Simple Sugars, Oligosaccharides, and Starch Concentrations in Raw and Cooked Sweet Potato

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Compositions of the soluble sugars extracted with 70% ethanol from five raw and five cooked Philippine sweet potato tubers were analyzed by HPLC. The sum of glucose, fructose, and sucrose accounted for 85–96% and 17–54% of the total soluble sugars identified in the extracted fractions of raw and cooked samples, respectively. Verbascose was present only in trace amounts, and stachyose was not detected in any of the samples. Starch content of raw and cooked samples determined enzymatically ranged from 33 to 73% and 32 to 61%. Starch degradation products, maltose and maltotriose, present in the cooked samples, coeluted with cellobiose and raffinose, respectively. GC analysis or amylase pretreatment of the extracted sugar solution before HPLC analysis revealed that the tubers contained 0.23–0.4% cellobiose and negligible raffinose. Unlike legumes, the concentrations of indigestible oligosaccharides in sweet potato were too low to account for the flatulence that accompanies ingestion of sweet potatoes as a staple food.

INTRODUCTION

Sweet potatoes (*Ipomoea batatas* (L.) Lam) are grown throughout the tropics and subtropics from the marginal to the cultivated areas. Despite the fact that they are viewed favorably for their high-yielding ability and wide ecological adaptability, sweet potatoes are usaully only consumed as substitutes or supplements to rice or corn when supplies of these staple foods are low, or as the main ingredient of some traditional but infrequently consumed delicacies (Truong and del Rosario, 1984). In developing countries, the consumption of sweet potato appears to be inversely proportional to income level, and they are typically viewed as a food to be consumed only for survival (Tsou and Villareal, 1982). The high sugar content of the tuber and the flatulence that occurs with the consumption of large amounts of sweet potatoes are other factors that hinder the use of sweet potato as a staple food (Tsou and Villareal, 1982).

Flatulence is a common phenomenon accompanying the ingestion of legumes, and the oligosaccharides raffinose, stachyose, and verbascose have been demonstrated to induce flatus (Rackis, 1975). Food legumes such as cowpea, chickpea, black gram, field bean and lentils contain these oligosaccharides in the range of 5.5–8.0%, with verbascose being the major saccharide (Hardinge et al., 1965; Christofaro et al., 1974; Reddy and Salunkhe, 1980; Mendoza et al., 1980). Soybeans contain about 1% raffinose and 2.5% stachyose (Rackis, 1975), and winged bean contains 1–2% raffinose, 2–4% stachyose, and 0.2–1% verbascose (Garcia and Palmer, 1980). These seed carbohydrates escape digestion and absorption in the upper digestive tract and instead are fermented by colonic bacteria to yield flatus gases, primarily H_2 and CO_2. Aside from the reserve oligosaccharides, Salyer et al. (1979) reported that a variety of polysaccharides constituting plant cell wall can also induce flatus. In addition, it has been recently demonstrated by direct measurement that as much as 20% of the starch in a mixed meal containing 20–60 g of starch escapes digestion in the small bowel (Stephen et al., 1983). Use of the sweet potato as a staple food would lead to the ingestion of substantial amounts of starch. A variety of cooked American cultivars have been reported to contain about 4% starch (fresh weight) (Reddy and Sistrank, 1980).

Changes in the carbohydrates of sweet potato tubers during maturity, storage, and processing have been reported (Hammett and Barrentine, 1961; Ali and Jones, 1967; Sistrunk, 1977; Reddy and Sistrunk, 1980; Kawabata et al., 1984), although none of these studies investigated the flatus-inducing substances in sweet potato. Further, these studies measured total soluble carbohydrates in sweet potatoes colorimetrically. Recently, Palmer (1982), using a combination of gas (GC) and thin-layer chromatography (TLC) techniques, has reported that tubers of American sweet potato contain a small amount of raffinose (0.5% fresh weight basis), 4.4% sucrose, 5.5% maltose, and <1% of fructose and glucose. Using a combination of HPLC and TLC, Picha (1985) has reported similar amounts of sucrose, glucose, and fructose but larger amounts of maltose from 4.0 to 14.1%, in baked American sweet potatoes.

There were two purposes to the present investigation: First, the simple sugars in raw and cooked Philippine sweet potatoes were measured by HPLC and compared to analysis by GC. Second, the concentrations of oligosaccharides, detected by HPLC and in some instances verified by GC, and starch, which was measured enzymatically, were determined in both raw and cooked sweet potatoes to assess their possible role in the clinically evident flatus that accompanies the ingestion of large amounts of sweet potatoes. HPLC, in which derivatization is not necessary prior to analysis, has been successfully employed in the analysis of oligosaccharides in other foods and food products (Conrad and Palmer, 1976; Black and Bagley, 1978).

MATERIALS AND METHODS

Sample Preparation. Four high-yielding varieties of sweet potato, namely VSP-1, VSP-2, VSP-3, and BNAS-51, which were developed by the Visayas State College of Agriculture (ViSCA, Philippines), were used in the study. The four varieties were planted in the dry season of 1983 at the ViSCA experimental field, and samples were harvested at 120 days after planting. Fresh tubers of a locally popular variety, i.e. Karinkit, which were bought at Baybay market, Leyte, Philippines, were also included in the analysis. The fresh tubers were washed, peeled, sliced, oven-dried at 50 °C, and ground into 40-mesh flour with a Wiley Mill. For the cooked samples, the same procedure

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0021-8561/86/1434-0421$01.50/0

was followed except that the tubers were steamed for 30 min after washing. The cooked sample of the American sweet potato was a vacuum-packed canned product from John W. Taylor Packing Co., Hallwood, VA, while the uncooked sample was prepared from Louisiana Yam tubers purchased at a store in Madison, WI. Preparation of the two samples was similar to that described above.

Sugar Extraction and Analysis by HPLC. The extraction process was carried out with the ground dried raw and cooked samples. Five grams of flour was extracted with 50 mL of an ethanol–water solution (70:30) by refluxing for 30 min (Schweizer et al., 1978). The hot solution was filtered through Whatman No. 54 filter paper. The residue was rinsed with four 10-mL portions of warm (~50 °C) 70% ethanol–water, and the rinsings were combined with the filtrate. The 70% ethanol, which is more polar than 80% ethanol, assured extraction of verbascose. To assess the extraction efficiency of the solvent with respect to the mono-, di-, and trisaccharides, aliquots of the Louisiana yam residue, which had been extracted with 70% ethanol, were reextracted with boiling water. An internal standard, 50 mg of erythritol, was added at this point. A preliminary experiment revealed that erythritol did not coelute with any compounds in the extracted solution. Extracts were then evaporated to approximately 10 mL in a rotary evaporator, centrifuged at 4300 RCF (Du Pont Sorvall Centrifuge 5B, Wilmington, DE) for 15 min and filtered through a 0.45-μm membrane filter (Gelman, Ann Arbor, MI). All samples were prepared in duplicate. Standard solutions were prepared with sugars from Sigma Chemicals (St. Louis, MO). Standard sugars were vacuum-dried (over P_2O_5, ≤40 °C), prepared in acetonitrile–deionized distilled water (30:70) in a concentration of 10 mg of each sugar/mL, and refrigerated when not in use. Verbascose was supplied by Dr. Schweizer, Nestle Research Department, Switzerland.

For HPLC analysis a Bio-Sil Amino 5S column (Bio-Rad Laboratories, Richmond, CA), which is an amino-bonded silica material with a dimension of 250 × 4 mm, was used. A 10-μL portion of each sample and 10–20 μL of the standard were injected. The eluant was 72:28 acetonitrile–water, with a flow rate of 1.0 mL/min at room temperature. Verbascose and stachyose were eluted with 60:40 acetonitrile–water to reduce analysis time; at this concentration of acetonitrile, fructose and glucose coeluted at ~4 min, as did sucrose, maltose, and cellobiose at ~4.7 min. Raffinose (room temperature, 6.2 min), stachyose (8.8 min), and verbascose (12.4 min) were well separated as sharp peaks that could be accurately integrated. A refractive index detector (Waters Associates, Medford, MA) attached to a HPLC unit (Milton Roy Co., Riviera Beach, FL) was used to detect the carbohydrates. Quantitation was obtained by peak areas using electronic integration (Hewlett-Packard Model 3390A, Avondale, PA). Every sample was analyzed in duplicate and with both concentrations of acetonitrile.

HPLC and GC analyses of the fructose, glucose, and sucrose in the samples were compared using samples prepared for GC separation as outlined below.

Verification of Coelution of Saccharides by HPLC. The coelutions of raffinose and maltotriose, and of maltose and cellobiose, were verified by two methods:

First, extracts of raw and cooked samples were subjected to enzymatic hydrolysis before HPLC analysis using amyloglucosidase from Rhizopus mold (10 000 units/g; Sigma, No. A-7255, St. Louis, MO) added as 1.0 mL of a 1 mg/mL solution of amylase in a 0.1 M acetate buffer, pH 5.0, containing 0.02% sodium azide, to 1.0 mL of concentrated extract. Samples were incubated at room temperature for 16 h, centrifuged, and filtered through a 0.45-μm membrane filter, as described above. A 20-μL aliquot was injected into the HPLC.

Second, the concentrations of raffinose, maltotriose, maltose, and cellobiose in the extracts were determined by GC. An aliquot (1 mL) of extract and rinses, prepared from 5 g of sweet potato flour as outlined above, was evaporated to dryness at room temperature at reduced pressure and silylated as described by Sweeley et al. (1963). The GC standard consisted of fructose, glucose, maltose, and myoinositol, the internal standard, in concentrations of 2 mg/mL, cellobiose and raffinose at 1 mg/mL, and sucrose at 5 mg/mL in deionized, distilled water. An aliquot of 0.25 mL was evaporated and derivatized. Standard was stored frozen (−20 °C). Injections of samples and standard were 1 μL. A Tracor GC (Model 560, Austin, TX) was used with an SPB-5 wide-bore capillary column (60 m; Supelco, Inc., Bellefonte, PA). The temperature program for the separations was 160 °C for 5 min and then 5 °C/min to 320 °C for 10 min. The carrier gas was hydrogen at a flow rate of 64 cm/s. Quantitation was as described for the HPLC chromatograms.

Starch Analysis. Starch was measured following enzymatic hydrolysis by glucose oxidase, using the procedure of the American Association of Cereal Chemists (1976) on 40-mg aliquots (dry weight). Sweet potato flour had been extracted twice with boiling ethanol (80%) and twice with 80% ethanol at 25 °C prior to enzymatic treatment.

Table I. Sequential Extraction of Sweet Potato Carbohydrates (Louisiana Yam) by Ethanol–Water (70:30) and Boiling Water (mg/g of Dry Weight)

component	ethanol–water extr	boiling water extr
fructose	37.3	0.27
glucose	43.9	0.37
sucrose	100.0	0.71
maltose/cellobiose	353.0	2.80
raffinose/maltotriose	1.1	0.07
stachyose	ND[a]	ND

[a] ND = not detectable, less than 0.2 mg/g.

RESULTS AND DISCUSSION

Extraction of Sweet Potato Carbohydrates. A 70% ethanol solution efficiently extracted the simple sugars and di- and oligosaccharides from sweet potato samples (Table I). Less than 1% of the total amount of any of the components extracted by the ethanol–water solution appeared in the sequential boiling water extract.

Separation of Sweet Potato Carbohydrates by HPLC. Representative HPLC chromatograms of the standard sugar solution and the extracts of one of the sweet potato varieties (VSP-1) are shown in Figure 1. The components were identified by comparing their retention times to those of the standard sugars. The concentrations of simple sugars measured by HPLC were generally similar to those obtained by GC analysis (Table II). The total amounts of fructose, glucose, and sucrose accounted for 85–96% and 17–54% of the soluble sugars identified in raw and cooked Filipino samples, respectively (Table II). Although sucrose was the most abundant sugar in raw sweet potato, accounting for 76–93% of the total simple sugars, the amounts of sucrose varied greatly from one cultivar to another. These data confirm the findings of Picha (1985) and Kawabata et al. (1984). The changes in sucrose concentration that occurred with cooking appear to be variety related. Tubers of VSP-1 and VSP-2 exhibited a large reduction in sucrose content after cooking. However, the trend was not the same with other cultivars

Table II. GC and HPLC Analysis[a] of Simple Sugars and Enzymatic Analysis of Starch in Filipino and American Sweet Potatoes (mg/g of Dry Weight)

variety	fructose	glucose	sucrose	starch[b]
VSP-1				
raw, HPLC	17.3 ± 0.0	20.7 ± 0.3	142.0 ± 0.3	579.5 ± 3.5
raw, GC	14.2 ± 1.1	16.9 ± 0.8	162.5 ± 2.1	
cooked, HPLC	18.2 ± 0.1	42.7 ± 2.1	12.2 ± 1.4	447.0 ± 7.1
cooked, GC	19.8 ± 0.7	39.2 ± 1.1	13.7 ± 0.1	
VSP-2				
raw, HPLC	12.4 ± 0.5	14.7 ± 0.9	126.0 ± 3.5	588.5 ± 7.8
raw, GC	9.9 ± 0.2	9.7 ± 0.2	140.0 ± 1.4	
cooked, HPLC	13.7 ± 0.5	25.7 ± 1.1	12.3 ± 0.3	371.0 ± 7.1
cooked, GC	14.3	23.6	13.3	
VSP-3				
raw, HPLC	8.1 ± 0.1	11.4 ± 0.8	62.0 ± 2.0	636.0 ± 1.4
raw, GC	13.6 ± 0.1	13.6 ± 0.1	74.0 ± 1.4	
cooked, HPLC	11.1 ± 0.6	14.9 ± 0.5	66.0 ± 2.4	550.0 ± 2.1
BNAS-51				
raw, HPLC	4.8 ± 0.3	6.2 ± 0.1	135.0 ± 8.5	584.3 ± 23.7
cooked, HPLC	0.9 ± 0.1	2.3 ± 0.0	114.0 ± 1.4	571.8 ± 12.4
Karinkit				
raw, HPLC	3.7 ± 0.0	4.5 ± 0.0	42.6 ± 1.8	729.3 ± 1.1
cooked, HPLC	3.9 ± 0.1	4.4 ± 0.0	41.1 ± 1.5	611.0 ± 14.8
Louisiana yam				
raw, HPLC	40.0 ± 0.7	42.0 ± 2.1	297.0 ± 3.5	331.8 ± 6.7
canned, HPLC	36.9 ± 0.5	44.4 ± 0.4	99.2 ± 0.7	317.3 ± 3.8

[a] Mean ± SD, n = 2 except for VSP-2, cooked, GC. [b] Determined enzymatically.

Table III. Oligosaccharides in 70% Ethanol Extract of Filipino and American Sweet Potatoes (mg/g of Dry Weight)

variety	maltose/ cellobiose	raffinose/ maltotriose	stachyose	verbascose
VSP-1				
raw, HPLC	7.4 ± 0.4[a]	0.5 ± 0.1	ND[b]	ND
raw, GC	2.2 ± 0.1/0.7 ± 0.0	1.4 ± 0.0/0.4 ± 0.0	NA[c]	NA
cooked, HPLC	47.9 ± 0.4	9.7 ± 1.4	ND	ND
cooked, GC	41.3 ± 2.9/1.2 ± 0.0	1.1 ± 0.1/8.2 ± 0.0	NA	NA
VSP-2				
raw, HPLC	8.1 ± 1.0	ND	ND	ND
raw, GC	1.8 ± 0.1/ND	0.9 ± 0.1/0.4 ± 0.0	NA	NA
cooked, HPLC	21.6 ± 1.6	16.2 ± 0.6	ND	tr
cooked, GC	23.9/ND	ND/14.6	NA	NA
VSP-3				
raw, HPLC	1.6 ± 0.1	ND	ND	ND
raw, GC	4.1 ± 0.1/ND	0.5 ± 0.0/ND	NA	NA
cooked, HPLC	118.0 ± 6.3	0.9 ± 0.1	ND	ND
BNAS-51				
raw, HPLC	6.5 ± 0.7	0.4 ± 0.0	ND	ND
cooked, HPLC	100.0 ± 0.2	0.2 ± 0.0	ND	ND
Karinkit				
raw, HPLC	5.0 ± 0.0	2.1 ± 0.4	ND	ND
cooked, HPLC	112.0 ± 1.7	2.1 ± 0.2	ND	ND
Louisiana yam				
raw, HPLC	4.0 ± 0.0	1.2 ± 0.0	ND	ND
canned, HPLC	35.8 ± 1.4	1.2 ± 0.1	ND	ND

[a] Mean ± SD, n = 2 except for VSP-2, cooked, GC. [b] ND = not detectable, less than 0.2 mg/g. [c] NA = not analyzed.

(Table II). Kawabata et al. (1984) reported a substantial decrease in sucrose concentration with cooking, from 54.8 mg/g (dry weight) in raw tubers to 30.8 mg/g in boiled sweet potato.

Oligosaccharide Content of Sweet Potato. When the analysis was accomplished by HPLC, raffinose and maltotriose coeluted, as did maltose and cellobiose (Table III). The peaks of both raffinose and maltose were greatly diminished on the HPLC chromatograms of the amylase-treated samples (Figure 1; Table IV). These data suggesting that maltotriose and maltose were responsible for most of the raffinose and cellobiose peaks were verified by GC analysis (Table III). Both methods demonstrated that the actual amounts of raffinose and cellobiose in the samples of VSP varieties were much less than what was determined in the HPLC analyses. Previous investigations of the soluble carbohydrates in sweet potato (Palmer, 1982; Kawabata et al., 1984), as well as of legumes (Garcia and Palmer, 1980; Sosulski et al., 1982), did not mention the possibility of the existence of interferring carbohydrates like maltotriose, cellobiose, and others. Picha (1985), however, has reported the coelution of maltose and sucrose on an HPX-87C (Bio-Rad) HPLC column.

Table IV. Raffinose and Cellobiose Content of Sweet Potato Extract Treated with Amyloglucosidase (mg/g of Dry Weight)

sample	cellobiose	raffinose
VSP-1 cooked	2.4 ± 0.1[a]	ND[b]
VSP-1 raw	2.5 ± 0.2	ND
VSP-2 cooked	4.4 ± 0.1	4.4 ± 0.4
VSP-2 raw	2.3 ± 0.2	ND
VSP-3 raw	ND	ND

[a] Mean percent SD, n = 2. [b] ND = not detectable, less than 0.2 mg/g.

Unlike legumes, in which raffinose, stachyose, and

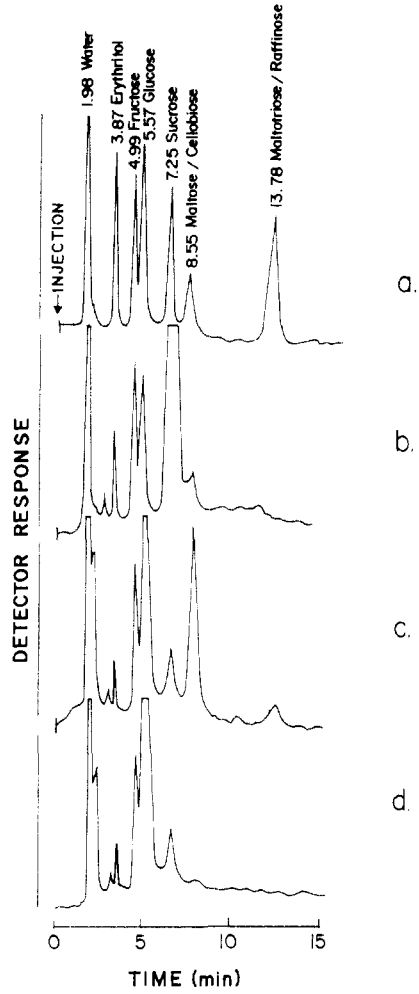

Figure 1. Chromatograms of standard sugars (a), VSP-1 raw sweet potato extract (b), VSP-1 cooked sweet potato extract (c), and VSP-1 cooked sweet potato extract treated with amyloglucosidase (d). Conditions: (a, b) 0.8×10^{-4} RIU FS; (c, d) 1.6×10^{-4} RIU FS.

verbascose account for 40–60% of the total soluble sugars (Hymowitz et al., 1972; Gardia and Palmer, 1980; Sosulski et al., 1982), the concentration of these oligosaccharides in sweet potato was negligible. Raffinose content of both the cooked and uncooked American sweet potato analyzed in this study was only 0.12%, in contrast to the value of 0.5% (fresh weight) reported by Palmer (1982) in baked samples using gas–liquid chromatography and thin-layer chromatography. In contrast, the raffinose content of Filipino sweet potato varied with the variety (Table III). Verbascose was present only in trace amounts, and stachyose was not detected in any of the samples we analyzed. The retention time of the small peak in the chromatograms of the cooked samples of VSP-1 and VSP-2 suggests it is likely a disaccharide; it did not have the same retention time as either melobiose or isomaltose. There were also some additional small peaks of the unknown sugars that account for about 5–10 mg/g of dried materials.

Various chromatographic techniques have been used to analyze oligosaccharides in legumes, and the use of different analytical techniques may be responsible for the contradictory results that have been reported for the effect of cooking on oligosaccharide content in beans. For example, Rao and Belavady (1978) found a significant increase in oligosaccharide content after cooking of the whole seeds of four Indian pulses. On the other hand, Silva and Braga (1982) reported that cooking of whole seeds led to a large decrease in oligosaccharide content.

The starch content of the raw and cooked samples ranged from 317 to 729 mg/g of dry weight (Table II). Starch decreased with steaming in all of the varieties we studied, although magnitude of the decrease varied with the sample. The GC analysis of VSP-1 and VSP-2 suggests that some starch was converted to maltose in these two samples (Table III). Walter et al. (1975) reported that about 42–95% of the starch in several cultivars of sweet potato was converted during baking; most (72–99%) of the converted starch accumulated as maltose. Boiling or roasting (200 °C) sweet potato tubers also resulted in a higher maltose and a lower sucrose concentration than those of the fresh samples (Kawabata et al., 1984).

Because there were only small amounts of oligosaccharides in the samples we analyzed, it is unlikely that they could be responsible for the flatus accompanying large intakes of sweet potatoes. Sweet potato varieties of Papua, New Guinea, also contain negligible amounts of raffinose (Bradbury, 1984). It is possible that some portion of the starch in sweet potato tubers is not hydrolyzable by the enzymes in the gastrointestinal tract until it reaches the large bowel, particularly if large amounts of sweet potato are consumed. Stephen et al. (1983) found that about 2–20% or 0.5–6.4 g of the 20–60 g of starch ingested in the form of rice, beans, bananas, or potatoes was not absorbed by the small bowel. One pound (454 g) of boiled American sweet potatoes (without skin) contains an average of about 500 kcal (Adams, 1975). If sweet potatoes were the staple food, it would be necessary to consume 3 lb to obtain 1500 kcal; on the basis of our starch analyses, about 120 g of starch would be ingested. This amount of starch is 2–6 times the 20–60 g fed by Stephen et al. This finding supports our hypothesis that some of the sweet potato starch may escape the small bowel and be responsible for the distress felt after eating sweet potatoes.

Palmer (1982) has argued correctly that more study is needed of the impression that flatus accompanies sweet potato ingestion. In his survey of the literature he was able to identify only one reference to this side effect. Fitzgerald (1976) reported that 14 of 100 respondents from North Carolina listed indigestion as a reason disliking sweet potatoes. It is highly likely that a survey of a population using sweet potatoes as their major staple food would reveal a higher frequency of complaints. Social and cultural practices in appropriate populations make such a survey difficult. Our data suggest that oligosaccharides are not likely to be the primary flatus-inducing component in sweet potatoes, as they are for the legumes.

Registry No. D-Glucose, 50-99-7; D-fructose, 57-48-7; sucrose, 57-50-1; verbascose, 546-62-3; stachyose, 470-55-3; starch, 9005-25-8; maltose, 69-79-4; maltotriose, 1109-28-0; cellobiose, 528-50-7; raffinose, 512-69-6.

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Received for review April 11, 1985. Revised manuscript received September 19, 1985. Accepted January 24, 1986. This work was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin—Madison, Hatch Project No. 2733, and by National Institute of Health Grant AM 21712. Dr. Marlett was a visiting professor at ViSCA from September to October, 1984, under the University of Wisconsin—Madison Title XII strengthening grant.

Polyphenol Concentrations in Grain, Leaf, and Callus Tissues of Mold-Susceptible and Mold-Resistant Sorghum Cultivars

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Sorghum cultivars exhibiting both resistance and susceptibility to grain mold were subjected to a variety of assays for polyphenols including flavan-4-ols and proanthocyanidins. In methanol and acidified methanol extracts of grains of mold-resistant cultivars, the levels of flavan-4-ols were two- to threefold higher than in mold-susceptible cultivars. Similar results were noted when the leaves of resistant and susceptible cultivars grown under greenhouse conditions were harvested at different stages of growth and analyzed for flavan-4-ols.

INTRODUCTION

The role of plant phenolics including their protective effects against fungi and other pathogens has been reviewed (Friend, 1981). Harris and Burns (1973) reported that sorghum seed tannin content was strongly and negatively correlated with preharvest seed-molding indices. The condensed tannins (proanthocyanidins, oligomers of flavan-3-ols) are depolymerized in acid solution to form anthocyanidin pigments. Cyanidin is the anthocyanidin produced from sorghum tannins (Gupta and Haslam, 1978), which are therefore referred to as procyanidins. Certain monomeric flavanols such as flavan-3,4-diols and flavan-4-ols can also give rise to anthocyanidins and are therefore distinguished from the oligomeric flavan-3-ols by the name "leucoanthocyanidin" (Watterson and Butler, 1983). We observed mold resistance in sorghum cultivars that have relatively low levels of tannin and examined a representative group of susceptible and resistant cultivars with a variety of assays for polyphenols including flavan-4-ols and condensed tannin.

EXPERIMENTAL SECTION

Grain Samples. Sorghum cultivars were grown at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, during the 1982 rainy season. Sorghum cultivars exhibiting both resistance and susceptibility to grain mold caused by a complex range of unspecialized fungi including *Fusarium moniliforme* and *Curvularia lunata* were selected according to established methods (ICRISAT, 1984). The seeds were exported to the U.S. and cleared through the Plant Protection and Quarantine Program of the U.S. Department of Agriculture. All analyses were carried out in the Department of Biochemistry, Purdue University.

All grain samples were ground in an Udy cyclone mill (U.D. Corp., Boulder, CO) to pass through a 0.4-mm screen. The color of the grain meal was measured in a Hunter Lab Colorimeter, Model D25-A2, using a yellow tile standard.

The sorghum meal was defatted by stirring with diethyl ether, 6 mL/g, for 30 min, and the mixture was filtered through a Whatman 541 filter paper. The residue was air-dried overnight and transferred to a screw-capped vial. The defatted sorghum meal was extracted with methanol, 6 mL/g, for 30 min in a screw-capped test tube. After centrifugation, the residue was reextracted with methanol, and the two methanol extracts were combined for analysis. The residue was further extracted twice with methanol containing 1% (v/v) concentrated HCl (H^+/methanol), and these two extracts were pooled together for analysis.

Total phenols were measured by a modification of the Prussian blue assay as described by Butler (1982). Protein-precipitable phenols were estimated by using a modified procedure of Hagerman and Butler (1978), as follows:

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0021-8561/86/1434-0425$01.50/0