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The Presence of 5,7-Dihydroxyisoflavone in Peanuts by High Performance Liquid Chromatography Analyses

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ABSTRACT

The defatted flours of 43 colored and one white testa peanut (*Arachis hypogaea* L.) genotypes were analyzed for flavonoids. Flavonoids were extracted from the flours with aqueous methanol, hydrolyzed, and the resulting aglycones analyzed by high pressure liquid chromatography with a variable wavelenth detector. The UV spectrum and retention time coupled with those of standards allowed for the tentative identification of the principal flavonoid aglycone as 5,7-dihydroxyisoflavone (DHI).

Key Words: Isoflavones, cotyledons, genotypes, *Arachis hypogaea* L., analysis.

Flavonoids are a ubiquitous group of plant phenols. Their large number and variety of structure have made them ideal chemical markers (8). There is also much interest in their biological functions and physiological properties. In peanut (*Arachis hypogaea* L.) a dihydroquercetin glycoside was isolated (5,7) and, recently, its properties as an antioxidant were confirmed by Pratt and Miller (6). Turner, *et al.* (9) reported 5,7 dimethoxyisoflavone as a component of peanut cotyledons. An important property of this flavonoid was that it inhibited growth of *Aspergillus flavus* (Link) Fr., and *Trichoderma viride* Pers. ex. Fr. Gibbs (4).

Because of its activity against these fungi the confirmation of 5,7 dimethoxyisoflavone in experimental peanut varieties should be helpful in differentiation within similar genotypes. In our previous work with white testa peanuts, there were two unidentified flavonoids (isoflavones) (3). To see if either one was 5,7 dimethoxyisoflavone or 5,7 dihydroxyisoflavone, standards were synthesized and used for comparison. The availability of high performance liquid chromatography coupled with a variable wavelength (scanning) UV detector provided the quantitative flavonoid analyses on the small quantities of each experimental peanut genotype.

Material and Methods

Preparation of Flour Extracts. The available quantity of genotype varied from 5 to 10g. Deskinned peanuts were de-oiled with hexane (1:10/W:V) by homogenizing twice in a Waring blender at high speed for 2 min. The second extraction used half the amount of hexane used in the first extraction. The flour was filtered after each extraction. Flavonoids were then extracted from the homogenate flour by refluxing a solution of methanol/water: 80/20 (V/V) and the flour (3-7g) for 4 hours. The methanol was evaporated at room temperature, and the resulting aqueous solution was adjusted to pH≳3.5 with 2N HCl. Polyvinylpyrollidone (PVP) (1.0g) was added and the mixture stirred for 1 hour at room temperature. The mixture was filtered, then the PVP was washed with water (pH = 6.0). The PVP was washed on a filter with basic (pH = 8-10) methanol until the filtrate was no longer

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yellow (50-100 mL). This extraction of the flavonoids with PVP was performed twice. The methanol solution was then adjusted to pH 6-7 with 2N HCl and evaporated to dryness with a rotary evaporator at room temperature. The dried material was dissolved in 2 mL of HPLC grade methanol and filtered. This PVP procedure is necessary to purify the flavonoid fraction.

Hydrolysis of Extracts. The flavonoid extract (0.5 mL) was sealed in a tube with 2N HCl (5 mL) and heated on a steam bath for 45 min. After ether extraction, the ether was removed on a rotary evaporator and the dried material taken up in 2 mL HPLC grade methanol, then filtered (.45 μ filter).

Quantitation of DHI by HPLC. A Waters Associates Liquid Chromatograph equipped with a Model 165 Beckman Variable Wavelenth Detector, two 6000A pumps and Model 660 solvent programmer was used. The output from the detector was monitored on a Hewlett-Packard 3380A Integrator. The detection wavelength was 254 nm. The scanning wavelengtth range was 230-400 nm. and the UV scan output was monitored on a Kipp and Zonen BD 40 recorder. The column was 30 cm by 3.9 mm. I. D. packed with μ Bondpack C18 (10 μ) preceded by a micro-guard silica column.

Solvents were filtered using a glass Millipore system with a 0.45 μ filter and degassed at room temperature under vacuum with magnetic stirring. The elution solvent was 58:42 mixture of water: acetic acid (495 mL: 5 mL) from pump A and methanol from pump B at a flow rate of 1.9 mL/min.

The aglycones were identified by comparing the UV spectra with standard spectra. Identification was confirmed by HPLC using retention times and spiking techniques. Aglycone ratios were determined by counts representing the area of each aglycone peak as measured by the Hewlett-Packard 3380A Integrator. The relative amounts of aglycone in each peanut was based on that peanut having the lowest count of identifiable isoflavone per gram of seed.

The standards 5,7 dihydroxyisoflavone (DHI) and 5,7 dimethyoxyisoflavone (DMI) were synthesized by the decarboxylation of their 2-carboxylic acid derivatives (1).

Results and Discussion

For purpose of flavonoid identification, comparison of unknowns with standards is preferred. Under the conditions outlined above, DHI had a retention time of 21.2 min. The dimethoxy derivative had a longer retention time - 24.9 min. In all of the peanuts analyzed, the UV spectrum, and retention time of DHI was the same as one of the two principal isoflavones to be identified. Control experiments in which one or both of the standards were added to the peanuts before extraction led to the same conclusion - DMI is not present in an indentifiable quantity compared to DHI.

Table I shows how the tan testa color is associated with an undetectable amount of Unk 1. in comparison with that of DHI. The larger peanuts such as Va 72R, T-2374, and Jenkins Jumbo contained much lower quantities of DHI in comparison to those amounts of DHI in the smaller peanuts such as Tifspan and Spancross. Work by Conkerton and Chapital (2) suggests that phenolic acids, in general, are stored in the embryo of the peanut. Therefore, the general overall size of the peanut may not be a true indication of how much DHI each peanut contains.

The other aglycone, Unk 1, (retention time 11.5 mins) whose UV spectrum indicates that it is an isoflavone remains unidentified. (Standards and other methods of identification are currently being pursued.) The column conditions did not allow for the confirmation of dihydroquercetin's presence or absence in these peanuts.

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Table 1. Flavonoid Analysis of Colored Testa Peanuts Flours.

Name or PI	Testa Color	Relative Quant. of DHI/10 seed	Ratio DHI:Unk:1
VA 72R	Pink	1	2.4:1
T-2374	Pink	2.2	4.3:1
Jenkins Jumbo	Pink	2.4	2.7:1
Va 61R	Pink	2.6	3.8:1
341879	Purple	3.9	-
T-2378	Red	3.9	I(Q):2.9(I):4(DHI)
109839	Pink	4.0	1.4:1
Tifton-8	Pink	4.2	2.4:1
Va Bunch G2	Pink	5.1	7.9:1
Tifrust 6	Tan	5.2	6.7:1
Tenn. Red	Red	6.0	-
Tifrust-11	Tan and Purple	6.2	1(Unk):1(K):5.9(DHI)
Tifrust-12	Red	6.3	1(Q):2.1(I):1.7(DHI)
CBR-R5	Tan	6.4	5.9:1
Tifrust-4	Tan	7.3	-
405915	Tan	7.6	-
Tifrust-3	Purple	7.9	-
Dixie Runner	Pink	8.2	10.1:1
221068	Purple and White	8.2	-
Tifrust-5	Tan and Purple	8.2	-
NC 6	Pink	9.1	6.7:1
CBR-R3	Tan	9.3	-
Va Bunch 67	Pink	10.5	5.2:1
T-2500	Purple	10.5	6.7:1
T-2359	Tan	11.5	-
Tifrust-2	Tan	11.8	-
Va Runner G26	Pink	12.0	-
CBR-R6	Tan	12.0	10.4:1
Krinkleleaf	Tan	12.1	-
T-1921	Tan	12.1	-
Tifrust-7	Purple	12.1	-
CBR-R2	White	12.6	7.2:1
Early Runner	Pink	13.0	6.4:1
414331	Tan	13.1	-
Florunner	Tan	13.1	5.9:1
Tifrust-10	Purple	14.1	-
T-2289	White and Red	14.3	2.5(Q):1(Unk.1):1.9(I) 6.1(DHI)
F334A-B-14	Pink	14.9	4.8:1
CBR-R1	Tan	15.4	-
NC 13	Pink	16.3	-
T-2501	Pink	20.3	1(Unk.1):2(I):7.1(DHI)
414331	Tan	21.9	-
Spancross	Tan	23.9	-
Tifspan	Tan	32.1	-

References

1. Baker, W., J. Chadderton, J. B. Harborne, and W. D. Ollis. 1953. A new synthesis of isoflavones. J. Chem. Soc. 1852-1860.
2. Conkerton, E. J. and D. C. Chapital. 1983. Personal Communication. Southern Region Research Center, P. O. Box 19687, New Orleans, La. 70179.
3. Daigle, D. J., E. J. Conkerton, R. O. Hammons, and W. D. Branch. 1983. A preliminary classification of selected white testa peanuts *Arachis hypogaea* L.) by flavonoid analysis. Peanut Sci. 10:40-43.
4. Lindsey, D. L. and R. B. Turner. 1975. Inhibition of growth of *Aspergillus flavus* and *Trichoderma viride* by peanut embryos. Mcopathologia. 55:149-152.
5. Masquelier, J. and P. Blanquet. 1948. Studies on the pigments of the peanut. A flavonone. Compt. Rend. 227: 641-642.
6. Pratt, D. E. and E. E. Miller. 1984. A flavonoid antioxidant in spanish peanuts. J. Amer. Oil. Chem. Soc. 61:1064-1067.
7. Skikora, R. J. 1977. An investigation of water soluble antioxidants in spanish peanuts. M. S. Thesis. Purdue University Lafayette, Ind. 40 pp.
8. Stewart, R. N., S. Asen, D. R. Massie, and K. H. Morris. 1980. The identification of poinsettia cultivars by HPLC analyses of their flavonol content. Biochemical Syst. and Ecol. 8:119-125.
9. 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.

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Errata:
A Preliminary Classification of Selected White Testa Peanuts (*Arachis hypogaea* L.) by Flavonoid Analyses (Peanut Science, 10:40-43: 1983).

The recent availability of the standard 5,7 dihydroxyisoflavone (DHI) prompted a reexamination of the flavonoids in the selected white testa peanuts. In Table II of the above publication the major flavonoid aglycone in groups I-IV identified as rhamnetin on reanalyses was found to be DHI. In groups VI-VIII, unknown 2 was found to be DHI.