G., Popoff, T., Theander, O., *J. Chromatogr. Sci.*, in press (1978).
Guenzi, W. D., McCalla, T. M., *Agron. J.* **58**, 303 (1966).
Harris, P. J., Hartley, R. D., *Nature (London)* **259**, 508 (1976).
Hartley, R. D., *Phytochemistry* **12**, 661 (1973).
Hartley, R. D., Jones, E. C., *J. Chromatogr.* **107**, 213 (1975).
Hartley, R. D., Jones, E. C., Wood, T. M., *Phytochemistry* **12**, 763 (1973).
Hartley, R. D., Jones, E. C., *Phytochemistry* **15**, 1157 (1976).
Jayasuriya, M. C. N., Owen, E., *Anim. Prod.* **21**, 313 (1975).
Kahnt, G., *Phytochemistry* **6**, 755 (1967).
Kuwatsuka, S., Shindo, H., *Soil Sci. Plant Nutr.* **19**, 219 (1973).
Lawrence, J. F., Laver, G. W., *J. Agric. Food Chem.* **23**, 1106 (1975).

Mason, B. S., Slover, H. T., *J. Agric. Food Chem.* **19**, 551 (1971).
Neish, A. C. *Phytochemistry* 1, 1 (1961).
Ribéreau-Gayon, P., in "Plant Phenolics, University Review in Botany", Hegwood, V. H., Ed., Oliver and Boyd, Edinburgh, 1972, pp 82.
Theander, O., Åman, P., unpublished work, 1977.
Wehtje, F., Swedish Patent No. 7407 230-7, Nov 17, 1977.

Received for review September 14, 1977. Accepted February 21, 1978.

Volatile Constituents of Prickly Pear (*Opuntia ficus indica* Mill., *de Castilla* Variety)

Robert A. Flath* and Juan M. Takahashi

Volatile components of a commercial variety of prickly pear were concentrated by vacuum codistillation with water, followed by ether extraction of the distillate. The concentrate was examined by large bore capillary gas chromatography–mass spectrometry, and 61 compounds were identified. Quantitatively, alcohols are the major class of compound represented, although numerous esters and carbonyl compounds are also present at low concentrations. Some of the more interesting compounds found are a group of saturated and unsaturated nine-carbon alcohols.

Opuntia cacti flourish in many hot, semiarid regions of the world, although they are natives of the Americas and were unknown in the Eastern Hemisphere before the beginning of the sixteenth century. In the succeeding years, *Opuntia* species were transplanted to the Mediterranean countries, Africa, and Australia. The rapid growth and abundant quantities of edible fruit produced by certain *Opuntia* species were major reasons for this spread.

Numerous trivial names—prickly pear, Barbary fig, indian fig—have been applied to the fruit. In certain parts of the world, *Opuntia* fruit comprises a significant fraction of the human daily food supply. Mexico has over twelve thousand hectares under commercial cultivation, with an annual production of approximately 120 000 metric tons (Barrera-Benitez, 1976). Eight varieties of prickly pear are grown in commercial quantities in Mexico. The most popular variety, *de Castilla*, is the subject of this study. Commercial production of *Opuntia* fruit in the U.S. is rather small at present; most of the fruit harvested in the country is grown to satisfy the demand for the commodity from the Mexican–American market in the West and Southwest, as well as from Americans of Mediterranean ancestry in the eastern U.S.

The fruit of the *de Castilla* variety is pale yellow-green, rather ellipsoid in shape, approximately 8 cm long and 5 cm in diameter. Most literature references to *O. ficus indica* describe the fruit as red, but the particular commercial variety studied contains no noticeable red pigment. The skin is relatively smooth, with spirally arranged areoles lacking spines distributed across the surface. In cross section, the fruit displays a pale-green interior, similar in texture to that of a ripe melon or Kiwi berry. In contrast to these two examples, however, the small dark seeds or stones are distributed rather uniformly throughout the interior tissue. These are eaten with the pulp, which has a light, somewhat melon-like flavor. It is mildly sweet with little acid character.

Western Regional Research Laboratory, U.S. Department of Agriculture, Berkeley, California 94710 (R.A.F.) and Comision Nacional de Fruiticultura, Palo Alto, Mexico 18, D.F. (J.M.T.).

Most of the Mexican crop is sold and used as fresh fruit, with some of this converted by the purchaser into fresh juice. On a smaller scale, a number of concentrated or partly dried products are prepared, including concentrated juices, syrup, preserves and quesos, or semisolid sweets.

All previous research work reported in the literature is confined to nonvolatile constituents of the fruit, including levels of certain nutrients. Total acid (as citric acid) values of 0.084–0.12% were found, with pH ranging from 4.85 to 6.3. The vitamin C level in the fruit was reported to be 42 mg/100 g of fresh fruit (Villareal et al., 1963, 1964; Nordal et al., 1966; Espinosa et al., 1973; Paredes Lopez and Rojos Burgos, 1973). Some work has been reported on the pigments in red and yellow-orange varieties as well (Minale et al., 1965; Impellizzeri and Piattelli, 1972).

EXPERIMENTAL SECTION

Starting Material. *Opuntia ficus indica* Mill., *de Castilla* variety, was grown at San Martin de las Pyramides, Estada de Mexico, Mexico. The fruit was harvested in late September, 1976, and air shipped in corrugated containers to the U.S.

Concentrate Preparation. Each end of the ripe fruit was cut off, then the skin was slit longitudinally and peeled back, yielding the intact central portion. The fruit tissue (4.93 kg) including seeds was coarsely chopped and placed in the glass 12-L pot of the stripping apparatus, which has been previously described (Forrey and Flath, 1974). The operation was basically a vacuum codistillation with water, the distillate being collected in a solid carbon dioxide–isopropyl alcohol-cooled receiver flask. Distilled water (3 L) and methyl silicone oil (2 drops; SF (96(50)) were combined in the 12-L pot with the fruit, and the system was evacuated to 40mm. After 3.25 h of distillation, 0.9 L of distillate had collected. The frozen distillate was melted and extracted for 4 h in a conventional liquid–liquid extractor, using freshly distilled ether (125 mL). After being chilled in a freezer, the ether solution was decanted from several drops of water and distilled, using a 30 cm × 1.2 cm glass helix-packed, vacuum-jacketed distillation column, to remove most of the solvent (a water bath was used for heating; maximum temperature = 45 °C). The residue (ca. 2 mL) was transferred to a small vial fitted with an 8 cm long air condenser, and most of the

0021-8561/78/1426-0835$01.00/0