Accumulation Pattern of Arachin in Maturing Peanut Seed[1]

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ABSTRACT

Accumulation pattern and compositional changes in peanut (*Arachis hypogaea* L. cv. Florunner) arachin were determined by monitoring arachin from seeds of different maturities by gel filtration, two dimensional gel electrophoresis and amino acid analysis. The results indicated that arachin deposition was maximum between Immature and Intermediate stages, and that the arachin monomer increased more than the polymer during seed maturation. Arachin polypeptides with apparent molecular weights of 70,000: 32,000 and 29,000 increased in abundance with increasing maturity while a 50,000 molecular weight polypeptide decreased during seed maturation. In addition, amino acid composition of arachin differed in seeds of different maturities. These data suggest that arachin polypeptides are not deposited in equal amounts but individual polypeptides accumulate in different proportions during peanut seed maturation.

Key Words: Peanut, arachin, maturity, accumulation, polypeptides, amino acids.

Arachin and con-arachin constitute the globulin fraction (11, 12, 15) of peanut seed. Of the globulins, arachin accounts for more than 50% of the total protein (12) and is composed of multiple subunits (4, 13, 21, 23, 26, 28). Non-arachin proteins primarily are synthesized shortly after pegging, while the arachin deposition is predominant in the later part of the seed development (6, 7, 27). In the seed, arachin exists in two molecular species from (monomer and polymer) which are known to possess the same subunits, but differ in their molecular weights. In addition, the arachin monomer has been shown to be composed of six different subunits in equimolar ratio (25-27). Yamada *et al.* (27) studied the arachin isolated from maturing peanut seeds by isoelectricfocusing and sodium dodecyl sulfate gel electrophoresis and found no difference in the subunit bands during seed development. Studies on the deposition pattern of reserve protein from *Vicia faba, Pisum sativum* and *Glycine max* have shown changes in the subunit ratios during seed development (14, 17, 24). While studying genetic variation in total seed proteins Basha and Pancholy (5) found major qualitative and quantitative differences in seeds of different maturities.

This study was conducted to understand the accumulation pattern of arachin in order to determine compositional differences in arachin from seeds of different maturities.

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Materials and Methods

Peanut (*Arachis hypogaea* L., cv. Florunner) seeds were grown in the experimental plots at the Florida A&M University during the 1987 crop season following the recommended cultural practices. Since the peanut plant is indeterminant in growth habit, plants from any one of the harvest dates usually contain seeds with a broad range of maturities (19, 20). Plants were harvested between 100 and 140 days after planting, pods were collected and split open to obtain the seeds. The seeds were then classified into Immature A (IM-A) Immature B (IM-B), Low Intermediate (LI), Intermediate A (IA), Intermediate B (IAB), High Intermediate (HI), Mature (M) and Over-mature (OM) categories based on the seed size, pericarp and testa color essentially as described earlier (6, 18). Seeds of the above categories were freeze-dried and ground into meals. The meals were then defatted with hexane (2) and the resulting fat-free meals were stored at -20 C for future analyses.

Isolation of Arachin

Protein from the defatted meal (3 g) was extracted with 10 mL of 2 M NaCl, 10 mM Tris-HCl (pH 8.2) in the presence of NaN_3 (0.002%) and phenylmethylsulfonylfluoride (0.2 mM) using a ploytron homogenizer (Brinkman Inst., NY). The homogenate was centrifuged at 20,000 x g for 20 min and the resulting supernatant was fractionated on a Sephacryl S-300 column (2.5 cm X 120 cm). The arachin (Peak III) was isolated according to the method of Basha and Pancholy (4). The pooled fractions were concentrated and re-chromatographed on a Sephacryl S-300 column under similar conditions. The resulting arachin fraction was pooled and dialysed against 0.01 M Tris-HCl (pH 8.2.). The dialysed sample was applied to a DEAE-Cellulose column (2.5 x 25 cm) and the protein was eluted with a linear gradient of salt from 0 to 0.5 M NaCl in 0.01 M Tris-HCl (pH 8.2), 0.02% NaN_3 (9). Fractions corresponding to the arachin peak were pooled, dialysed against several changes of deionized water and freeze-dried. Purity of the arachin was checked by gel electrophoresis (4).

Amino acid analysis

Amino acid composition of arachin was determined employing HPLC, following the PICO-TAG amino acid analysis method of Waters (1). One mg of lyophilized protein was hydrolized at 110 C for 24 h with 6 N HCl in a PICO-TAG workstation (Waters, Milford, MA). Following hydrolysis, the sample was dried and phenylisothiocyanate-amino acid (PITC-amino acid) derivatives were prepared. An aliquot of the derivatized sample was analyzed using an HPLC system equipped with a PICO TAG stainless steel column (3.9 mm x 15 cm), a UV/VIS dector, a U6K injector, two model 510 pumps, and a 840 Data Station. The amino acids were quantified using an external amino acid standard (Pierce, Rockford, IL) and expressed as relative mole percent of amino acids.

Two-dimensional Gel Electrophoresis (2-D PAGE)

Lyophilized arachin samples were dissolved in a buffer containing 9.3 M urea, 5 mM K_2CO_3, 0.5% (w/v) dithiothreitol and 2% (v/v) Nonidet P-40 and subjected to two-dimensional gel electrophoresis as described earlier (2). After electrophoresis, the slab gels were stained with Coomassie Blue R-250 to visualize the polypeptide spots.

High Performance Liquid Chromatography of Proteins

To monitor the accumulation pattern of arachin during seed development, seed protein was extracted from defatted meals of various maturities, with 0.5 M NaCl, 0.01 M sodium phosphate buffer, (pH 7.0) 0.2% NaN_3 using a polytron homogenizer. The homogenate was centrifuged at 20,000 g for 20 min and a 20 mL aliquot was analyzed by HPLC (3). The HPLC system consisted of a model 510 pump, a UV/VIS detector, PROTEIN PAK SW 300 column and a 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% of NaN_3. The flow rate was 1.0 mL/min, the gradient was isocratic and the detector was set at 280 nm with the range of 1 AUFS. Arachin was identified among the total seed proteins by co-chromatographing purified arachin, and also comparing the retention times of the purified arachin prepared by the method of Basha and Pancholy (4).

Molecular Weight Estimation

The HPLC column was calibrated using thyroglobulin (669,000), ferritin (500,000), aldolase (161,000), igG (156,000), maleic dehydrogenase (70,000), BSA (67,000), ovalbumin (43,000), and ∝-chymotrypsin (21,000). The 2-D gel was calibrated using the following protein standards: thyroglobulin (334,000), ß-galactosidase (130,000), phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100) and lysozyme (14,300).

Results and Discussion

Arachin and Non-arachin Proteins

Changes in the arachin content with respect to other seed proteins (non-arachin) was determined by examining the protein profiles of seeds from different maturities by HPLC. Following HPLC, peanut seed proteins resolved into ten peaks (Fig. 1). The observed protein profile and molecular weights are consistent with our previous reports (4, 5) in which we had reported occurence of arachin monomer (parent or native molecule) and polymers. Among the ten peaks, Peak III contained arachin monomer (MW 380,000 Daltons) while Peaks I and II represented the arachin polymers (> 500,000 Daltons). In Immature seed, Peak VIII (MW 70,000 Daltons) a non-arachin protein was the dominant species, while arachin peaks (I, II, III) were relatively small. However, arachin peaks increased gradually with increasing maturity with maximum increase occurring between Immature and Intermediate "B" stages. After Intermediate stage, there was only a moderate increase in the arachin. In Immature seed the ratios (peak heights) for peaks I/VIII, II/VIII and III/VIII were 0.15, 0.17 and 0.11, where as, in the Intermediate "B" seed the ratios increased to 0.29, 0.29 and 0.43, respectively. Using only peak heights as a criteria for concentration, this would indicate that compared to Peak VIII proteins, arachin monomer and polymer proportions of the seed increased with increasing maturity.

Arachin Monomer and Polymer

Arachin accumulation pattern was also monitored by quantifying the amount of protein in the arachin monomer (III) and polymer (I and II) peaks following separation of total seed proteins by gel filtration on Sephacryl S-300 column. Table I shows the variation in the amount (the sum of protein present in the whole peak) of arachin monomer and polymer in seeds of different maturities. The amount of arachin monomer was higher in more mature seeds while the polymer amount decreased with higher maturity. For example, the arachin monomer and polymer content of the immature seed were 9% and 11%, respectively, while in the mature seed they were 24% and 5.2%, indicating that arachin monomer (the parent molecule or the 380,000 molecular weight form) is the predominant species of the mature seed. In contrast, the polymer content of the overmature seed was higher than the mature seed and was close to the level of the immature seed. These differences may be due to the changes in the association-dissociation behavior attributed to the arachin molecule (10,11,22)

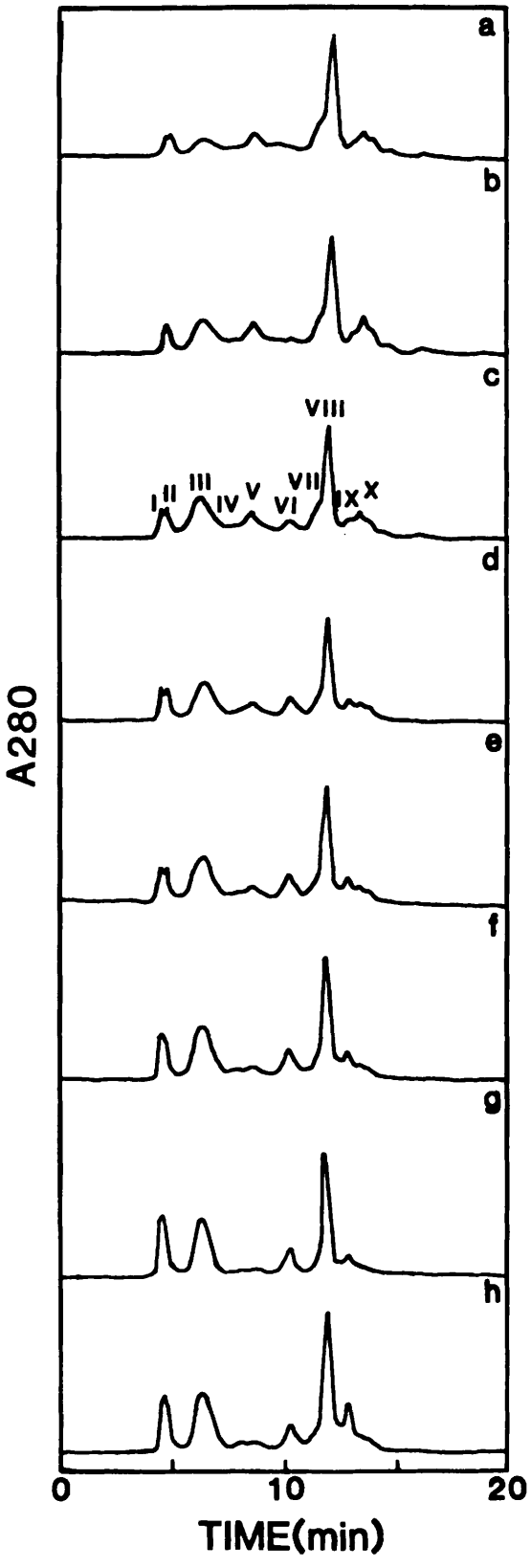

Fig. 1. Changes in the arachin protein content with respect to non-arachin proteins during peanut seed maturation, as shown by high performance liquid chromatography.
a: Immature A, b: Immature B, c:Low-Intermediate, d:Intermediate A, e:Intermediate B, f:High Intermediate, g:Mature, h:Over Mature.Peaks I and II arachin polymers, III:arachin monomer, IV through X:non-arachin proteins.

Table 1. Arachin Monomer and Polymer content in Peanut Seeds of Different Maturities

Maturity Stage	Protein (g/100 g Defatted Meal)	
	Polymer (Peaks I + II)	Monomer (Peak III)
Immature B	11.0	9.1
Low Intermediate	9.3	10.6
Intermediate A	5.6	11.9
Intermediate B	6.5	20.7
High Intermediate	6.0	23.2
Mature	5.2	24.0
Over Mature	10.7	13.5

Changes in Arachin Poltpeptide Composition

To monitor changes in the arachin polypeptide composition during seed development, purified arachin from all the maturity stages were analysed by 2-D PAGE. Polypeptide pattern from six maturity stages showing developmental trend in arachin during seed maturation are shown in Figure 2. The 2-D PAGE data indicated that the polyeptide composition of arachin changed quantitatively during seed maturation. Three polypeptides with molecular weights around 70,000 (A), 32,000 (B) and 29,000 (C) gradually increased with increasing seed maturity, while a 50,000 molecular weight polypeptide decreased with increasing seed maturity. The electrophoresis data indicated that arachin molecule undergoes structural alterations during seed maturation as evidenced by quantitative increases in several polypeptides on the 2-D gels.

Amino Acid Composition

Arachin from Immature seed was relatively high in aspartate, glutamate, arginine, proline and leucine (Table II). Likewise arachin from mature seed was also rich in these amino acids. However, aspartate, glutamate, methionine, and cystine increased with increasing seed maturity while the content of threonine, alanine, isoleucine, and leucine decreased with higher seed maturity. The observed amino acid composition is consistent with the amino acid composition reported for arachin by Dawson (8) and Neucere and Conkerton (16).

Table 2. Relative Mole Percent of Amino Acids in Seeds of Different Maturities.

Amino Acids	Immature B	Low Intermediate	Intermediate A	Intermediate B	High Intermediate	Mature	Over Mature
ASP	9.3	13.4	12.1	12.4	13.9	12.6	12.3
GLU	10.6	15.6	14.5	13.0	16.3	15.4	16.5
SER	5.9	6.2	5.5	5.2	5.9	5.6	5.7
GLY	7.5	7.6	7.0	7.5	8.0	7.2	7.7
HIS	0.8	1.0	1.0	1.5	1.6	1.5	1.3
ARG	10.2	9.5	8.9	8.4	8.2	8.4	9.0
THR	3.6	3.1	2.0	3.0	2.7	2.7	2.5
ALA	7.3	6.8	6.7	6.7	6.4	5.9	5.4
PRO	8.3	6.1	8.5	7.4	7.5	8.4	7.0
NH_3	3.4	1.9	3.1	3.6	2.0	1.7	1.2
TYR	4.7	4.2	4.2	4.7	3.7	4.0	4.3
VAL	5.3	4.0	4.0	5.9	4.3	4.9	4.5
MET	0.1	0.3	0.4	0.6	0.6	0.6	0.6
CYS	0.0	0.2	0.2	0.3	0.2	0.3	0.2
ILE	4.2	3.5	3.4	3.9	2.6	3.2	3.0
LEU	9.0	8.3	8.4	9.7	7.3	8.3	6.2
PHE	5.1	4.2	5.0	6.3	5.2	5.3	3.6
LYS	3.2	3.1	2.7	2.2	2.6	2.9	3.5

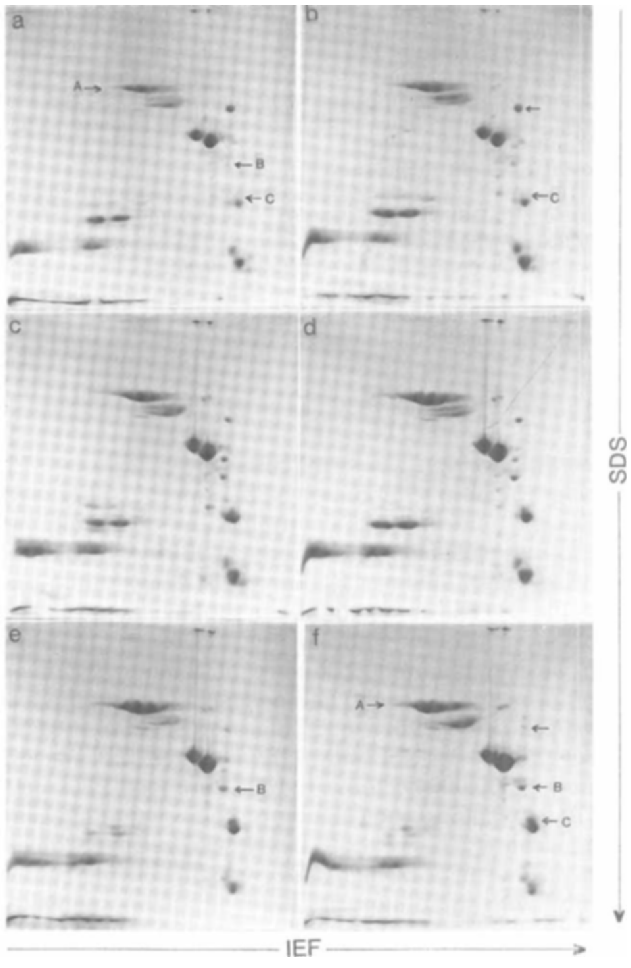

Fig. 2. Two-dimensional gel electrophoretic profiles of arachin from peanut seeds of six different maturities. Polypeptides showing changes during seed maturation are shown with arrows.
a:Immature B, b:Low-Intermediate, c:Intermediate A, d:High Intermediate, e:Mature, f:Over Mature.

The results of this study suggest that arachin composition changes with seed maturity and that the arachin polypeptides are not synthesized in equal amounts but the individual polypeptides are deposited in varying amounts and undergo structural modifications during seed maturation.

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