Free, Esterified, and Insoluble-Bound Phenolic Acids. 3. Composition of Phenolic Acids in Cereal and Potato Flours

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The total phenolic acid content of wheat, rice, and oat flours ranged from 71 to 87 ppm while corn flour contained 309 ppm and potato flour 410 ppm. *Cis*- and *trans*-ferulic and -*p*-coumaric acids and syringic acid were the principal phenolic compounds in the free acid or soluble ester fractions of the cereal flours. Alkaline hydrolysis of the insoluble residue of the cereals released the major proportion of phenolic acids, principally *trans*-ferulic acid. Free chlorogenic acid was the principal phenolic acid in potato flour, and a significant proportion of caffeic acid was released on hydrolysis of soluble esters. Wheat flour which had been stored for 6 months contained only 26 ppm of total phenolic acids, primarily in the bound form.

Maga and Lorenz (1973) have shown that phenolic acids can contribute objectionable flavors, especially astringency, at taste threshold levels of 40–90 ppm. Several phenolic acids and their derivatives are known to contribute grey, brown, or green colors to food products, which may also be undesirable (Cater et al., 1972; Clark et al., 1957). Despite their influence on the organoleptic characteristics of foods, little is known about the quantitative distribution of the phenolic acids and their derivatives in primary food products prepared from cereals, oilseeds, and legumes. A rapid procedure for the accurate quantitation of free, esterified, and insoluble-bound phenolic acids in rapeseed has been described (Krygier et al., 1982a,b). In the present study these procedures were followed, with modifications, to assess the phenolic composition of a series of cereal and potato flours.

Wheat kernels have been reported to contain free phenolic compounds and their derivatives (el-Basyouni and Towers, 1964). Several unidentified phenolic compounds were released from wheat flour by acid and alkali hydrolysis, but ferulic acid was not present (Gallus and Jennings, 1971). The contents of soluble-bound ferulic acid in wheat and triticale flour was reported to be in the range of 20–30 ppm (Maga and Lorenz, 1974).

Durkee and Thivierge (1977) estimated that oat (groat) meal contained 300 ppm of ferulic acid in the free, esterified, and bound forms, the latter fraction showing the highest concentration. Oats also contained vanillic, sinapic, *p*-coumaric, and *p*-hydroxybenzoic acids, but the concentrations were not determined. In potato, the total polyphenolic compounds are commonly measured spectrophotometrically (Clark et al., 1957), and data on the composition of specific phenolic acids are rarely available (Amberger and Schaller, 1975).

The objectives of the present investigation were to fractionate the phenolic constituents of flours into free, soluble, and insoluble forms and, after hydrolysis, to determine the relative proportions of the various phenolic acids by gas–liquid chromatography (GLC). Identification of all phenolic compounds in the chromatograms was confirmed by gas chromatography–mass spectroscopy (GLC–MS). The products investigated in this study were the flours of wheat, rice, oats, corn, and potato. The phenolic composition of the freshly milled wheat flour was compared with that of a flour which had been stored in the laboratory for 6 months.

EXPERIMENTAL SECTION

Plant Materials. The samples analyzed for phenolic composition included Neepawa wheat, Neepawa wheat flour, Harmon oats, yellow dent corn, long grain brown rice, and Netted Gem potatoes.

The seed samples were mature, sound, and, except for rice, had been harvested about 2 months previously.

The cereals, after dehulling in the case of oats, were debranned by roller milling or pearling and ground into a fine flour. The potatoes were peeled, sliced, freeze-dried, and ground.

Methods. The 10-g flour samples were extracted 6 times each with 50 mL of methanol–acetone–water (7:7:6) at room temperature. The combined extracts were evaporated to remove the organic solvents, and the aqueous phase was adjusted to pH 2 before extraction with hexane to remove interfering lipids (Krygier et al., 1982a). The free phenolic acids were then extracted with diethyl ether–ethyl acetate (1:1) and prepared for GLC as described previously. The esters remaining in the aqueous phase were hydrolyzed with 4 N NaOH, and the liberated phenolic acids were extracted with ether–ethyl acetate for analysis. The Krygier et al. (1982a) procedure was modified for the alkaline hydrolysis of the residue because of problems with starch galatinization. The residues were initially dispersed in 200–300 mL of 2 N NaOH and stirred for 4 h under nitrogen. The solution was then acidified slightly (pH 5), centrifuged, and stored under nitrogen. The residue was washed twice with water and centrifuged. The combined supernatants were concentrated under vacuum to 40 mL, adjusted to pH 2, and extracted with ether–ethyl acetate as above.

The procedures for GLC and GLC–MS were previously described (Krygier et al., 1982a). Concentrations of phenolic acids are expressed in ppm, dry basis.

RESULTS AND DISCUSSION

Compared to those of rapeseed flours (Krygier et al., 1982b), the concentrations of free phenolic acids in the cereal flours were very low; the totals ranged from 2.3 ppm in wheat flour to 16.5 ppm in corn flour (Table I). The fresh wheat flour contained measurable quantities of *trans*-ferulic, syringic, and vanillic acids. Rice contained significant levels of five phenolic acids, and eight were present in oats and corn. *trans*-Ferulic was an important free phenolic acid in each cereal; the following acids were also major components: *p*-hydroxybenzoic in rice, syringic in oats, and *p*-coumaric in corn. The cis isomer of ferulic acid was present in three of the cereal flours, possibly as

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Table I. Free Phenolic Acids in Flours (ppm)

phenolic acid	wheat fresh	wheat stored	rice	oats	corn	potato
p-hydroxybenzoic		0.1	1.9	0.7	0.3	
(*p*-hydroxyphenyl)acetic			trace	0.4	1.1	
vanillic	0.6	0.2	1.3	0.7	1.0	
protocatechuic				0.5	1.1	
syringic	0.5		trace	2.3	1.1	
quinic						trace
trans-*p*-coumaric	trace		1.3	0.7	6.2	
cis-ferulic	trace		0.3		0.6	
trans-ferulic	1.2		2.4	2.4	5.1	
caffeic				1.0	trace	trace
trans-sinapic				trace	trace	
chlorogenic						341.3
total	2.3	0.3	7.2	8.7	16.5	341.3
% of total phenolics	3.2	1.2	8.4	10.0	5.3	83.2

Table II. Phenolic Acids Liberated from Soluble Esters (ppm)

phenolic acid	wheat fresh	wheat stored	rice	oats	corn	potato
p-hydroxybenzoic	trace	trace	2.7	0.7	1.0	
(*p*-hydroxyphenyl)acetic				trace	trace	
vanillic	3.0	0.6	0.8	3.5	2.7	
protocatechuic		trace		trace	1.9	
syringic	2.3	0.9	0.2	3.0	10.4	
cis-*p*-coumaric	trace		trace		trace	
trans-*p*-coumaric	trace	trace	trace	0.5	12.7	5.1
cis-ferulic	trace	0.6	1.6	1.9	5.1	
trans-ferulic	3.8	1.7	9.6	6.7	44.9	4.8
caffeic					trace	58.4
cis-sinapic	trace		trace	trace		
trans-sinapic	trace	trace	trace	4.3	trace	
total	9.1	3.8	14.9	20.6	78.7	68.3
% of total phenolics	12.8	14.8	17.4	23.7	25.5	16.6

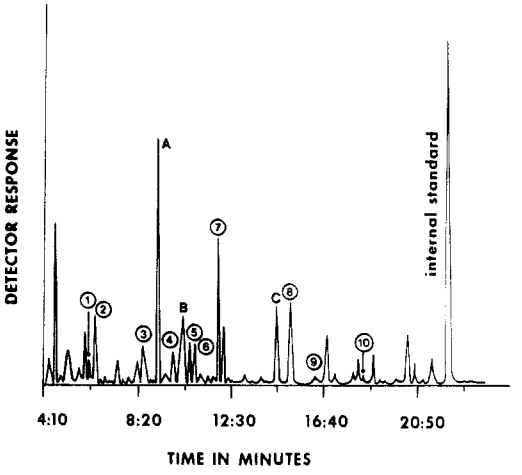

Figure 1. GLC–MS chromatogram of free phenolic acids in corn flour separated on a WCOT capillary column of fused silica (0.2 mm × 24 m) coated with OV-101. 1 = *p*-hydroxybenzoic, 2 = (*p*-hydroxyphenyl)acetic, 3 = vanillic, 4 = protocatechuic, 5 = syringic, 6 = *cis*-ferulic, 7 = *trans*-*p*-coumaric, 8 = *trans*-ferulic, 9 = caffeic, and 10 = *trans*-sinapic acids. Main contaminants: A = azelaic acid, B = citric acid, and C = palmitic acid.

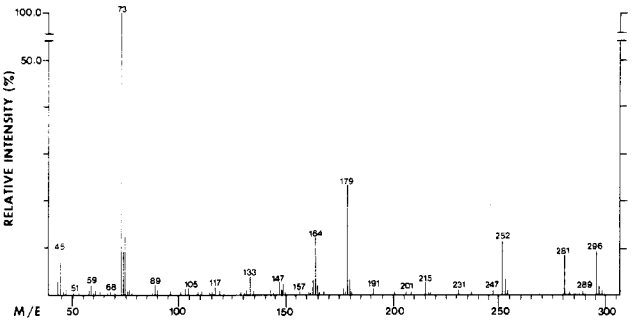

Figure 2. Mass spectrum of the Me_3Si derivative of (*p*-hydroxyphenyl)acetic acid.

an artifact of the extraction (Krygier et al., 1982a). In rice, oats, and corn, (*p*-hydroxyphenyl)acetic acid was identified by GLC–MS (Figures 1 and 2). Nonphenolic acids appeared as contaminants in the chromatograms, especially fatty acids and their derivatives (Figure 1). Potato flour differed from the cereals in its high concentration of chlorogenic acid and its lack of other free phenolic acids except for traces of the constituents of chlorogenic acid—caffeic and quinic acids (Table I).

As in rapeseed (Krygier et al., 1982b), the levels of soluble esters and glycosides of the phenolic acids in the cereals flours were 2–5 times greater than the free phenolic acid levels, with corn again showing the highest concentrations (Table II). Ferulic, syringic, and vanillic acids were the principal phenolic aglycons, but greater than trace levels of *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, and sinapic acids were present in some cereals. Caffeic acid was the principal phenolic aglycon liberated in potato flour which also contained 5 ppm each of ferulic and *p*-coumaric acids (Table II).

Less than 1 ppm of caffeic acid was present in the hydrolysate of the insoluble-bound phenolics in the potato residue (Table III), indicating that the potato flour contained primarily chlorogenic acid and a lesser quantity of ferulic acid glycosides. While rapeseed (Krygier et al., 1982b) and the potato were almost devoid of insoluble–

Table III. Phenolic Acids Liberated from the Insoluble Residue (ppm)

phenolic acid	wheat fresh	wheat stored	rice	oats	corn	potato
p-hydroxybenzoic	trace		0.4	trace	trace	trace
vanillic	trace		trace			trace
protocatechuic	trace			trace		
syringic	1.4			trace	trace	
trans-*p*-coumaric	trace		trace	0.8	trace	trace
cis-ferulic	trace		trace	0.7	0.8	trace
trans-ferulic	58.6	21.6	63.1	54.6	208.6	trace
caffeic	trace			1.6	4.5	0.8
trans-sinapic	trace			trace	trace	
total	60.0	21.6	63.5	57.7	213.9	0.8
% of total phenolics	84.0	84.0	74.2	66.3	69.2	0.2

Table IV. Total Phenolic Acids in Flours (ppm)

phenolic acid	wheat fresh	wheat stored	rice	oats	corn	potato
p-hydroxybenzoic	trace	0.1	5.0	1.4	1.3	trace
(*p*-hydroxyphenyl)acetic			trace	0.4	1.1	
vanillic	3.6	0.8	2.1	4.2	3.7	trace
protocatechuic	trace	trace		0.5	3.0	
syringic	4.2	0.9	0.2	5.3	11.5	
quinic						trace
cis-*p*-coumaric			trace		trace	
trans-*p*-coumaric	trace	trace	1.3	2.0	18.9	5.1
cis-ferulic	trace	0.6	1.9	2.6	6.5	trace
trans-ferulic	63.6	23.3	75.1	63.7	258.6	4.8
caffeic	trace			2.6	4.5	59.2
cis-sinapic	trace		trace	trace		
trans-sinapic	trace	trace	trace	4.3	trace	
chlorogenic						341.3
total	71.4	25.7	85.6	87.0	309.1	410.4
% of ferulic acid	89.1	93.0	90.0	76.2	85.1	1.2

bound phenolic acids, the cereal residues contained quite high levels, especially in corn (Table III). The total bound phenolic acids represented from 66% (oats) to 84% (wheat) of the total phenolic compounds in the flours. *trans*-Ferulic acid was the predominant phenolic acid released by hydrolysis of the cereal residues, corn having almost 4 times the concentrations found in the other cereals. There were only a few other phenolic acids recovered in concentrations of 2–5 ppm.

The sum of *cis*- and *trans*-ferulic acids represented 76% (oats) to 90% (wheat; rice) of the total phenolic acids present in the three fractions (Table IV). In potato, the soluble ester, chlorogenic acid, accounted for 83% of the total phenolic acids (Table I). Previously, Amberger and Schaller (1975) found that the average chlorogenic and caffeic acid levels in potato cultivars were 258 and 245 ppm, respectively, but there were wide variations due to location and cultivar.

The reactive nature of hydroxy-substituted cinnamic acids is due to the presence of an acrylic acid group conjugated with the aromatic ring, which facilitates oxidation of ring-hydroxy groups to the corresponding *o*-quinone (Sosulski, 1979). The oxidation potential of the monocyclic phenolics is highest in the 2,4,5-trihydroxy compounds and least reactive among the monophenols. The *o*-dihydroxyphenols like caffeic and chlorogenic acids are more susceptible to oxidation to *o*-quinone than partially methylated phenolics like ferulic acid. In the present study, the high level of free chlorogenic acid in potato would provide an excellent substrate for phenol oxidase when cell structures are disrupted. On the other hand, most of the phenolic acids in the cereals were bound to insoluble residues and would have little immediate effect on the color or flavor of aqueous slurries of cereal flours. These results are consistent with the common experience of rapid discoloration of potato flesh after peeling but the limited degree of astringency and cereal flavor in cereals when incorporated into moist food products. The taste threshold of 90 ppm for ferulic acid (Maga and Lorenz, 1973) was not exceeded in the two soluble fractions of flours (Tables I and II). However, bound phenolic acids could be liberated during wet processing, especially under alkaline conditions, and during cooking or baking. In addition, taste thresholds for mixtures of phenolic acids are much lower than for individual acids (Maga and Lorenz, 1973). Corn appeared more susceptible to the development of objectionable flavors and colors than other cereal flours.

The fresh and stored samples of wheat flour contained essentially the same phenolic acids in each fraction (Tables I–III). However, the stored sample contained only one-third that of the fresh flour. Apparently ferulic acid, even in the bound form, underwent destructive oxidation reactions during storage. The loss in polyphenols during storage has been observed for chlorogenic acid and rutin in tobacco (Sheen and Calvert, 1969) and hydroxylated derivatives of benzoic acid in pecans (Senter et al., 1980).

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Detection of Floc-Producing Sugars by a Protein Dye-Binding Method

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Trace amounts of protein have been shown to be major contributors to the ability of granulated cane sugar to cause floc in carbonated beverages. This research was designed to develop a protein dye-binding technique for determining the quantitative presence of the proteins in suspected floc-causing sugars. The procedure was standardized for variables of reagent concentrations, time, colors, and volumes. The results showed a significant correlation between the protein levels determined by the dye-binding method and the Kjeldahl procedure. Floc-positive sugars showed protein concentrations ranging from 0.3 to 0.4% whereas the floc-negative samples ranged from 0.004 to 0.006%. On the basis of these results, it can be concluded that this dye-binding method can serve as a reliable and rapid procedure for predicting the ability of a sugar to produce floc.

The occurrence of floc in acidified carbonated beverages, bottler's concentrates, and pharmaceutical syrups has been a major production problem for many years. Eis et al. (1952) identified the causative agent for floc production in beet sugar as a saponin. However, this was not the case when floc-producing cane sugar was analyzed. Efforts to identify the floc-causing substance (FCS) in cane sugar has led to the analyses and identification of several impurities in the sugar: namely, starch, wax, ash constituents, decolorizing carbon, protein, and silicon dioxide (Roberts and Carpenter, 1974; Stansbury and Hoffpauir, 1959).

Research to detect the FCS has narrowed the possibilities to protein and an amylose derivative (Cohen et al., 1970; Liuzzo and Hsu, 1975). Liuzzo et al. (1977) reported that the amino acid content of floc was directly related to the flocking occurrences of sugar. These workers concluded that the FCS in granulated cane sugar was due to an amylose-related substance which can complex a number of other compounds to enhance the floc formation, especially trace protein.

The removal of the FCS from the tons of cane sugar usually purchased by a commercial firm for use in production does not presently seem to be economically feasible. A more practical approach to the problem would be to detect the FCS in a shipment by a rapid and reliable method in order to divert the sugar to an industry whose products are not affected by the sugar tendency to floc.

Protein dye-binding techniques applied to detection of protein in several food products have been used since they were first reported by Fraenkel-Conrat and Cooper (1944). The purpose of this research was to develop a rapid dye-binding technique for detection of the protein responsible for flocculation, thus providing a screening procedure for flocking sugars.

EXPERIMENTAL SECTION

Samples. All sugar samples used were granulated cane sugars. Three floc-positive sugars and a floc-negative sample were obtained from the American Society of Soft Drink Technologists. Three floc-negative sugars were purchased from local supermarkets. Chemically pure sucrose was used for comparative purposes.

Protein Separation. The amount of protein in refined sugars is extremely small. Therefore, this presents a problem in its separation from the sugars. Several methods were attempted, but a modification of the Sevag method (Staub, 1965) proved most suitable.

Three hundred grams of cane sugar was dissolved in 300 mL of distilled water and filtered through a coarse-porosity fritted glass funnel to remove gross foreign materials. The solution was placed in centrifuge bottles, and chloroform at 20% of the water volume of the sugar solution was added. This was followed by 1-butanol at 20% of the chloroform volume. The contents were mixed on a rotary shaker (Lab-Line Junior Orbit Shaker) set a 200 rpm to facilitate the denaturation of protein in the chloroform emulsion. After 30 min, the mixture was centrifuged at 16300 g (Sorvall type GSA high-speed centrifuge rotor) for 5 min. A gellike layer appeared in the water–chloroform interface. The aqueous phase was separated in a separatory funnel, and the chloroform phase was washed with water to remove all the gellike interface which contained the trace protein.

Protein Dye Binding. The sugar impurities in the gellike interface were collected in a test tube, and 0.02 mL was diluted to 0.27 mL with distilled water. The diluted sample was placed in an 8 × 60 mM test tube to which was added 0.03 mL of 1 M Tris-HCl at pH 7.5 containing 1% sodium dodecyl sulfate (Long, 1961).

Trichloroacetic acid (0.06 mL of 60% was added to each tube to give a final concentration of 10%. The samples were mixed on a Vortex mixer for 2 min. The contents of each tube was spot filtered under suction through a Millipore membrane of 0.22-μm pore size. The tube was rinsed with about 0.3 mL of 6% Cl_3AcOH. The entire filter area was flooded twice with 2 mL of the Cl_3AcOH solution.

The membrane was stained with 0.05% Amido Black 10B for 15 min. The dye solution was prepared by dissolving the Amido Black in a mixture of methanol, glacial acetic acid, and distilled water (45:10:45 vol %). Acid Orange 12, Orange G, and Coomassie Blue were not rec-

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