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Received for review June 11, 1987. Accepted April 27, 1988. Support in part by a grant from the National Live Stock and Meat Board. Contribution from the Missouri Agricultural Experiment Station. Journal Series No. 10328.

Peanut Hull Flavonoids: Their Relationship with Peanut Maturity

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As peanuts mature, the color of the mesocarp layer of the peanut shell changes from yellow to orange to brown and finally to black. On the assumption that the flavonoid content of the shells could be associated with these color changes, a study of the 5,7-dihydroxychromone, eriodictyol, and luteolin contents of peanut shells was initiated. Total flavonoid content increased as the mesocarp color changed from yellow to black. Relative concentrations of the three flavonoids also changed with maturity. Eriodictyol was the predominant flavonoid in the shells from immature peanuts whereas luteolin was the predominant flavonoid in shells from mature peanuts.

Because of the indeterminate fruiting characteristics of peanuts, seeds of varying maturity are on the plant at harvest time. The most appropriate time to harvest is when the largest number of sound mature kernels is present. Mature peanuts produce better products, and a high percentage of mature kernels increases the value of the peanut crop. Over the years subjective and objective methods for determination of maturity have been developed and used by the peanut industry (Sanders et al., 1982). Probably the oldest method for estimating maturity is based on the color of the internal surface of the hull. As the peanut matures, this internal hull color changes from white to dark brown (Schenk, 1961). Pattee et al. (1974) used seed coat and internal pericarp characteristics, including color, to develop the Physiological Maturity Index. In 1979, Drexler and Williams devised a nondestructive method for predicting harvest date and determining maturity of fresh Florunner cv. pods. The method, which involved scraping off a small portion of the hull exocarp to expose the color of the mesocarp, is described in detail by Williams and Drexler (1981). It is referred to as the Pod Maturity Profile or the Hull-Scrape Method and segregates peanuts into six maturity classes based on mesocarp color. Flavonoids are compounds that could be responsible for or contribute to the color of the mesocarp. Using column chromatography Pendse et al. (1973) isolated three flavonoids—5,7-dihydroxychromone, eriodictyol, and luteolin—from peanut hulls. Daigle et al. (1983) used high-performance liquid chromatography (HPLC) to separate and identify flavonoids in peanut flour and testa. This paper extends the use of HPLC to the flavonoid content of peanut hulls and demonstrates how the amounts of these compounds vary in peanuts of increasing maturity.

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MATERIALS AND METHODS

Peanut Hull Samples. In mid-May 1984, Florunner, cv. peanuts, were planted in a research plot in Tifton, GA. To obtain peanuts at increasing stages of maturity, plants were harvested at 79, 93, 107, 121, and 135 days after planting (DAP). Each time, plants were hand-dug, hand-picked, packed in dry ice, and shipped to the Southern Regional Research Center, USDA—ARS. Peanut hulls were removed by hand, washed, and then freeze-dried.

In 1986, Florunner cv. peanuts planted in early May were grown in three closely monitored research plots (6.1 m × 12.3 m), one at ambient temperature (mean 25.6 °C), one at 2–3 °C above ambient, and the third maintained at 2–3 °C below ambient. The mean temperatures were determined by averaging the 8:00 a.m. and 4:00 p.m. temperature from 28 DAP (start of treatment) until harvest at 148–155 DAP. At harvesting time, the fields were hand-dug and all pods of harvestable size were hand-picked and then washed. Approximately 10 kg of peanuts from random field and plot locations was used in each sample. By the method developed by Williams and Monroe (1986), the exocarp of the peanut hulls was scraped off by gentle abrasion in a slurry of small glass beads in water. Removal

Table I. Flavonoid Analysis of Hulls from Six Maturity Classes of Peanuts

maturity class	5,7-dihydroxychromone hull, mg/g	SE	total flavonoids, %	eriodictyol hull, mg/g	SE	total flavonoids, %	luteolin hull, mg/g	SE	total flavonoids, %
Yellow 1	0		0	0		0	0		0
Yellow 2	0		0	0.141	±0.001	100	0		0
Orange A	0		0	0.339	±0.013	51.8	0.315	±0.027	48.2
Orange B	0.032	±0.010	0.63	2.389	±0.081	47.3	2.625	±0.049	52.0
Brown	1.053	±0.017	10.2	4.313	±0.171	41.9	4.922	±0.10	47.8
Black	1.488	±0.109	13.2	3.765	±0.128	33.4	6.009	±0.594	53.3

of the exocarp exposed the mesocarp color for use in the Hull-Scrape maturity class determination. Pods were visually sorted into increasing maturity classes on the basis of mesocarp colors designated as Yellow 1, Yellow 2, Orange A, Orange B, Brown, and Black. Pods from each class were dried with ambient air until mean kernel moisture was 7–8% and then hand-shelled.

Extraction of Flavonoids. Peanut hulls were ground to a fine powder in a Wiley mill. One gram of the powder was placed in a cellulose thimble and extracted with 80% methanol for 3 h on a Soxtec System HT (1043). The solution was reduced to 5–10 mL on a rotary evaporator. Three 10-mL portions of ether were used to extract the flavonoids from the solution. After the ether was removed by evaporation at ambient temperature, the residual material was dissolved in 2 mL of HPLC-grade methanol and then filtered (0.45-μm filter).

Chromatography of Flavonoids. A Waters Associates liquid chromatograph equipped with a Model 440 absorbance detector, 254-nm wavelength; two 6000A pumps; and a Model 660 solvent programmer was used. Retention times and peak areas of eluting compounds were determined by a Hewlett-Packard 3380A integrator. The column was 30 cm × 3.9 mm (i.d.) packed with 10-μm μ-Bondapak C_{18}. Solvents were filtered through a glass Millipore system with a 0.45-μm filter and degassed at room temperature under vacuum magnetic stirring. The elution solvent was water–acetic acid (495 mL:5 mL) from pump A and methanol from pump B. The flow rate was 1.9 mL/min, programmed at initial 80% A and 20% B to 60% A and 40% B in a 15-min linear gradient.

To quantitate flavonoids in the extracts, standard solutions of 5,7-dihydroxychromone, eriodictyol, and luteolin were prepared. Dilutions were made so that the range of concentrations correlated with the estimated content of flavonoids in the samples. Peak area data were used for calculation, and a regression analysis was used to quantitate the amount of each flavonoid in the peanut hulls. Duplicate samples were run for each set.

RESULTS AND DISCUSSION

Results from both the 1984 and 1986 studies confirm the observation of Pendse et al. (1973) that peanut hulls contain three flavonoids, 5,7-dihydroxychromone, eriodictyol, and luteolin.

In the 1984 season, peanuts were harvested at times selected to provide samples at varying stages of maturity. Sanders et al. (1982) have shown that peanuts planted in mid-May mature between 129 and 139 DAP. Since the most immature peanuts, those harvested at 79 and 93 DAP, were too wet to air-dry, none of the samples were air-dried. The samples were not visually sorted into maturity classes, but hulls from each group of peanuts harvested at 79, 93, 107, 121, and 135 DAP were pooled for analysis. At 79 and 93 DAP, the flavonoid content of the hulls was not measurable. At 107 and 121 DAP, 5,7-dihydroxychromone represented 9% of the total flavonoid content, luteolin represented 41%, and eriodictyol represented 50%. At 135 DAP, the 5,7-dihydroxychromone content remained at a low percentage, the luteolin content rose to 52%, and the eriodictyol content declined to 38%. These data indicate that flavonoid components of the hull are not measurable until after 93 DAP and that, of the three flavonoids present in peanut hulls, luteolin is predominant when the peanuts are mature.

Since the 1986 study was planted in early May, the peanuts were harvested 148–155 DAP (Sanders et al., 1982). The Hull-Scrape method was used to sort the peanuts, visually, into the six maturity classes. Duplicate samples of each maturity class from three plots were analyzed. Statistical analysis of the data indicated no significant differences in the individual maturity classes from the plots at ambient, 2–3 °C above ambient, or 2–3 °C below ambient temperature. Therefore, data for each maturity class, as shown in Table I, represent the mean of duplicates from three research plots. Eriodictyol is the only flavonoid that appears earlier than the orange color stage, and at that point it is in very low concentration. Total flavonoid content continues to increase as maturity increases, from 5.1 mg/g at the Orange B stage to 11.3 mg/g at the Black stage. Eriodictyol is the predominant flavonoid at the Orange A stage. Luteolin, however, is the predominant flavonoid present at the more mature stages. There is actually a decrease in the eriodictyol content as the color of the mesocarp changes from brown to black. These data substantiate the observation of the changes in flavonoid content of peanut hulls as the peanuts mature noted in the 1984 studies.

Determination of peanut crop maturity has been made by (1) indirect methods, i.e. days after planting; (2) weight and weight relationships; (3) specific component quantitation, i.e. the Arginine Maturity Index; or (4) some relative color evaluation (Sanders et al., 1982). While all methods are useful, the first three are not always accurate because they do not adapt to nonoptimum environmental conditions. The most recent color evaluation technique, the Hull-Scrape method developed by Drexler and Williams (1979), is advantageous because it can be used at any stage of plant development to predict a harvest date. In addition, because it is nondestrucive, it can be used after harvesting to segregate fully mature peanuts from less mature ones without affecting total yield. The data reported here demonstrate that the flavonoid components found in peanut hulls change as the peanuts mature. There is a direct correlation between each maturity class and specific flavonoid content of the hull. This study, therefore, provides a chemical rationale for the color classification of peanut maturity by the Hull-Scrape method.

Registry No. 5,7-Dihydroxychromone, 31721-94-5; eriodictyol, 552-58-9; luteolin, 491-70-3.

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Received for review January 19, 1988. Accepted May 16, 1988.

Removal of Phytic Acid from Soybean and Cottonseed Meals

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Soybean and cottonseed meals were subjected to various treatments with enzymes, heat, and chemicals, and their effects on hydrolysis and removal of phytic acid were studied. Soybean meal and cottonseed meal contain about 2.2 and 4.4% (w/w) phytate, of which 60 and 50%, respectively, were in water-soluble forms. The water-insoluble portion of the phytate was further hydrolyzed and removed by extraction with acid, application of enzymes, precipitation by divalent cations, and autolysis by endogenous phytase. Washing with 1 N HCl removed about 87% of phytate in soybean meal, while treatment with cationic agents had little effect on removing phytate from the plant seed. Coapplication of phytase with cellulose showed a synergistic effect in the hydrolysis of phytate. Incubating cottonseed meal in the presence of water in the temperature range 30–60 °C significantly reduced phytate content in the seed meals.

Plant seeds contain certain antinutrient substances that have an adverse effect on the nutritive properties of the seed protein. Phytic acid in soybean and cottonseed meal is an example. Phytic acid and its derivatives are known to bind essential dietary minerals, thus making them unavailable or only partially available for absorption by animals. Soybean and cottonseed meals are the major protein supplement in poultry feeds and also are a source of phosphorus. However, two-thirds of soybean meal phosphorus is bound as phytate and is unavailable for poultry (Whitaker and Brunnert, 1977). Although phytase activity has been shown to be present in the small intestine of various animals, its activity is not sufficient to utilize dietary phytic acid. Hydrolysis and/or removal of phytic acid prior to animal consumption would increase the availability of inorganic phosphorus and other minerals in the animal diet. Thus, efforts have been made to remove or reduce phytic acid level in seed protein by application of microbial enzymes (Han, 1988; Whitaker and Brunnert, 1977; Liener, 1977; Nelson et al., 1968; Rojas and Scott, 1969), cationic metal salts (Brooks and Morr, 1984), and autolysis (hydrolysis of phytate by endogenous phytase) (Chang and Schwimmer, 1977). Although beneficial effects of the enzyme treatment were evident, the high cost of enzyme production and lack of a practical method for enzyme application were cited as limiting factors in using the enzyme in animal diets (Han, 1988). The objective of this study was to investigate the effect of various treatments (washing, autolysis, enzyme and cation applications) in reducing phytate content in soybean and cottonseed meals.

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MATERIALS AND METHODS

Materials. Soybean meal and cottonseed meals were obtained from a local feed store and stored at 18 °C and 50% relative humidity until used. The moisture content of the meals was about 11%, and the total phytate contents of the soybean meal and cottonseed meal were 2.27 and 4.40%, respectively. Phytase, cellulase, hemicellulase, and proteinase (bromelain) were obtained from Sigma Chemical Co., St. Louis, MO.

Treatment. Two-gram portions of soybean and cottonseed meals were mixed with 30 mL of a solution containing 1 unit each of various enzymes, a combination of such enzymes, or various concentrations of cationic salts. The substrate mixed with the same amount of water was used as a control. The enzyme solutions were adjusted to pH 5.4 with acetate buffer, whereas the pH of the mineral solutions was not adjusted. The mixtures were then incubated at room temperature (about 25 °C) for a predetermined time period. For study of autolysis, 2 g of substrate was mixed with 2 or 30 mL of water and the resultant mixture incubated at various temperatures for the preset time period. Sodium hypochlorite (0.2%) was added to soybean meal to prevent microbial growth during the incubation period. At the end of incubation, phytate content in water-soluble and water-insoluble portions was determined. The phytate loss (%) was calculated on the basis of the total amount of phytate lost by the treatment compared to that of untreated control. All the treatments were replicated (two to five times), and the values reported are the average of the replicated samples.