Protein as an Indicator of Peanut Seed Maturity

Sheikh M. Basha

Peanut Research Laboratory, Division of Agricultural Sciences, Florida A&M University, Tallahassee, Florida 32307

Protein as an indicator of peanut seed maturity was determined by examining protein profiles from seeds at different maturities. The results showed that peak II (arachin) and peak V increased with maturity. In contrast, the percent of peak IV protein in relation to other proteins decreased with increasing maturity and remained unchanged toward later stages (brown and black) of seed maturity. Examination of HPLC protein profiles of different peanut cultivars showed the presence of peak IV protein in all cultivars, and the cultivars also exhibited typical "mature seed protein profile" with respect to peak IV protein. The data suggested that peak IV protein would be a potential indicator of peanut seed maturity since it is found consistently in similar amounts in mature seeds of all the peanut cultivars. Because of its significance as an indicator of peanut seed maturity this protein is tentatively named Maturin.

The indeterminate fruiting characteristics of peanuts result in seeds of varying maturity on the plant as harvest time approaches. The proper time to harvest is when the greatest weight of sound mature kernels is available, since this affects the dollar value of the crop and relates directly to yield and overall high-quality peanuts (Sanders et al., 1982). Methods to determine the time to dig peanuts have been used for many years and range from digging a certain number of days after planting to highly sophisticated techniques (Holley and Young, 1963; Pattee et al., 1969; Young and Mason, 1972; Gilman and Smith, 1977; Williams and Drexler, 1981; Henning, 1983; Sanders et al., 1980). All methods have some value under a given set of environmental conditions. However, some of these methods are so significantly affected by environmental conditions that their general use is precluded. Among the maturity methods, the physiological maturity method in combination with specific-component quantitation methods appears to be the most reliable because of the relationship of flavor and seed component composition. The relationship of various seed components with maturity generally indicates that, at a relatively consistent stage in development, many seed parameters reach a plateau and a crop of seed predominantly in this biochemical and physiological plateau will be of consistently high quality (Sanders et al., 1982).

Among the seed components, protein constitutes 23–30% of seed weight and is less influenced qualitatively and quantitatively by the varying climatic conditions of the peanut belt (Altschul, 1964; Pancholy et al., 1978). In addition, protein deposition is rapid and reaches a plateau well before harvest time (Cherry, 1974; Basha et al., 1976; Yamada et al., 1980).

Peanut seed proteins have been generally separated into arachin and conarachin or non-arachin proteins (Jones and Horn, 1930; Neucere, 1969; Basha and Cherry, 1976; Basha and Pancholy, 1981b). Arachin and conarachin together constitute approximately 87% of the seed proteins (Irving et al., 1946). Recently Basha (1988) separated peanut seed proteins into 10 components based on their molecular weight using HPLC. This method was found to be rapid and gave excellent resolution of arachin and non-arachin proteins. Application of this method to study developmental changes in seed proteins showed the existence of distinct protein profiles for mature and immature seed.

This study was aimed at determining the possibility of employing protein as an indicator of peanut seed maturity. It is hoped that once a specific protein is identified as a indicator of seed maturity, an assay such as enzyme-linked immunosorbent assay (ELISA) can be developed to test the peanut seed maturity status and predict the harvest date. Such an assay will be useful for both the farmer and the peanut industry for obtaining quality peanuts.

In this paper we report identification of a protein with the potential as an indicator of peanut seed maturity status.

MATERIALS AND METHODS

Materials. Peanut (*Arachis hypogaea* L. cv. Florunner) seeds were grown in experimental plots at Florida A&M University during the 1987 crop season following recommended cultural practices. Plants were harvested between 100 and 140 days after planting, and pods were collected and classified into various maturity categories by two methods. In the first method (method 1), the peanut hulls were scraped and then grouped into white, yellow 1, yellow 2, orange, brown, and black categories as per the method of Williams and Drexler (1981). In this method most mature seed will be black while the most immature seed will be white. In the second method (method 2), the pods were split open and the seeds were then classified into immature (I), low-intermediate (LI), intermediate A (IA), intermediate B (IB), high-intermediate (HI), and mature (M) categories based on the pericarp and testa color (Pattee et al., 1974; Basha and Pancholy, 1981a). Peanut entries from the 1987 Uniform Peanut Performance Tests were supplied by Dr. T. A. Coffelt, USDA—ARS, Suffolk, VA. Additional cultivars were provided by Dr. A. J. Norden, IFAS, University of Florida, Gainesville, FL. Seed samples were freeze-dried and ground into meals. The meals were then defatted with hexane (Basha and Pancholy, 1981a), and the resulting fat-free meals were stored at −20 °C for further analyses.

High-Performance Liquid Chromatography (HPLC) of Proteins. Seed proteins were extracted from the defatted meals (25 mg) of various maturities with 2 mL of 0.5 M NaCl, 0.01 M sodium phosphate (pH 7.0), and 0.02% NaN_3 on a Polytron homogenizer. The homogenate was centrifuged at 20000*g* for 20 min, and a 20-μL aliquot was analyzed by HPLC as described by Basha (1988). The HPLC system consisted of a Model 510 pump, a UV/vis detector, a Protein Pak SW 300 column, and a 820 Data Station (Waters, Milford, MA). The column was equilibrated and eluted with 0.01 M sodium phosphate buffer (pH 7) containing 0.5 M NaCl and 0.05% sodium azide. Flow rate of the column was 1.0 mL/min, the gradient was isocratic, the detector was set at 280 nm, and the range was 1 AUFS.

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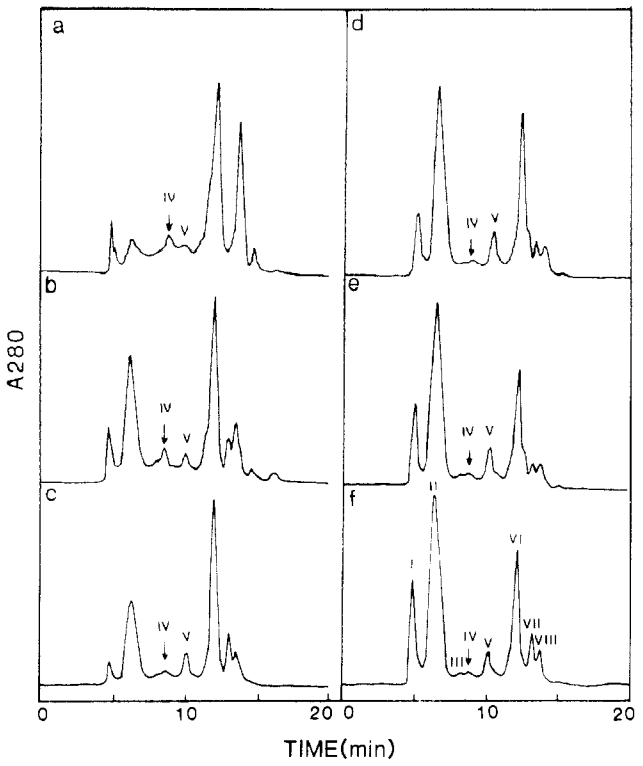

Figure 1. HPLC protein profiles from peanut seeds of different maturities obtained by method 1: a = white, b = yellow 1, c = yellow 2, d = orange, e = brown, f = black. Arrow points to changes in peak IV protein content during seed maturation.

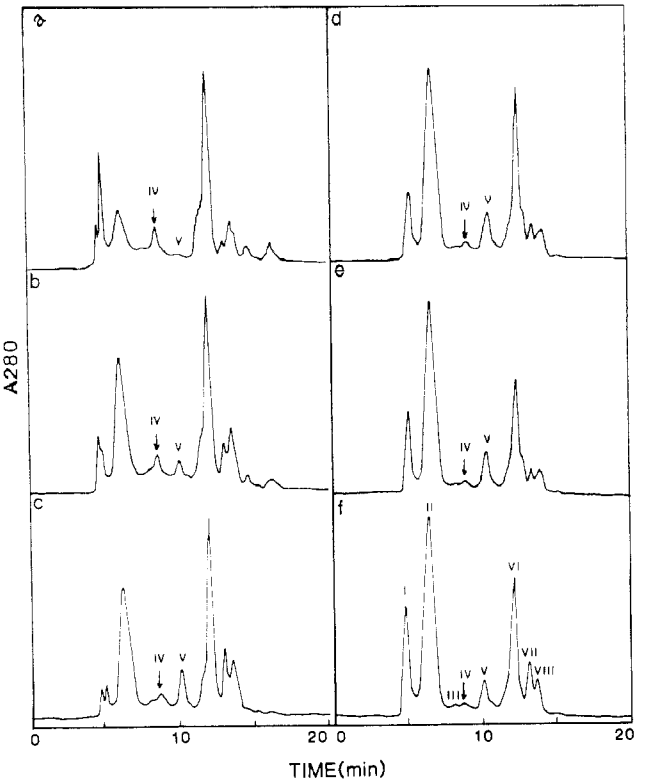

Figure 2. HPLC protein profiles from peanut seeds of different maturities obtained by method 2: a = immature, b = low-intermediate, c = intermediate A, d = intermediate B, e = high-intermediate, f = mature. Arrow points to changes in peak IV protein content during seed maturation.

RESULTS

Protein profiles from peanut seeds of different maturities were obtained by HPLC to determine a typical "mature seed protein profile" and to identify a protein(s) marker that would be useful as a indicator of mature seed. Following HPLC, peanut seed proteins were resolved into eight peaks (Figure 1). Previously we identified (Basha, 1988) peaks I and III as arachin polymers ($>10^6$ Da) and monomer (380 000 Da), respectively. Figure 1 shows protein composition of seeds of various maturities obtained by method 1. As seen in the figure, in the white (immature) seed, peaks VI and VII were the dominant proteins while the other peaks were relatively small. However, from the yellow 1 stage onward, peak II (arachin monomer) was present in increasing amounts and exceeded peak VI by the black (mature) stage. For example, the ratio of peaks II/VI was 0.14 in the white seed while it was 1.5 in the black seed. Likewise, the ratio of peaks V/VI changed from 0.04 in the white seed to 0.22 in the black seed. In contrast, the percent of peak IV protein in relation to total seed protein declined with increasing maturity, and by the Black stage it constituted only around 0.8% of the total seed protein. In addition to peak II, peak V also increased rapidly to the orange stage and then remained similar. Figure 2 shows changes in the protein profiles of seeds of different maturities obtained by classifying the seeds on the basis of testa and pericarp color (method 2). The protein composition of seeds of different maturities obtained by this method was similar to the one obtained by the hull–scrape method (Figure 1), indicating that, irrespective of the method of classification, changes in seed protein composition followed the maturity status of the seed.

Developmental Changes in Peak IV Protein. Quantitative differences in peak IV protein content of the seed at different maturities are shown in Table I. As seen in the table, peak IV protein content significantly ($P < 0.05$) differed between immature and mature seeds. For example, peak IV protein content decreased from 3.86% (in white seed) to 0.79% by black stage. Likewise in method 2 also the decrease was from 4.6% to 0.8% between immature and mature categories.

Table I. Quantitative Differences in the Peak IV Protein Content of Peanut Seeds of Different Maturities

maturity stage	peak area, μV/s	peak height, μV	area,[a] %	weight,[a] %
	Method 1			
white	221.637	6.003	3.86	3.72
yellow 1	257.169	7.740	2.71	3.46
yellow 2	210.236	7.691	2.53	2.64
orange A	174.223	5.260	2.02	2.07
orange B	70.003	2.767	0.83	1.24
brown	72.140	2.614	0.87	1.14
black	72.144	2.301	0.79	0.96
	Method 2			
immature	255.900	8.922	4.66	5.14
low-intermediate	263.703	9.560	4.78	5.80
intermediate A	263.058	.643	3.30	4.39
intermediate B	270.896	8.118	2.97	3.10
high-intermediate	168.787	5.795	1.25	1.50
mature	106.777	3.486	0.81	0.90

[a] Represents the percent of peak IV protein in relation to total peak area and total peak height of all the protein peaks present in the chromatogram.

Peak IV Protein Distribution among Peanut Cultivars. To determine the occurrence of peak IV protein among the peanut cultivars, and to compare the observed mature seed protein profile of Florunner with other peanut cultivars, protein profiles of 20 peanut cultivars and breeding lines were obtained by HPLC. The results showed (Figure 3) that all the cultivars and lines examined contained peak IV protein and also exhibited a typical peak IV profile of mature seed. In addition, in

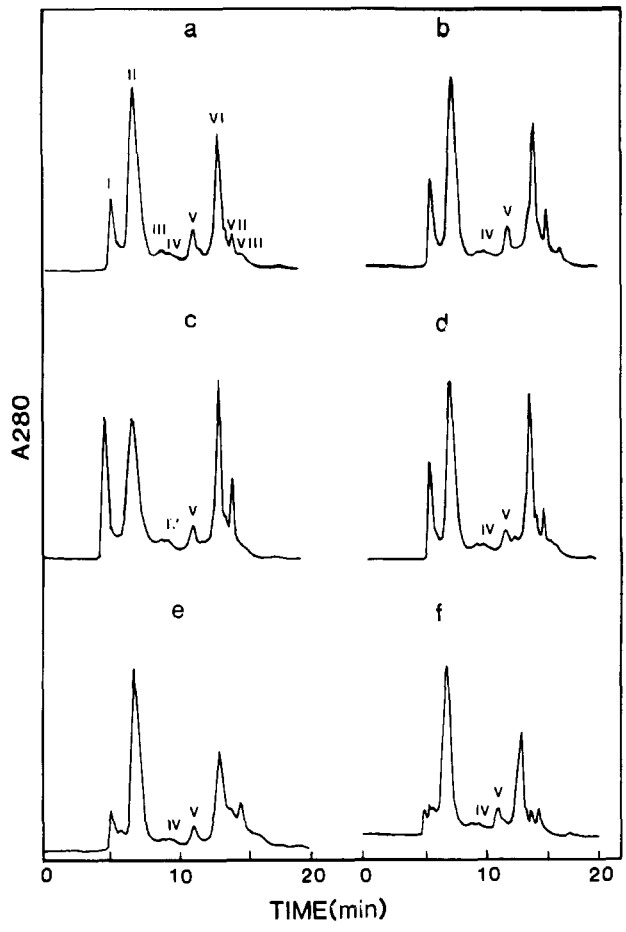

Figure 3. HPLC profiles of seed proteins from various peanut cultivars and lines: a = Florigiant, b = TP 107-11, c = VP 8140, d = UF 82107, e = Altika, f = Early Bunch.

all entries the area and height percents of peak IV protein were between 0.8 and 0.9. In contrast, the amount of protein in peaks I, II, V, and VI differed among the cultivars, indicating genetic variation in these proteins.

DISCUSSION

Protein as an indicator of peanut seed maturity was evaluated by determining compositional differences in seed proteins at different maturities. The data indicated that only three proteins, viz., peaks II, IV, and V, showed significant quantitative differences among the maturity stages. Although peak II (arachin) content increased with increasing maturity, it was not chosen as a indicator of seed maturity, because of its abundance in the seed (>63%), polymeric nature, and complex association kinetics that depends upon pH, ionic strength, protein concentration, time lapse after extraction, and temperature (Irving et al., 1946; Johnson and Shooter, 1950; Johnson et al., 1950; Tombs et al., 1974). In addition, the ratio of peaks II/VI differed with the maturity method and among the peanut cultivars. This would suggest that peak II may not be a reliable parameter for use in measuring the maturity of peanut seeds in general and also may be affected by various physiological and environmental factors. Likewise, peak V, a methionine-rich protein, also increased between white (immature in method 2) and orange (intermediate stage in method 2) stages and then remained unchanged. Because of genetic variation in the amount of methionine (Young et al., 1973; Pancholy et al., 1980) and methionine-rich protein (peak V) content and composition (Basha and Pancholy, 1984), peak V was also not selected as a maturity marker.

In contrast, the percent of peak IV protein in relation to total seed protein decreased between white (immature stage in method 2) and orange (high-intermediate stage in method 2) stages, and afterward the decrease was smaller. However, by black stage (mature stage in method 2) the height of peak IV always reached that of peak III, suggesting that peak III may serve as a reference to confirm seed maturity. Thus, when the height of peak IV reaches that of peak III, the seeds can be considered most mature. Alternatively, peak IV protein can be quantified and compared with a maturity curve prepared on the basis of the amount of peak IV at different developmental stages of the seed. Since the area and height percents of peak IV protein decrease with increasing maturity, it would be easier to visualize the decrease in the seed protein profiles, possibly with a highly sensitive technique such as the ELISA method, which is widely used for detecting extremely small amounts of proteins.

The amount of protein in peak IV would be no problem in developing the ELISA method for use in predicting peanut maturity based upon the amount of peak IV. ELISA will give a higher positive reading in immature seed, which should decrease with increasing maturity. As the seed matures, there will be lesser amounts of antigen, and hence, color intensity will change with increasing maturity. Though the ELISA method is not as simple as the hull–scrape method of Williams and Drexler (1981), it will be more sensitive, accurate, and reliable than the manual methods. This method should be great for industry, seed-procuring companies, and exporters who could monitor the maturity status of the peanuts before they are processed or purchased, to ensure the quality. This method could be of major interest to those who are critically concerned with the maturity status of the peanuts.

Examination of seed protein profiles of 20 different peanut cultivars and lines showed typical peak III and IV profiles (in which the two peaks were found to be of similar heights) of mature seed, indicating that these proteins are present in similar proportions in mature seeds of all of the peanut cultivars examined. Because of its possible role as a maturity indicator, peak IV protein is tentatively named Maturin.

Additional studies are in progress to determine the nature of Maturin and the influence of agronomical and environmental factors on Maturin content of the seed.

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Cooking Quality of Lentils: The Role of Structure and Composition of Cell Walls

Rattan S. Bhatty

Crop Development Centre, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

Good- and poor-cooking samples of Eston lentil, grown under field conditions, were compared for physical (microscopic) and chemical composition of seed, cell walls (CW), and pectin isolated from the CW. Hulled and dehulled, good- and poor-cooking samples of the lentil generally had similar hydration coefficients (water uptake). The poor-cooking sample had 44.1% lower seed phytic acid (PA) than the good-cooking sample. The CW were isolated in similar yields (3.8–4.2%) from the good- and poor-cooking samples and contained 5.8–6.5% protein, 0.5–1.2% starch, 1.2–1.7% lignin, and 17.7–18.1% galacturonic acid. Arabinose, glucose, galactose, and xylose were the major CW monosaccharides. Transmission electron micrographs of cotyledons and CW of uncooked poor-cooking lentil stained with $KMnO_4$ showed some evidence of a "lignification-like" mechanism at the cell junctions in the middle lamella. The CW of poor-cooking lentil appeared multilayered under a scanning electron microscope. Pectic substances of the two samples had similar methoxyl content, degree of esterification, and galacturonic acid content (colorimetric) and were thus of the low-ester variety. Seed PA content seemed to play a critical role in affecting the cooking quality of lentil.

Canada is now the fourth largest producer and second largest exporter of lentil in the world. Lentil, primarily used in human foods, has excellent nutritional quality. Although low in methionine and sometimes tryptophan, lentil protein complements proteins of wheat and rice with which it is commonly eaten in the developing countries. Lentil contains negligible levels of antinutritional factors, is low in flatulence-causing sucrose α-galacto-

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