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Changes in Tannin-Like Compounds of Peanut Fruit Parts During Maturation[1]

Timothy H. Sanders[2]

ABSTRACT

This study served a twofold purpose in that it investigated the relationship of tannins to maturity of peanuts and provided some information on the possible importance of tannins in resistance of peanuts to invasion by various fungi. Peanut fruits were separated into selected physiological maturity stages and divided into hull, seed coat and seed. The fruit parts in each maturity stage were analyzed for tannin using Folin-Denis reagent. Examination of physical and chemical characteristics of hull and seed coat tannins confirmed they are both condensed tannins. Presented as mg tannin per fruit part, hull tannins increased significantly after stage 9, seed coat tannins increased significantly to stage 9 then decreased, and seed tannins did not change. Fruit parts of cured peanuts containing several maturity stages were similar in tannin content to the more mature uncured fruit parts. Calculated as % fresh weight, tannins in the hull and seed coat were significantly higher in the three most mature stages. The results show a close relationship of tannin and maturity and also indicate that tannin concentrations may be sufficient to provide some measure of resistance to microbial attack.

Additional index words: Tannin, *Arachis hypogaea*, Maturation, Peanuts.

Phenolic compounds are a chemically diverse group of natural constituents of significance in many different fields of research. They have been reported in many plant species and effects on fungi are well documented (2). There is a lack of information on these compounds in peanuts and their possible importance in peanut resistance to invasion by various fungi. Tannins are precisely defined as high molecular weight polyphenolic compounds able to tan leather; however, the term is commonly used to refer to polyphenolic compounds in general (10) and is used as such in this paper.

Various phenols, tannins and related pigments, have been reported to be present in peanut seed coats (4, 6, 9, 11). Schenk (7) attributed the brown coloration in peanut hulls to tannin. Using Folin-Denis reagent, he reported 1.4% tannin in mature hulls of Dixie Spanish and Virginia Bunch 67 peanuts and slightly less in younger, but full-sized hulls with white inner surfaces. Information in the literature on peanuts concerning changes that occur in phenolic compounds with maturity is extremely limited. Lindsey and Turner and their coworkers(3, 12) recently reported four compounds in peanut cotyledons inhibitory to growth of *Asperfillus flavus* and *Trichoderma viride* and identified one of them as a flavone.

This study was performed as part of an investigation of the possible importance of tannins in resistance of peanuts to invasion by various fungi. Fungitoxicity of tanins was not measured in this portion of the study as the primary objective was to determine if quantitative changes occurred with maturity. An examination of tannin characteristics was made and is reported along with changes in methanol-extractable tannin-like compounds in hulls, seed coats and seeds of Florunner peanuts at selected physiological maturity stages.

Materials and Methods

During September and October 1976, 6 samples of peanuts (*Arachis hypogaea* L. var. Florunner) from a plot at the Coastal Plain Experiment Station, Tifton, Georgia were hand picked and brought immediately to the laboratory, washed, towel-dried and separated into physiological maturity stages according to Pattee *et al.* (5). The various stages were quickly separated into hull, seed coat and seed. Peanuts from the same plot were later harvested and cured; and the fruits visually judged mature were likewise separated into hull, seed coat and seed. Fruit parts (20-30) were immediately ground in 150 ml methanol for 1 min in a Waring Blendor and statically extracted for 48 hours. The supernatant was decanted and 2-3 similar static extractions were performed.

Tannin content of each extract was measured with Folin-Denis reagent by the Association of Official Analytical Chemists' method (1). Aromatic hydroxyl groups react with the Folin-Denis reagents to form a blue color. Assays were performed on 0.5 ml of methanol extract, and when clouding occurred, the test solution was filtered through Reeve Angel 934H glass fiber filter. Absorbance of the test solution at 760 nm was determined on a Bausch and Lomb Spectronic 70.

The residues from the methanol extractions were air dried 2 hr and then extracted 24 hr with 85 ml water. Folin-Denis reagents were added directly to the flasks containing the ground material and water. After 20 min, samples were filtered, and absorbance was determined. Assay of water extracts indicated that in all samples, over 90% of the tannin was extracted with methanol. Tannin data are the total of all extracts and are presented as mg/fruit part and % fresh weight.

Chemical and physical characteristics were determined on methanol extracted, water soluble tannins from hulls and seed coats of cured and green peanuts (stages 9-12 combined). The methods of Stansbury *et al.* (9) were used for purification of the extracted tannins before characteristics were determined. Absorption spectra of the tannins and related compounds were determined in methanol on a Beckman DB-G Grating Spectrophotometer. Thin-layer chromatography (TLC) was performed with glass plates coated with 0.5 mm silica gel G-HR. The solvent system was toluene:ethyl acetate:formic acid (5:4:1). Plates were sprayed with methanolic 10% H_2SO_4, heated and viewed under UV light to elucidate components.

Results and Discussion

Extractable-tannin content of hulls and seed coats changed significantly as peanut fruits developed from stage 6 to stage 12 (Table 1). Hull tannin content was not significantly (0.05 level) different in stages 6-9 but increased significantly in the more mature stages.

The first significant increase in hull tannin content occurred at stage 10, which corresponds with the development of a substantial number of brown splotches on the inner surface of the peanut hull. From stage 10 to stage 12, tannin per hull almost doubled, and the inner hull turned brown with some black splotches. In spite of this dark color at stage 12, tannins accounted for only about 0.4% of the hull on

[1]Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

[2]Plant Physiologist, Southern Region, ARS, USDA, National Peanut Research Laboratory, Dawson, Georgia 31742.

Table 1. Methanol-extractable tannin content of hulls, seed coats and seeds of maturing and cured Florunner peanuts.

Stage of Development	Hulls		Seed Coats		Seeds	
	mg Fruit Part	% Fresh Weight	mg Fruit Part	% Fresh Weight	mg Fruit Part	% Fresh Weight
6	0.31 a	0.06 a	0.81 a	0.92 a	0.12 a	0.04 a
7	0.31 a	0.06 a	2.02 bc	1.29 a	0.15 a	0.04 a
8	0.24 a	0.11 a	2.50 cde	1.85 a	0.23 a	0.04 a
9	0.25 a	0.11 a	3.02 e	3.05 a	0.27 a	0.04 a
10	0.64 b	0.31 b	2.77 de	6.95 b	0.28 a	0.04 a
11	0.89 c	0.37 bc	2.32 bcd	6.91 b	0.26 a	0.03 a
12	1.16 d	0.43 c	1.79 b	6.04 b	0.30 a	0.04 a
cured	0.86 c		2.38 cd		0.49 b	

Each value is the mean of 3-6 replications.

Means in a column followed by the same letter are not significantly different (0.05 level, Duncan's Multiple Range Test).

a fresh weight basis. Schenk (7) reported 1.4% tannins in hulls of Dixie Spanish and Virginia Bunch 67 peanuts without specifying wet or dry weight basis, but did indicate that the tannins decreased in solubility as the hulls increased in age. The complexes that form between tannin and proteins are resistant to decomposition and the dark color inside the hull may reflect the extent of the formation. Oxidized tannins are relatively insoluble and thus, our values and Schenk's (7), may underestimate tannin content in the later maturity stages. Though providing no data, Schenk (7) stated that the tannin concentration was particularly high in very young and in mature hulls. Our data do not substantiate high concentrations in young hulls and thus do not substantiate Schenk's suggestion that the middle stages of rapid development might be a period of higher susceptibility to microbial attacks than very young stages because of lower tannin levels in the middle stages.

Seed coats contained considerably more extractable tannin than hulls and seeds combined (Table 1). Tannin content of the seed coat increased significantly to a peak in stages 8-10 then decreased. Although tannin content per seed coat was the same in stages 8-10, the fresh weight changed; thus tannin expressed as % fresh weight increased from 1.85% at stage 8 to 6.95% at stage 10 and did not change significantly in stages 11 and 12 (Table 1). Pattee *et al.* (5) and Schenk (8) indicated that fresh weight of seed coats declines rapidly through these maturity stages. Although dry weight of seed coats was not determined in this work, a conservative estimate from Schenk (8) indicated that moisture in the seed coat was about 70% at stage 10 and about 50% at stage 12. If moisture ranges from 60-70% at stage 10 and about 40-50% at stage 12, the percent tannin on a dry weight basis would be, respectively 18.2-24.3% and 9.3-11.2%. Although these tannin values may seem large, the concentration of water soluble tannin encountered by an invading fungus would depend on the quantity of tannin and the moisture content at the particular stage of development.

Tannin content of the seed was relatively low and did not change significantly during the maturity stages observed (Table 1). Possibly, little or no tannin is actually produced in the seed, and the amounts detected may have been due to contact with the seed coat.

The data from cured fruits (Table 1) reflect several maturity stages and are a representative sample of the test plot. Stages 10-12 are most prevalent in the cured fruit and averages of hull and seed coat tannin in these stages (0.90 and 2.29 respectively) are very similar to the values for cured hulls and seed coats. The data for cured seed indicate a slight but significant (0.05 level) increase in tannin content from uncured seed. We can give no explanation for the increase except possibly that seed coat tannins diffused into the seed during curing.

Properties of the tannins are outlined in Table 2. Tannin was isolated from seed coats as a light brown amorphous powder, and that from hulls was similar but darker. Hull tannin was slightly less soluble than seed coat tannin in water, methanol and acetone. Tannin from both sources produced a green to greenish black color in methanolic and aqueous ferric chloride and gave a dark brown precipitate with potassium dichromate. Seed coat tannin precipitated 1% gelatin in 10% aqueous NaCl, as indicated by Stansbury *et al.* (9), whereas hull tannin did not, as indicated by Schenk (7).

Table 2. Characteristics of peanut hull and seed coat tannins.

Physical and Chemical Properties	Hull	Seed Coat
Color	medium brown	light brown
Solubility	methanol acetone water	methanol acetone water
Folin-Denis Test	positive	positive
Aqueous or methanolic ferric chloride	olive green color	olive green color
Aqueous potassium dichromate	dark brown precipitate	dark brown precipitate
Aqueous lead acetate	precipitate	precipitate
1% gelatin in 10% NaCl	no precipitate	precipitate
Red phlobaphene formation	no	yes

Boiling seed coat tannin in methanolic 10% HCl resulted in production of a red phlobaphene as

indicated by Stansbury *et al.* (9). Hull tannin did not give this reaction; instead, the material changed from brown to reddish brown, like the hull tannin that Schenk (7) heated at 100 °C with 6N HCl for 1 hr. Because the hull tannin did not form a phlobaphene exactly characteristic of condensed tannins, the hydrolysis products were tested for sugar characteristic of hydrolyzable tannins. The Molisch test for carbohydrates was negative. The results thus indicate that the tannins from both seed coat and hull were condensed or catechol type tannins even though all reactions of the two were not the same.

The visible-ultraviolet spectrum of seed coat tannin in methanol showed a single maximum at 280 nm and strong absorption in the region of 220 nm. The phlobaphene exhibited the strong absorption near 220 nm and peaks at 278 and 580 nm. The tannin spectrum was identical to that reported by Stansbury *et al.* (9); however, they reported that the spectrum of the phlobaphene in ethanol showed absorption at 280, 420 (inflection), and 530 (inflection) nm. The hull tannin in methanol showed absorption peaks at 286 and 330 nm.

TLC indicated differences between the seed coat and hull tannins. Spraying with methanolic 10% H_2SO_4 and heating revealed five mobile components in hull tannin and two in seed coat tannin (stages 9-12 combined). Much of the seed coat tannin and a lesser amount of hull tannin remained in a more or less unresolved area at the origin. In UV light, all the mobile compounds and material at the origin, except three regions from the hull tannin (Rf 0.81, 0.39 and 0.31), exhibited a rather intense self absorption typical of most phenol derivatives. Rf values for mobile components were as follows: hull tannin, 0.81, 0.64 (red in white light), 0.58 (yellow in white light), 0.39 and 0.31; seed coat tannin, 0.13 and 0.04.

Characteristics of tannins from cured and green hulls appeared to be the same as did tannins from cured and green seed coats. However, it was visually obvious during purification that extracts of cured hulls and seed coats contained considerably more water insoluble tannin-like material (positive Folin-Denis reaction) than the corresponding extracts of green fruit parts. This indicates that as peanuts mature, and consequently dehydrate and are cured, the hull and seed coat tannins become less water soluble. Such action could explain why green peanuts are less susceptible to colonization by certain fungi than cured peanuts.

The results of this study show a close relationship between tannin and maturity and also indicate that tannin concentrations in peanuts in the later stages of maturation may be sufficient to provide some measure of resistance to microbial attack. Studies to further investigate the relationship of tannin concentration and resistance to fungus invasion are in progress. Applicability of tannin concentration in determining peanut crop maturity is also under investigation.

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References

1. AOAC. 1975. Official Methods of Analysis of the Association of Official Analytical Chemists. 12th ed., W. Horowitz, ed. Washington, D.C. pp. 164-165.
2. Cruickshank, I.A.M. and D.R. Perrin. (1964). *In* "Biochemistry of Phenolic Compounds," (J.B. Harborne, ed.) pp. 511-544. Academic Press, New York.
3. Lindsey, D.L. and R.B. Turner. 1975. Inhibition of growth of *Aspergillus flavus* and *Trichoderma viride* by peanut embryos. Mycopathologia 55:149-152.
4. Masquelier, J. and P. Blanquet. 1948. Recherches sur les pigments de la graine d'Arachide. Mise en evidence d'un flavanone. Compt. Rend. Acad. Sci., Paris. 222:641-642.
5. Pattee, H.E., E.B. Johns, J.A. Singleton and T.H. Sanders. 1974. Composition changes of peanut fruit parts during maturation. Peanut Science 1:57-62.
6. Robinson, G.M. and R. Robinson. 1933. XXXI. A survey of anthocyanins. Biochem. J. 27:206-212.
7. Schenk, R.U. 1960. Source of the inner brown color of the peanut shell. Bot. Gaz. 121:191-192.
8. Schenk, R.U. 1961. Development of the peanut fruit. Ga. Agric. Expt. Stn. Tech. Bull. N.S. 22.
9. Stansbury, M.F., E.T. Field and J.D. Guthrie. 1950. The tannin and related pigments in the red skins (testa) of peanut kernels. J. Am. Oil Chem. Soc. 27:317-321.
10. Swain, T. (1965). *In* "Plant Biochemistry," (J. Bonner and J.E. Varner, eds.) pp. 552-580. Academic Press, New York.
11. Tayeau, F. and J. Masquelier. 1948. Recherches sur les pigments de la graine d'Arachide. I. Mise en evidence d'un chromogene. Compt. Rend. Acad. Sci., Paris, 227:602-604.
12. Turner, R.B., D.L. Lindsey, D.D. Davis and R.D. Bishop. 1975. Isolation and identification of 5, 7 dimethoxyisoflavone, an inhibitor of *Aspergillus flavus* from peanuts. Mycopathologia 57:39-40.