determination on 70–80 samples per day. Thus, the described procedure combining enzymatic hydrolysis and colorimetric assay is suitable for research projects that generate substantial numbers of samples.

ABBREVIATIONS USED

HCN-p, hydrocyanic acid potential; *p*-HB, *p*-hydroxybenzaldehyde.

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Registry No. CN^-, 57-12-5; dhurrin, 499-20-7.

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Resolution of Peanut Seed Proteins by High-Performance Liquid Chromatography

Sheikh M. Basha

Peanut (*Arachis hypogaea* L.) seed proteins were extracted from the defatted meal with 0.01 M sodium phosphate buffer, pH 7.0, containing 0.5 M NaCl and 0.05% NaN_3 and resolved into four major and six minor fractions by HPLC. The molecular weight of these fractions ranged from 80 000 to 480 000. By this technique, variation in protein composition was detected in peanut seeds of different genotypes, boiled seeds, and seeds of different maturities. The data showed that HLPC can be successfully used to detect qualitative and quantitative differences in peanut seed protein composition.

Peanut seed proteins have been broadly classified into arachin, con-arachin (globulins), and albumins (Johnson et al., 1950; Dawson, 1968; Neucere, 1969; Basha and Cherry, 1976). Arachin and con-arachin together comprise approximately 87% of the seed proteins (Irving et al., 1946). Classical methods for separation of peanut proteins include ammonium sulfate precipitation (Jones and Horn, 1930; Dawson, 1968), $CaCl_2$ precipitation (Tombs, 1965), cryoprecipitation (Neucere, 1969; Basha and Pancholy, 1982), NaBr precipitation (Shetty and Rao, 1974), and ion-exchange chromatography (Dechary et al., 1962; Cherry et al., 1973; Neucere and Conkerton 1978). However, these methods are tedious and time-consuming. Recently, Basha and Pancholy (1981) separated peanut proteins into 10 arachin and non-arachin fractions employing gel filtration on a Sephacryl S-300 column. However, this method requires about 2 days to achieve the desired resolution, making it less attractive for use in large-scale sample monitoring such as screening of germplasm, following compositional changes due to environmental factors, and during processing. Hence, an HPLC method has been developed for seed protein fractionation and characterization that would greatly increase sample-screening capabilities. This paper describes an HPLC method that would yield a peanut seed protein pattern similar to a conventional gel filtration column and require only 15 min/analysis.

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MATERIALS AND METHODS

Instrumentation. The HPLC system consisted of a Model 510 pump, a variable-wavelength Model 490 UV/vis detector, Model 840 data station, and a manual U6K injector (Waters Chromatography Division, Millipore Corp., Milford, MA). The 7.8 mm × 30 cm PROTEIN PAK 300 SW column was obtained from Waters.

Materials. Peanut seeds of different lines and species [*Arachis hypogaea* L. (Nambiquare, Jenkins Jumbo, T2376, Chico); *Arachis batizogaea*, *Arachis stenosperma*; *Arachis monticola*, *Arachis villosulicarpa*] were gifts from Drs. R. O. Hammons and D. W. Gorbet, University of Georgia and University of Florida, respectively. For the boiling study, 25 g of freshly harvested green peanuts (*A. hypogaea* L., cv. Florunner) was boiled for 0–120 min in

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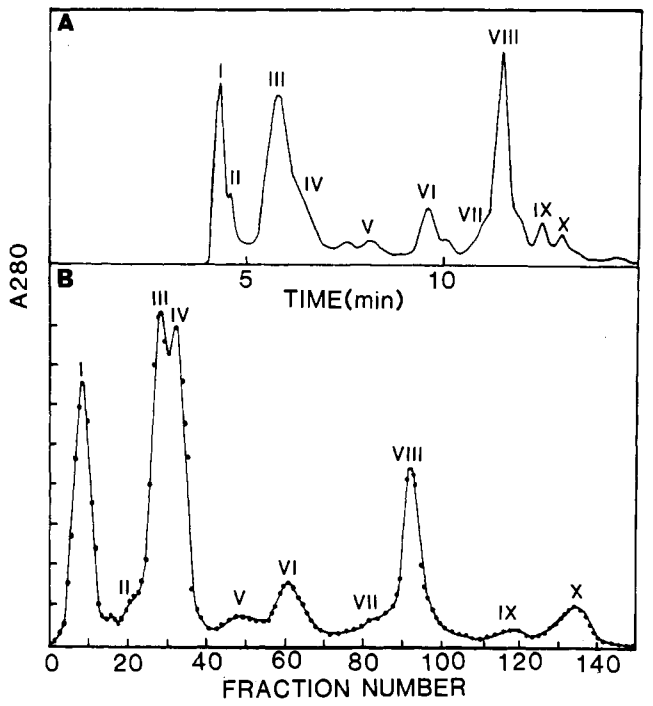

Figure 1. Chromatographic profiles of peanut (*A. hypogaea* L., cv. Florunner) seed proteins following separation by HPLC (A) and column chromatography (B). HPLC: column, protein PAK 300 SW (7.8 mm × 30 cm); mobile phase, 0.5 M NaCl, 0.01 M sodium phosphate, pH 7.0, 0.05% sodium azide; flow rate, 1.5 mL/min; detection, UV at 280 nm (1 AUFS); injection volume, 20 μL. Column chromatography: column, Sephacryl S-300 (2.5 × 135 cm); column buffer, 0.5 M NaCl, 0.01 M sodium phosphate, pH 7.0, 0.05% sodium azide; flow rate, 0.5 mL/min; detection, UV at 280 nm; sample volume, 8 mL. Peaks: I, void volume; II, arachin dimer; III, arachin A; IV, arachin B; V, non-arachin; VI, methionine-rich protein; VII–X, non-arachin proteins.

500 mL of water containing 1% (w/v) NaCl. The boiled pods were shelled and the seeds freeze-dried. For the developmental study, peanuts (*A. hypogaea* L., cv. Florunner) were collected from the field plots of Florida A&M university, separated into different maturity groups on the basis of testa and shell colors (Basha et al., 1976), and freeze-dried.

Sample Preparation. Freeze-dried peanut seeds were ground into a meal after seed coats were removed and then defatted with hexane (Basha and Pancholy, 1981). Protein was extracted from the defatted meal (50 mg) with 2 mL of 0.01 M sodium phosphate buffer, pH 7.0, containing 0.5 M NaCl on a Polytron homogenizer (Brinkman Instruments Co., Westbury, NY). The homogenate was centrifuged at 20000*g* for 20 min. The supernatant was filtered through a 0.45-μc filter, and a 20-μL aliquot was injected into the HPLC column.

Run Conditions. The column was equilibrated with 0.01 M sodium phosphate buffer, pH 7.0, containing 0.5 M NaCl and 0.05% NaN_3. The same buffer was used as the mobile phase. Flow rate was 1.5 mL/min under isocratic conditions. The detector was set at 280 nm, and the range was 1 AUFS.

Calibration. The column was calibrated with the following protein standards (Sigma Chemical Co., St. Louis, MO) of known molecular weights: thyroglobulin (669000), ferritin (500000), aldolase (161000), IgG (156000), maleic dehydrogenase (70000), BSA (67000), ovalbumin (43000), pepsin (35000), carbonic anhydrase (30000), trypsin inhibitor (24000), α-chymotrypsin (21000), cytochrome *c* (19000), lysozyme (18500).

Column Chromatography. Five grams of defatted peanut (cv. Florunner) meal was extracted with 10 mL of 0.01 M sodium phosphate buffer, pH 7.0, containing 0.5 M NaCl (1:2, w/v) and 0.05% NaN_3. The extract was centrifuged at 20000*g* for 20 min, and 8 mL of the supernatant was loaded on a Sephacryl S-300 (pharmacia Fine Chemicals, Piscataway, NJ) column (2.5 × 135 cm). The protein was eluted with the same buffer in 5-mL fractions by a peristaltic pump. Protein content of the fractions was determined by measuring the absorbance at 280 nm.

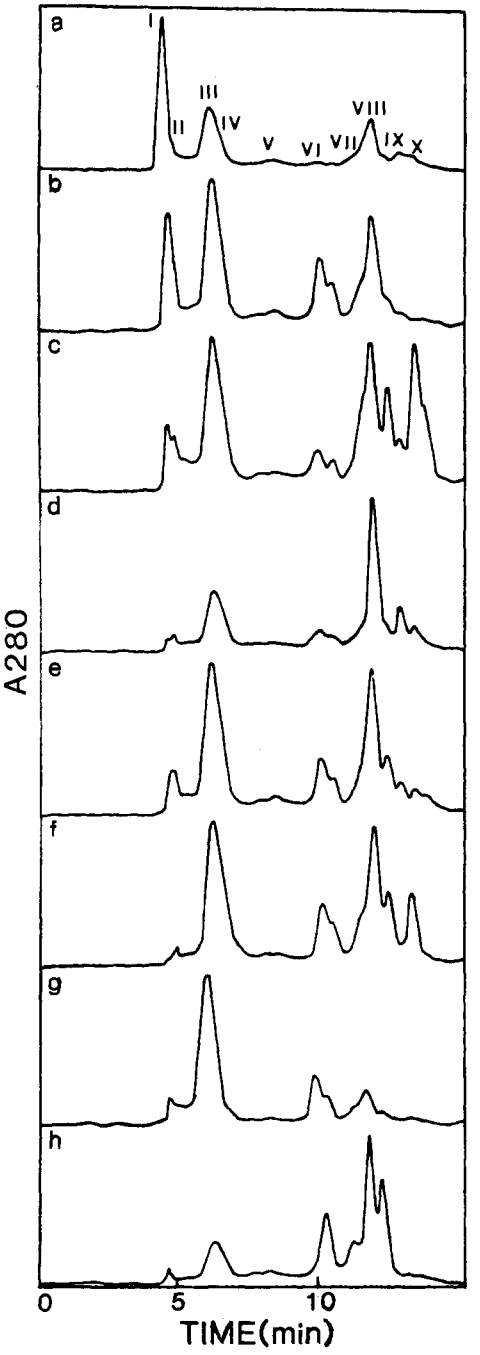

Figure 2. Genetic variation in the protein composition of peanut seed as determined by HPLC: a, Nambiquare (*A. hypogaes*); b, Jenkins jumbo (*A. hypogaea*); c, T 2376 nonnodulating line (*A. hypogaea*); d, Chico (*A. hypogaea*); e, *A. batizogaea*; f, *A. stenosperma*; g, *A. monticola*; h, *A. villosulicarpa*.

RESULTS AND DISCUSSION

Protein from the defatted peanut meal was extracted and fractionated by HPLC using a Waters Protein PAK 300 SW column. Under the experimental conditions, peanut seed proteins resolved into four major (I, III, IV,

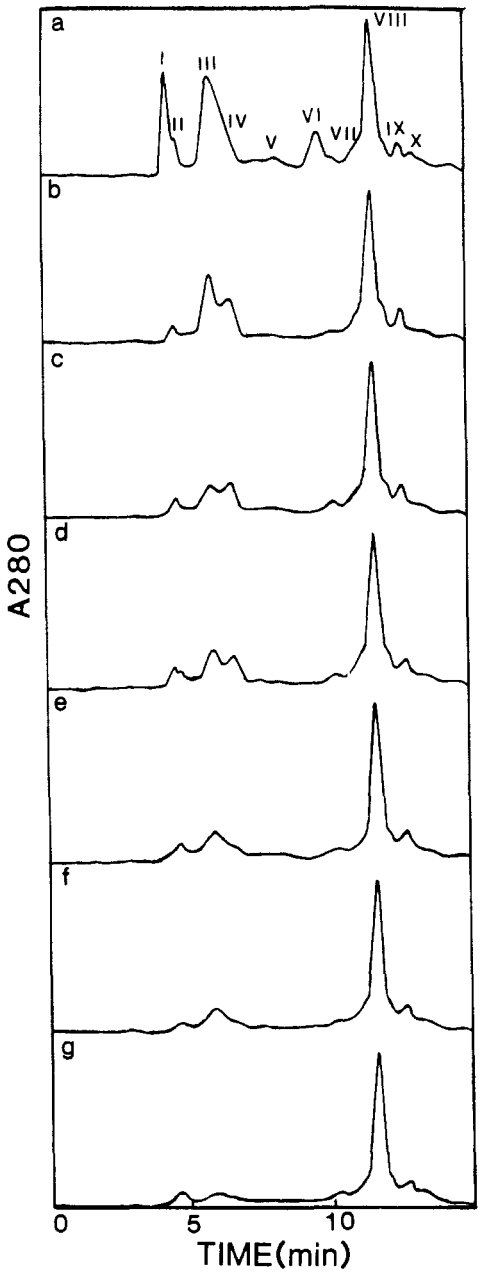

Figure 3. Changes in the protein composition of peanut (*A. hypogaea* L., cv. Florunner) seed following different periods of boiling: a, 0 min; b, 10 min; c, 20 min; d, 30 min; e, 40 min; f, 60 min; g, 120 min.

VIII) and six minor (II, V–VII, IX, X) peaks (Figure 1). Apparent molecular weights of peaks II–X ranged between 80 000 and 480 000 while peak I eluted in the void volume (molecular weight >500 000) of the column. The observed protein pattern is consistent with report of Basha and Pancholy (1981) who resolved peanut seed proteins into 10 peaks by Sephacryl S-300 column chromatography. Comparison of the protein profiles obtained by HPLC (Figure 1A) and Sephacryl S-300 chromatography (Figure 1B) showed them to be similar in terms of peak number, molecular weights, elution pattern, and protein composition. This is to be expected since both the columns have similar fractionation capability (10 000–500 000) and are eluted with the same buffer. On the basis of molecular weight data, peaks I–IV were found to represent monomer and polymers of arachin (major storage protein of peanut), while peaks V–X represent the non-arachin proteins. These data indicated that HPLC gives satisfactory resolution of arachin and non-arachin proteins and may be

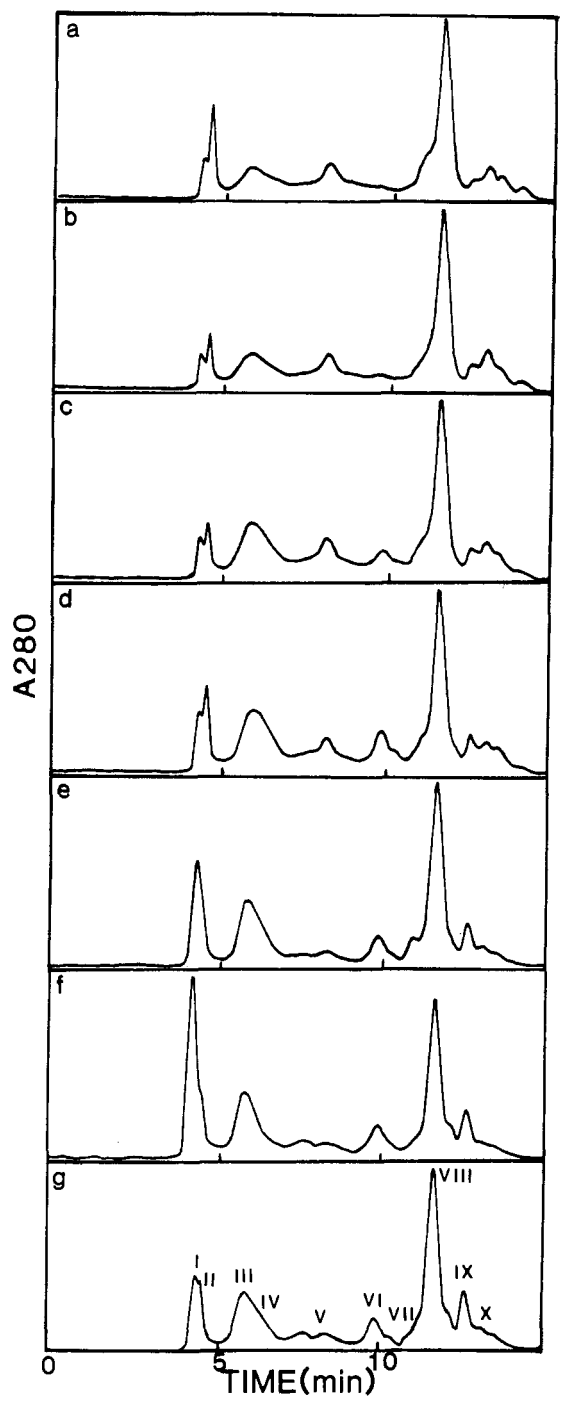

Figure 4. Developmental changes in the peanut (*A. hypogaea* L., cv. Florunner) seed protein composition during seed maturation: a, very immature; b, immature; c, low intermediate; d, intermediate; e, high-intermediate; f, mature; g, over-mature.

used to identify variations in seed protein composition.

Application to Seed Protein Research. In order to determine applicability of the HPLC method in seed protein research, this technique was employed to monitor peanut seed protein composition under various conditions.

Genetic Screening. Peanut seed proteins from different breeding lines and various species of genus *Arachis* were extracted and subjected to HPLC as described in Materials and Methods. Figure 2 shows the protein patterns of selected peanut genotypes. As seen in the figure, HPLC revealed significant differences in the protein composition among the peanut cultivars. For example, Nambiquare (*Arachis hypogaea*, Figure 2a) contained the highest amount of peak I proteins (polymers of arachin) while *A. batizogaea*, *A. stenosperma*, *A. monticola*, and

A. villosulicarpa (Figure 2e–h) had less protein in peak I. Nambiquare, Chico, and *A. villosulicarpa* also contained lower amounts of peak III (arachin monomer) protein than the other lines. In addition, Nambiquare and Chico (figure 2a,d) contained the lowest amounts of methionine-rich protein (peak VI) (Basha and Pancholy, 1981). *A. monticola* (Figure 2g) contained the lowest amount of low molecular weight (<80 000) proteins (peaks VIII–X) while *A. villosulicarpa* had the highest amount of these proteins.

Seed Processing. Boiling of green peanuts for various periods caused major changes in the seed protein composition (Figure 3). The data showed that during the first 10 min of boiling peaks I and VI proteins disappeared while the arachin peaks (III, IV) decreased gradually with increasing periods of boiling. Peak VIII, which contained the low molecular weight (<80 000) proteins, remained unaffected throughout the 2-h boiling period. Thus, the ratios of peaks VIII/I and VIII/III changed from 1.4 and 1.5 (at 0 min of boiling) to 10.8 and 13.5 after 120 min of boiling, indicating that peaks I and III proteins were degraded due to boiling while peak VIII proteins are unaffected.

Seed Development. Examination of the seed protein pattern during seed development revealed (Figure 4) significant changes in seed protein composition. The data showed that the amount of protein in peaks III, IV, VI, and IX increased during seed maturation while the peak V proteins decreased with increasing maturity. These data suggest that high molecular weight (>300 000) proteins such as arachin and its polymers increased during seed maturation. This finding is consistant with the previous report of Basha et al. (1976) who employed gel electrophoresis to determine the peanut seed protein deposition patterns during seed maturation. In contrast, peak I (void volume), which contains high molecular weight (>500 000) polymers of arachin and other components (Basha and Pancholy, 1981), gradually increased up to a mature stage and then decreased at over-mature stage. This decrease in peak I may be due to the protein turnover resulting in dissociation of polymers into smaller components as evidenced by the relatively higher amount of peaks II and IV proteins in the over-mature seed than the mature seed. It should be noted that the over-mature seed often germinates in the field upon exposure to favorable conditions, indicating active metabolic status of the seed.

Overall the above findings suggest that HPLC can be used to detect variation in protein composition, follow developmental changes, and monitor seed quality during processing. The method is precise, reproducible, and signficantly faster than the conventional techniques, making it attractive to employ in seed protein research.

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