Polyphenols of the Bran–Aleurone Fraction of Buckwheat Seed (*Fagopyrum sagitatum*, Gilib)

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A qualitative survey of certain classes of polyphenols in the bran–aleurone layer fraction of buckwheat (*Fagopyrum sagitatum*, Gilib) showed that syringic acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid occurred in a bound form and were liberated by either alkaline or acid hydrolysis, indicating the possibility of both ester and glycosidic linkages. These acids were obtained by hydrolysis of the soluble (alcohol–water) fraction and the residues. Chromatography of the alcohol–water extracts and digestion of these extracts with alcoholic–acid (5:1) and subsequent chromatography showed that flavan-3,4-diols and soluble condensed tannin were present. No flavone or flavonol glycosides were identified in the extracts. The presence of tannin and other phenolic derivatives could have an adverse effect on the quality of this fraction as a food especially with respect to taste and color.

Recently emphasis has been placed on naturally occurring oxidizable phenols and their deleterious effect on the preparation of protein from plant material for use as a food (Synge, 1975). This could be especially significant with respect to the bran–aleurone layer fraction from buckwheat seed. The fraction is high in protein but contains much of the seed coat material and may contain tannin. Nevertheless, according to Synge, reactions of even simple phenolic compounds with proteins have been almost entirely overlooked, yet these phenolic substances occur ubiquitously in plants.

Little information is available on polyphenolic compounds in seeds, including buckwheat. Rutin (quercetin 3-rutinoside) is a well-known glycoside of the leaves (Schunk, 1858) and quinones have been reported in *Fagopyrum* and other members of the *Polygonales* (Mathis, 1966). Apart from some new phenolic amine glycosides and amides recently characterized in buckwheat seed (Koyama et al., 1973, 1974), there is not much information available on the bound phenolic acid and tannin components of buckwheat seed. Since these particular types of polyphenolics are probably responsible for discoloration, astringency, and reaction with proteins, it is of interest to analyze the buckwheat seed fraction for these polyphenols.

EXPERIMENTAL SECTION

Materials. The bran–aleurone fraction was separated from the cotyledon fraction and both were provided by Dr. R. Wasik of the Food Research Institute. Cyanidin, pelargonidin, kaempferol, quercetin, and the phenolic acids, used as authentic markers for chromatography, were obtained from commercial sources and were not further purified.

Methods. *Phenolic Acids.* The phenolic acids were extracted as the combined forms with 70% ethanol and liberated by either acid (2 N HCl at 100 °C for 30 min) or alkaline hydrolysis (2 N NaOH at room temperature, 4 h), separated, and identified on paper chromatograms by methods previously described (Durkee and Thivierge, 1975).

Flavonols. The 70% ethanolic extracts were concentrated and subjected to acid hydrolysis with 2 N HCl at 100 °C for 45 min and the aglycons extracted into ethyl acetate. The concentrated extracts were chromatographed on Whatman No. 1 paper and developed overnight in Forestal solvent (acetic acid–concentrated hydrochloric acid–water, 15:1.5:5). The air-dried chromatograms were examined under UV light to detect yellow fluorescence due to flavonol aglycons and fumed with ammonia to detect flavone aglycons. The dark brown spots of the latter turn to a yellow color in the presence of ammonia.

Proanthocyanidins (Condensed Tannins and Precursors). The proanthocyanidins were detected in the bran–aleurone fraction by digestion of the meal with hot butanol–concentrated HCl (5:1). Upon addition of water the red pigment separated entirely in the butanol layer which was concentrated in vacuo and applied as a streak on Whatman No. 3MM paper and developed in Forestal overnight.

The separated anthocyanidins were eluted with acidic methanol and their spectral properties observed. Spots of the eluates were re-run in Forestal and BAW (butanol–acetic acid–water, 4:1:5) for comparison with authentic anthocyanidins, by color, R_f value, and cochromatography. Soluble proanthocyanidins or oligomeric tannins were also detected, when 70% ethanol extracts were acidified (5:1) and heated in the water bath at 100 °C. To detect the soluble flavans untreated 70% ethanol extracts were chromatographed and developed in BAW. The positions of the flavan bands were located by spraying a section of the chromatogram with diazotized sulfanilic acid (Block et al., 1958) and another with vanillic reagent (Ribereau-Gayon, 1972). The unsprayed portions were eluted with methanol–concentrated HCl (5:1) and the eluates heated on the water bath for 5 min. Flavan-3,4-diols were identified by the production of red pigments. The partial structure and nature of the condensed tannin are determined by identification of the anthocyanidins.

Spectra. All absorption maxima were obtained using a Bausch and Lomb 502 recording spectrophotometer.

RESULTS AND DISCUSSION

The phenolic acids liberated by hydrolysis are shown in Table I. Their identity was based on chromatography described earlier (Durkee and Thivierge, 1975). Syringic acid, *p*-hydroxybenzoic acid, and vanillic acid, which occurred as soluble bound constituents in the bran–aleurone fraction, were liberated by alkaline or acid hydrolysis. A sample of the flour extract was chromatographed and essentially the same phenolic acids were noted, except that *p*-coumaric was also present but syringic acid was absent.

The absence of hydroxycinnamic acids in the bran–aleurone layer fraction and the presence of the hydroxybenzoic acids may be an indication of the presence of lignin in the seed coat (Harborne and Simmonds, 1964). Ferulic acid, present in most cereals and oilseeds, is absent in the bound soluble and insoluble form in buckwheat bran and flour.

It has been shown recently that most oilseeds and cereals contain free and bound phenolic acids (Maga and Lorenz,

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Table I. Phenolic Acids in Buckwheat Bran-Aleurone and Flour Fraction Hydrolysates

Substance	R_f[a] TAW(5)	R_f[a] TAW(48)	Color[a] Diaz S	Identity	Hydrolysis	Fraction
Compd A	0.29	[b]	Red	Syringic acid	HCl	Bran only
Compd B	0.08	0.32	Yellow	*p*-Hydroxybenzoic acid	HCl + NaOH	Both
Compd C	0.48	[b]	Orange	Vanillic acid	HCl + NaOH	Both
Compd D	0.15	0.70	Red	*p*-Coumaric acid	HCl + NaOH	Flour only
Unknown 1	0.40	[b]	Yellow		HCl + NaOH	Both
Unknown 2	0.15	0.20	Pink		HCl + NaOH	Both

[a] TAW(5), toluene-acetic acid-water (4:1:5), 5-h development; TAW(48), toluene-acetic acid-water (4:1:5), 48-h development; Diaz S, diazotized sulfanilic acid, followed by 20% sodium carbonate. [b] Completely run off the paper.

Table II. Flavones, Flavonols, and Derived Anthocyanidins from the Buckwheat Bran-Aleurone Layer Meal Fraction

Substance	R_f BAW	R_f Forestal	Color UV[a]	Color Van	Color Diaz S[b]	Spectra max, nm	Identity
Band 1	0.15			Pink	Yellow	530[c]	Flavon-3,4-diol
Band 2	0.35			Pink	Yellow	534[c]	Flavon-3,4-diol
Pigment 1	0.70	0.64				523	Pelargonidin
Pigment 2	0.45	0.50				535	Cyanidin
Unknown flavonol		0.85	y				?
Quercetin		0.40	y				Standard
Kaempferol		0.58	y				Standard

[a] y = yellow fluorescence in UV. [b] Van = vanillin reagent; diaz S = diazotized sulfanilic acid. [c] Maximum after treatment with hot BuOH-HCl (5:1).

1974; Van Sumere et al., 1972). Maga and Lorenz (1973) determined the taste threshold values for many of these acids and found that certain combinations of acids had lower threshold values than the acids alone. The potential for astringency characteristics in the protein rich bran–aleurone fraction of buckwheat is high, since some of the combinations occur.

Soluble proanthocyanidins (flavans) which occur in the bran–aleurone fraction are shown in Table II. They confirm the presence of soluble oligomeric condensed tannin. Their presence, along with the phenolic acids, increases the chance for astringency (Goldstein and Swain, 1963). The buckwheat tannin is based on both pelargonidin and cyanidin structures. The identity of these anthocyanidins was established by cochromatography and comparisons of R_f values in two solvent systems using authentic substances.

No real evidence was obtained for the presence of quercetin, kaempferol, or myricetin on the Forestal chromatograms of the extracts subjected to 48-min hydrolysis at 100 °C with 2 N HCl. Table II shows that a yellow fluorescent spot was noted at R_f 0.85. The 7-glycosides which are fairly resistant to hydrolysis are yellow under UV light and the R_f values with Forestal solvent do not rule out the 7-glycosides, but all the 3-glycosides would be hydrolyzed and the results show that rutin (quercetin 3-rutinoside) was therefore absent in the bran–aleurone fraction. The latter has been reported in immature buckwheat seed by Sato and Sakamura (1975), along with quercetrin and hyperin. If they exist in these seeds, they are likely constituents of the flour fraction, which was not examined for flavonol aglycons at this time.

The chemistry of the common phenolics in the dicot buckwheat differs to quite an extent from the cereals. In buckwheat, ferulic acid and other hydroxycinnamic acids are low, if not absent, but condensed tannin is present. Some cereals, for example, *Sorghum vulgare* (Strumeyer and Malin, 1975) and barley (Bate-Smith and Rasper, 1969), contain tannins, but generally they are absent in the *Graminae*.

From the standpoint of undesirable colors and taste in buckwheat protein concentrates prepared from the bran–aleurone fraction, the soluble tannins would be the most troublesome. In rape, soya, and the usual cereal grains, which are possible protein sources, there is very little tannin, but the seeds and grains do contain hydroxycinnamic and hydroxybenzoic acids, probably both free and bound. Certainly soybeans contain these acids in bound form and soya concentrates have been shown to be fairly acceptable at the present time. More research on the effect of phenolics on color, taste, and nutritive value of plant proteins is therefore necessary.

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