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Polypeptide Composition of Arachin and Non-arachin Proteins From Early Bunch Peanut (*Arachis hypogaea* L.) Seed[1]

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ABSTRACT

Peanut (*Arachis hypogaea* L.) seed proteins were resolved into arachin and non-arachin fractions, and composite two-dimensional polypeptide maps were prepared. Seed proteins were extracted with a buffer containing 2 M NaCl, 10 mM Tris-HCl (pH 8.2), 0.2 mM phenylmethyl sulfonyl fluoride and 0.002% NaN_3 and resolved into ten peaks by gel filtration on a Sephacryl S-300 column. Gel filtration of total protein extract yielded three molecular weight variants (490,000., 400,000, and 365,000) of arachin. Gel electrophoresis showed quantitative and qualitative differences in the protein and polypeptide composition of the three arachin variants. Nonarachin proteins obtained by this method were heterogeneous and distinct from the arachin. Two-dimensional gel electrophoresis revealed several differences in the polypeptide composition between arachin fraction IV and fractions II and III. Composite two-dimensional polypeptide maps of arachin and non-arachin revealed the presence of several polypeptides with similar isoelectric points and molecular weights between them. Arachin contained six molecular weight (between 15,500 and 68,000) classes of polypeptides with isoelectric points between 4.7 and 8.4 while nonarachin contained nine molecular weight (between 16,000 and 170,000) classes of polypeptides having isoelectric points between 4.7 and 7.9.

Key Words: Peanut, Protein, Arachin, Non-arachin, Two-dimensional gel electrophoresis, Composite polypeptide map.

Peanut proteins have been broadly classified into arachin, con-arachin (globulins) and albumins (2, 6, 10, 13, 14, 17). Arachin and con-arachin together comprise approximately 87% of the seed proteins (9). Separation methods for arachin and con-arachin proteins include either ammonium sulfate precipitation (6, 10, 14), $CaCl_2$ precipitation (25), cryoprecipitation (2,17), NaBr precipitation (22) or ion-exchange chromatography (3,7,18). The protein fractions obtained by the above methods have been studied extensively by several investigators (3, 4, 7, 9, 18, 19, 24). Although the terminology arachin and con-arachin has been widely used in the literature to describe peanut proteins, they are not well defined as to their protein and polypeptide composition and molecular weights. This is especially true with the con-arachin and albumins for which very little information is available. The peanut globulins, arachin and con-arachin, undergo a reversible association phenomenon depending on pH, ionic strength, protein concentration and storage period of the protein (7,11,12,13,27). Further, proteins derived from different peanut species are known to show differences in their polypeptide composition (1, 21) and exhibit different behavior in the dissociation-association properties of the arachin molecule (17,23,27). Thus, the insolubility, association-dissociation phenomenon, cryoprecipitation, poor resolution by conventional methods, and lack of proper definition of the molecules make identification and characterization of arachin, con-arachin and albumins difficult and often unreliable. Arachin dominates the peanut seed proteins and tends to interfere with the characterization of non-arachin proteins. Hence, it is essential to distinguish the arachin and non-arachin proteins, and to determine their composition and characteristics. The main objective of this study was to separate the arachin and non-arachin proteins from the seed extracts and to construct composite maps for arachin and non-arachin showing the polypeptide composition, and their molecular weights and isoelectric points.

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Materials and Methods

Seed Material. Cotyledons of peanut (*Arachis hypogaea* L.) cultivar "Early Bunch" were ground into a meal and repeatedly extracted with cold diethyl ether to remove the oil (1). The resulting defatted meal was air dried and stored at -20 C.

Chemicals. Sephacryl S-300 and Blue Dextran 2000 were from Pharmacia Fine Chemicals, Uppsala, Sweden. The protein standards for molecular weight determination were from Sigma Chemical Co., Sodium dodecyl sulfate and Nonidet P-40 were purchased from BDH Chemicals Ltd. Poole, UK; Coomassie brilliant blue R-250 Agarose and N, N diallyltartardiamide were obtained from Bio-Rad Laboratories. Acrylamide, glycine and Tris were products of Eastman Kodak Co. Ampholines were purchased from LKB, Uppsala, Sweden; Urea (Sequanol grade) was from Pierce Chemical Co. Other inorganic chemicals used were of reagent grade or better.

Protein Extraction. Three grams of defatted peanut meal were extracted with 10 mM tris-HCl (pH 8.2) buffer containing 2 M NaCl and 0.2 mM of phenylmethyl sulfonyl fluoride (PMSF) and 0.002% NaN_3 by grinding in a mortar with a pestle and then stirred for 10 min. The homogenate was then centrifuged at 20,000g for 10 min at 18 C and the resulting supernatant was used for protein analysis.

Protein Fractionation by Gel Filtration. Pre-swollen Sephacryl S-300 gel was washed thoroughly with water and equilibrated with the column buffer containing 0.5 M NaCl, 10 mM Tris-HCl (pH 8.2), and 0.002% (w/v) sodium azide. The equilibrated gel was packed in a 2.5 X 135 cm column at room temperature. Flow rate (10 ml/hour) of the column was regulated using a peristaltic pump. An 8 ml aliquot of the protein extract was applied to the column and eluted with the column buffer. Five milliliter portions were collected and their protein contents were routinely monitored by measuring the absorbance at 280 nm. Fractions under each peak were pooled and designated as follows: tube numbers 2-18 = fraction I, 19-23 = fraction II, 24-31 = fraction III, 32-41 = fraction IV, 42-55 = fraction V, 56-70 = VI, 71-89 = Fraction VII, 90-110 = Fraction VIII, 111-125 = Fraction IX and 126-145 = Fraction X. The pooled fractions were dialyzed and concentrated by lyophilization. These pooled fraction numbers will be used throughout the manuscript for description of the peaks. Protein content of the pooled fractions was determined by the method of Lowry *et al.* (16) using peanut protein isolate as the standard.

One-dimensional Gel Electrophoresis. Small amounts (45 μg) of the lyophilized protein from each fraction (I to X) were electrophoresed under non-denaturing conditions at a constant current of 1 mA/gel in 7.5% (w/v) polyacrylamide gels following the method of Davis (5). After electrophoresis, proteins were stained with 0.125% Coomassie blue R-250 in 7% acetic acid and 40% methanol and destained with 7% acetic acid and 10% methanol. The destained gels were scanned in a Beckman Model 25 spectrophotometer equipped with a gel scanner, using a 0.05 mm slit. For dolium dodecyl sulfate gel electrophoresis (SDS-PAGE) protein fractions were dissolved in a buffer (1.8 mg/ml) containing 2% (w/v) SDS, 1.5% (w/v) dithiothreitol and 1.2% (w/v) Tris and boiled for 3 min. SDS-PAGE was carried out in 10% (w/v) acrylamide gels using Tris-Glycine-SDS buffer (pH 8.3) system of Laemmli (15). About 90 μg of protein was loaded on each gel and electrophoresed at a constant current of 2 mA/gel. After electrophoresis, the proteins were stained with Coomassie blue R-250 and destained with 7% acetic acid containing 10% methanol. The destained gels were scanned as described earlier.

Two-dimensional Gel Electrophoresis. Lyophilized protein fractions were dissolved in a freshly prepared 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 eletrophoresis (2-D PAGE) by the method of Basha (1) and Horst and Roberts (8). The first dimension was isoelectric-focusing (IEF) in 4% acrylamide gels containing 9.3 M urea, 2% Nonidet P-40 and 2% (v/v) ampholine mixture (pH 3.5-10, pH 5-7 and pH 9-11 ampholines, 10:7:3). About 180-270 μg of protein was loaded on each gel and focused for 18 hr toward anode. After IEF, the gels were equilibrated for 10 min with a buffer containing 1% SDS, 1% 2-mercaptoethanol and 65 mM Tris-HCl (pH 6.9). The equilibrated gels were transferred on to 10% acrylamide slab gels and subjected to SDS-PAGE following the method of Laemmli (15). After electrophoresis, the slab gels were stained with Coomassie blue R-250 for 2 hr and destained with 7% acetic acid containing 10% methanol.

Molecular Weight Estimation. The molecular weight (MW) of the protein fractions was estimated by gel filtration on a Sephacryl S-300 column, using immunoglobulin M (970,000), thyroglobulin (669,000), ferritin (500,000), catalase (232,000), alkaline phasphatase (140,000) and transferrin (76,000) as molecular weight markers. The void volume and salt volume of the column were determined using Blue Dextran 2000 and sucrose, respectively. For estimation of molecular weights of the polypeptides in SDS gels, proteins of known MW's were boiled with 1% SDS, 1.5% dithiothreitol and 1.2% Tris and subjected to SDS-PAGE. Protein standards used for calibration of the gel were thyroglobulin (334,500), β-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).

Preparation of Arachin. Crude arachin was prepared by the method of Jones and Horn (14) with partial modification. The defatted meal was extracted with 10 mM Tris-HCl buffer (pH 8.2), containing 2 M NaCl, 0.2 mM PMSF and 0.002% NaN_3 using mortar and pestle. The homogenate was centrifuged at 20,000g for 20 min at 18 C. The supernatant was made to 20% saturation with ammonium sulfate and the arachin precipitate was collected by centrifugation. The crude arachin was purified by DEAE-cellulose chromatography using a linear NaCl gradient from 0 to 0.5 M. Major protein fractions (eluted between 0.2 to 0.3 M) were pooled and rechromatographed on DEAE.

Protein Hydrolysis. Five milligrams of lyophilized protein were hydrolyzed at 110 C for 18 hours using 6 N HCl. The hydrolyzates were neutralized with sodium acetate buffer and appropriately diluted with 10 mM HCl. An aliquot of the hydrolyzate was analysed in a JEOL amino acid analyzer equipped with a long and a short column following the method of Pancholy *et al.* (20).

Results and Discussion

Fractionation of Peanut Proteins. The peanut proteins were separated into five major (I, III, IV, VI, VIII) and five minor (II, V, VII, IX, X) peaks by gel filtration on a Sephacryl S-300 column (Fig. 1). Most of the peaks were

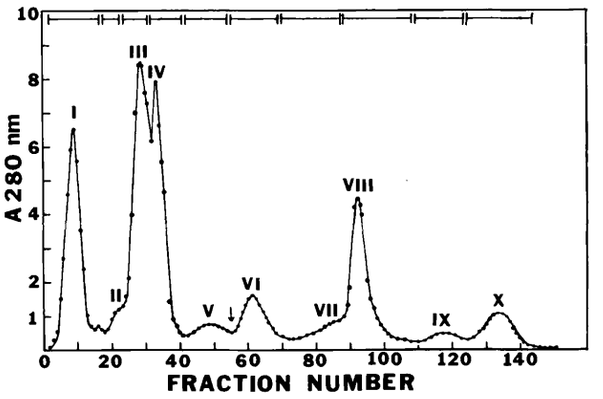

Fig. 1. Gel Filtration of peanut (cv. Early Bunch) seed proteins on Sephacryl S-300 column (2.5 x 135 cm). Following application of the sample (2 g) on the column 150 ml buffer was collected first and then 5 ml fractions were collected. The fractions shown by horizontal bars were pooled, dialyzed and lyophilized for further analysis. Fraction I represents the void volume and fraction VIII represents the salt volume of the column. Vertical arrow shows the position of arachin monomer (MW 180,000).

sharp and symmetrical without excessive trailing. Occasionally, peak IV eluted as a shoulder to peak III. However, in all cases peaks III and IV were easily distinguishable from each other. Fractions under each protein peak were pooled and designated as fractions I through X as described in Methods section. Percent protein distribution (Based on five replications) in the fractions I through X respectively, were: 17, 6, 19, 18, 6, 8, 5, 13, 3, and 4. The position of arachin in the column elutes was identified by chromatographing purified arachin on the Sephacryl S-300 column. Purified arachin eluted mainly in the regions of fractions II, III and IV and approximately 90% of the protein was present in the fractions III and IV. Similar results were obtained when the purified arachin was co-chromatographed either with total extract or with fractions II, III and IV. Except for the protein in fractions II, III and IV, none was found in the other regions of the column following chromatography of pure arachin. Further, the electrophoretic patterns of the purified arachin were identical with those reported earlier for arachin (17, 27). Thus, this would indicate that fractions II, III and IV contain the arachin proteins while the other fractions represent the non-arachin proteins. Fraction I was turbid and had a milkish white color, while fractions VIII, IX and X had a light yellow coloration. Arachin and non-arachin fractions obtained by this method had distinct protein compositions and hence, we decided to use this terminology throughout the manuscript. Additional studies are

being conducted to determine the purity and cryoprecipitation properties of the fractions II, III and IV and will be reported elsewhere.

Molecular Weight Estimation by Gel Filtration. Identity of arachin and non-arachin fractions was also checked by estimating the molecular weight (MW) of the ten fractions. It was found that fraction I eluted in the void volume of the column, while fraction VIII eluted in the salt volume of the column. Since fractions IX and X were retarded longer and eluted after the salt volume as broad peaks, it is likely that these proteins may be interacting with the gel matrix. Fractions IX and X constituted approximately 3% and 4%, respectively, of the total seed protein and were present consistently in each run. Hence, we decided to include them in further analysis. The molecular weights (MW) of the fractions included in the column fractionation range are in Table 1. As shown in the table, MW's for the major arachin fractions III and IV (400,000 and 365,000, respectively) are in close agreement with the reported values (350,000) for the parent molecule (12) and dimer form of arachin (26,27). Interestingly, we did not find the monomer or the dissociated form of arachin (MW 180,000) as reported by Johnson and Shooter (12), Tombs (25) and Yamada *et al.* (27), in our column eluates (tube number 55 of Fig. 1). Additionally, rechromatography of fractions III and IV on Sephacryl S-300 column showed no change in elution volume or any new peaks, suggesting that in our preparation arachin existed mainly in the parental or associated form. Shetty and Rao (23) and Neucere (17) also reported that their purified arachin with a sedimentation coefficient of 14 S (MW 350,000) did not undergo dissociation to the 9 S (MW 180,000) component even under the dissociation conditions used by Johnson and Shooter (12). Then the arachin fractions II, III and IV, which differ slightly in their MW's may represent the MW variants of arachin, as reported by Tombs (25), Tombs and Lowe (26) and Yamada *et al.* (27). Since the exclusion limit of Sephacryl S-300 is 1.5 X 10^6 and fraction I eluted in the void volume of the column, it may contain mainly the high MW aggregates of arachin and non-arachin.

Table 1. Apparent molecular weights of arachin and non-arachin protein fractions derived by gel filtration on Sephacryl S-300 column (2.5 x 135 cm)

Fraction Number	Molecular Weight ± 5,000	
I	> 1.5 x 10^6	(void volume)
II	490,000	
III	400,000	
IV	365,000	
V	255,000	
VI	120,000	
VII	75,000	
VIII	< 75,000	(salt volume)
IX	< 75,000	
X	< 75,000	

Amino acid Composition of the Arachin Fractions. The amino acid composition of the three arachin fractions (II, III and IV) was analyzed to determine their possible structural differences. As seen in Table 2, no major differences are apparent in amino acid composition of the arachin fractions. However, minor differences were present in certain amino acid content. For example, fraction II was slightly higher in aspartic acid (16.58%) while fraction III had more serine and tyrosine. Similarly, fraction IV was rich in lysine and valine (5.5% and 4.1%). In view of the closeness in amounts of most of the amino acids, it appears that the three fractions have similar amino acid composition.

Table 2. Amino Acid composition (g amino acid/100g protein) of arachin fractions II, III, and IV derived by gel filtration on Sephacryl S-300 column (2.5 x 135 cm)

Amino Acids	Arachin fractions from Sephacryl S-300		
	II	III	IV
Lysine	4.20	3.42	5.54
Histidine	2.22	2.14	2.58
NH_3	3.93	3.63	3.86
Arginine	16.58	14.20	14.62
Aspartic acid	12.93	12.60	12.85
Threonine	4.31	4.92	3.20
Serine	3.66	5.67	4.21
Glutamic acid	17.94	18.42	18.62
Proline	4.01	4.56	4.77
Glycine	4.59	3.78	4.05
Alanine	4.69	3.99	3.83
Cystine	T	T	T
Valine	3.04	3.06	4.11
Methionine	T	T	T
Isoleucine	3.59	3.46	3.91
Leucine	6.02	5.97	6.18
Tyrosine	3.79	5.44	3.17
Phenylalanine	5.17	4.87	4.96

Gel Electrophoresis. Gel electrophoresis of the three arachin fractions (II, III and IV) revealed interesting differences in their protein composition (Fig. 2). Fraction III showed a single protein band (B) with a relative mobility (Rm) of 0.39 while fractions II and IV contained two protein bands with Rm values of 0.27 (A) and 0.39 (B). However, ratios of A and B proteins were different for the fractions II and IV (0.39 and 1.11, respectively). Thus the three arachin fractions appeared to differ quantitatively in their protein composition. Electrophoresis of purified

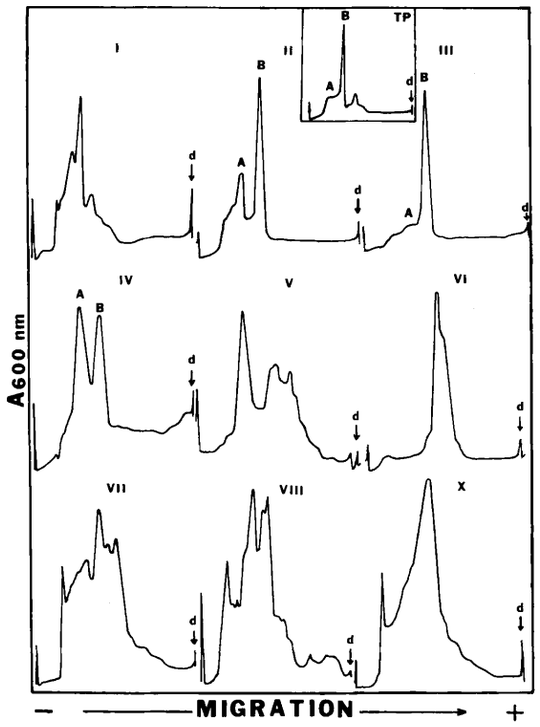

Fig. 2. One-dimensional polyacrylamide gel electrophoretic profiles of peanut (cv. Early Bunch) protein fractions (I, II, III, IV, V, VI, VII, VIII and X) derived after Sephacryl S-300 chromatography. About 45 μg of protein was applied on each gel and electrophoresed at 4 C. After electrophoresis the proteins were stained with Coomassie blue R-250 and the gels were scanned at 600 nm using a 0.05 mm slit. Insert in the figure represents the electrophoretic profile of total protein (TP) from Early Bunch. d = tracking dye.

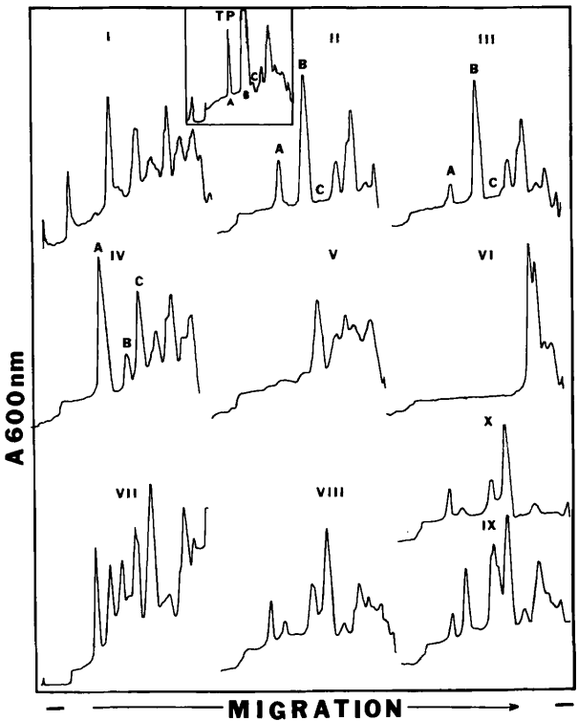

Fig. 3. Sodium dodecyl sulfate gel electrophoretic patterns of peanut (cv. Early Bunch) protein fractions (I, II, III, IV, V, VI, VII, VIII, IX and X) derived after Sephacryl S-300 chromatography. Before electrophoresis samples were treated with 2% SDS at 100 C for 3 min in the presence of 1.5% dithiothreitol. After electrophoresis proteins were stained with Coomassie blue R-250 and scanned at 600 nm using a 0.05 mm slit. The insert in the figure shows the electrophoretic profile of total protein (TP) from peanut cultivar Early Bunch. About 90 μg of protein from fractions I through X were loaded on each gel. In the case of total protein about 180 μg of protein was loaded on the gel to reveal the minor components.

arachin (unfractionated) from Early Bunch showed (data not shown) mainly one component with an Rm value (0.39) identical to fraction III. Likewise, total seed protein showed (insert in Fig. 2) one major band (B) with an Rm value (0.39) similar to that of fraction III. In addition to this component, two other minor bands (non-arachin) were seen in the gel profiles.

It is likely that since component B is present in all the three fractions (Fig. 2), it would be the predominant component in total arachin (II + III + IV) of the seed. Thus, the broad band seen before the component B may represent the component A. Unlike the arachin proteins, the non-arachin proteins were more heterogeneous and showed two to eight protein bands (Fig. 2). Protein composition of fractions VI, IX (not shown) and X appeared to be very simple (one broad band) compared to the other non-arachin fractions (V, VII and VIII). In addition to the protein composition, polypeptide composition of the fractions were also checked by sodium dodecyl sulfate (SDS) gel electrophoresis. Like the non-denaturing gels, the SDS-gels also showed both quantitative and qualitative differences in the polypeptide composition of the ten fractions (Fig. 3). Comparison of polypeptide profiles of arachin fractions (II, III and IV) revealed that, except for polypeptide C (present in fraction V only), all the other polypeptides were common to all the three fractions. The ratios of polypeptides A and B in fractions II, III and IV were 0.38, 0.13 and 3.79, respectively. Except for the quantitative difference in polypeptide A, the polypeptide composition between fractions II and III are very similar. Fraction IV on the other hand, showed both qualitative and quantitative differences in its polypeptide composition and hence, appeared to be distinct. Thus, the observed differences in polypeptide composition of the arachin fractions might be responsible for the slight variations in their MW's. The polypeptide number obtained after dissociation of the arachin fractions (Fig. 3) is consistent with the reported value (six) by Tombs and Lowe (26) and Yamada *et al.* (28). Hence, the arachin obtained by our method appears to be pure and similar to the one obtained by the previous investigators. The SDS-gel pattern of total seed protein was similar to that of arachin fractions with component B being the major polypeptide. Even when the gel was overloaded with the protein (200 to 300 μg), only trace amount of protein was seen in the region of component C. Similarly, component A was seen as a minor peak (not shown) when normal quantity (50 μg) of protein was loaded on the gel. Thus it appears that in total arachin, protein B (Fig. 2) and polypeptide B (Fig. 3) are dominant. Similar results were obtained with the purified arachin after SDS-PAGE.

Two-Dimensional Gel Electrophoresis. The one-dimensional gel electrophoresis facilitated identification of the arachin and non-arachin proteins in the column eluates and also revealed differences in their protein and polypeptide composition. It also showed the existence of several proteins and polypeptides with similar electrophoretic mobilities between arachin and non-arachin. However, by 1-D PAGE it was difficult to ascertain similarities in the protein and polypeptide composition of arachin and non-arachin. In order to further examine the compositional differences in the arachin fractions and to check for the possible existence of common polypeptides between the arachin and non-arachin proteins, the ten fractions were studied by two-dimensional gel electrophoresis (2-D PAGE). The 2-D PAGE, which separates proteins based on their isoelectric point (pI) and molecular weight (MW), increased the protein resolution and revealed a complex polypeptide composition of the fractions (Fig. 4 and 5). In general, the polypeptide components of the ten

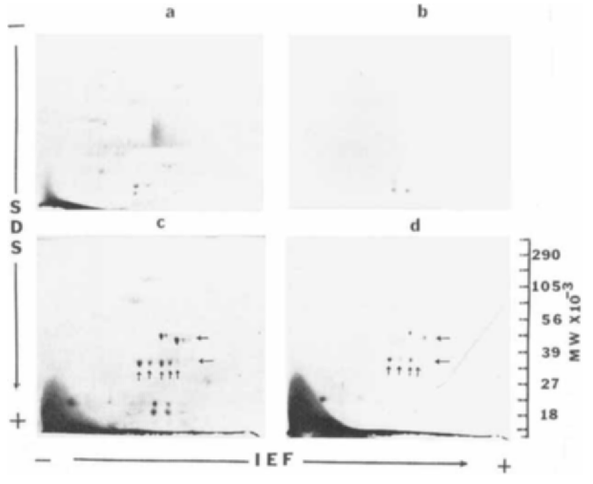

Fig. 4. Two-dimensional gel electrophoretic patterns of arachin fractions. The protein fractions derived after Sephacryl S-300 chromatography were concentrated and subjected to isoelectricfocusing in the first dimension and SDS-gel electrophoresis in the second dimension. About 180 to 270 μg of protein was applied on each gel. For comparison a two-dimensional polypeptide map of purified arachin from peanut cultivar Early Bunch is included. a = purified arachin, b = fraction II, c = fraction III, d = fraction IV. The proteins that differ among the fractions are indicated with arrows. Similar patterns were obtained consistently for each fraction derived from different experiments.

fractions had pI's between 4.4 and 8.5 and MW's between 15,000 and 70,000. Comparison of polypeptide maps of arachin fractions II, III and IV showed a similar polypeptide composition between them (Fig. 4). Interestingly, the quantity of a component with pI's between 6.0 and 6.4 and MW around 68,000 was relatively higher in fraction IV than in fractions II and III (arrowed horizontally in Fig. 4-d). This is consistent with our observation in Fig. 3, in which the amount of polypeptide A (MW 68,000) was higher in fraction IV than in fractions II and III. Further, fraction IV also contained a row of polypeptides with MW around 68,000 and pI's between 5.8 and 6.2 (arrowed in Fig. 4-b). But, these components were present in trace amounts in fractions III and IV. Except for these, no other major differences in the polypeptide composition were

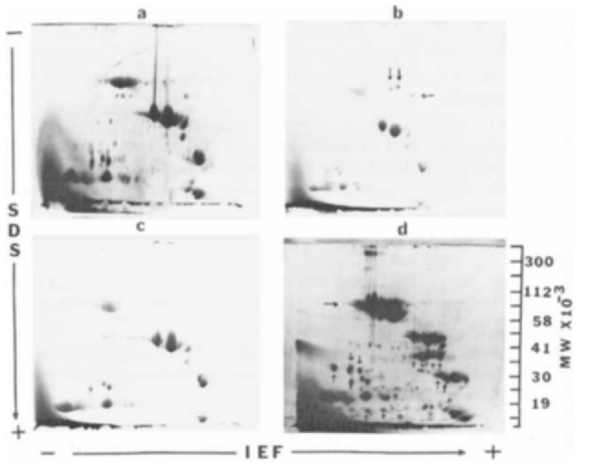

Fig. 5. Two-dimensional polypeptide maps of non-arachin fractions. The protein fractions derived after Sephacryl S-300 chromatography were subjected to isoelectricfocusing in the first dimension and SDS-gel electrophoresis in the second dimension. a = fraction V, b = fraction VI, c = fraction VIII, d = fraction X. The differences in the polypeptide compostion among the fractions are indicated with arrows. About 90 to 180 μg of protein was loaded on each gel. Similar patterns were obtained consistently for each fraction derived from different experiments.

observed between the three arachin fractions, suggesting that qualitatively fraction IV differs slightly from the fractions II and III. Among the non-arachin proteins, fraction V was more heterogeneous and contained several polypeptides with a wide range of MW's and pI's (Fig. 5-a). Fraction VI, on the other hand showed a simple polypeptide pattern and contained four polypeptides with MW's between 15,500 and 20,000 and pI's between 5.6 and 6.2. In general, fractions VII, VIII, IX and X showed slight differences in their polypeptide composition. For example, fraction VII (not shown) contained a row of polypeptides with MW around 34,000 and pI's between 5.5 and 6.4. Fractions VIII, IX (not shown) and X contained two rows of polypeptides with pI's between 5.5 and 6.2 and MW's around 34,000 and 43,000 (arrowed horizontally). However, the polypeptide quantity and number were different in each row among these fractions. For example, fractions VIII and IX contained five polypeptides in the 34,000 MW class while fractions VII and X showed only four components (arrowed vertically in Fig. 5-c and 5-d). Although fractions VII, VIII, IX and X contained small amounts of fractions V and VI proteins, they did not contain any arachin proteins. The 2-D polypeptide patterns obtained for each fraction was consistent from run to run, and among the fractions derived from different gel filtration runs.

Composite 2-D Polypeptide Maps. Figure 6 shows the composite polypeptide maps of arachin and non-arachin proteins prepared after including all the polypeptides found in the arachin (fractions II to IV) and non-arachin (fractions V to X) protein fractions. For easy identification of the polypeptides, the map has been divided into alphabetically labeled quadrants. The vertical bars represent different pI's and the horizontal bars represent different

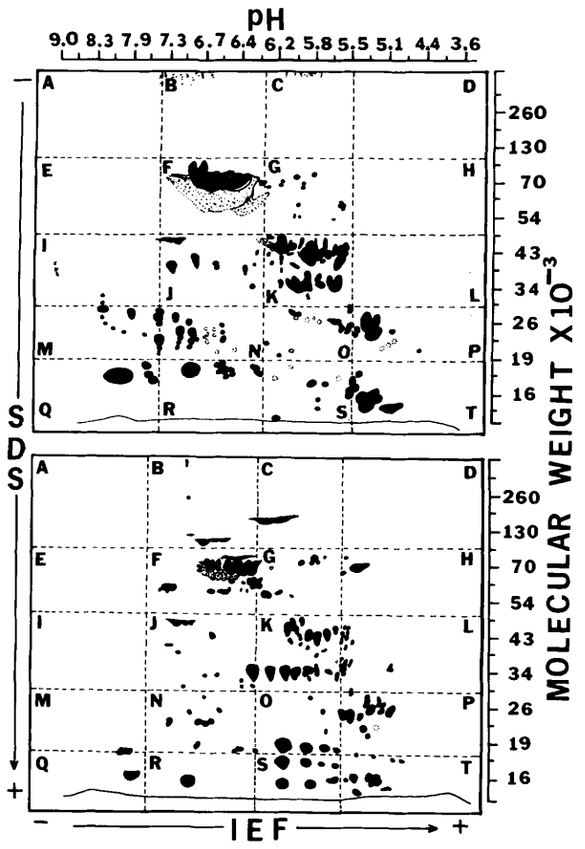

Fig. 6. composite two-dimensional polypeptide maps of arachin (a) and non-arachin (b) proteins from peanut cultivar Early Bunch, prepared after examining several 2-D maps of each fraction. Seed proteins were fractionated on a Sephacryl S-300 column into arachin and non-arachin fractions and then the two-dimensional polypeptide patterns of these fractions were obtained. The composite maps of arachin and non-arachin were then constructed after including all the polypeptide found among the arachin (II, III, IV) and non-arachin (V, VI. VII, VIII, IX, X) fractions.

MW's. Examination of the composite maps suggests that the arachin polypeptides can be grouped into six molecular weight classes (Fig. 6-a). They are 68,000; 44,000; 34,000; 26,000; 18,500 and 15,500 MW classes. The polypeptides of each class had different pI's. For example, the 18,500 and 26,000 MW class polypeptides had pI's between 4.7 and 8.4, while the 68,000, 43,000, 34,000 and 15,500 MW polypeptides showed narrow pI ranges between 6.3 to 7.3, 5.5 to 6.3, 5.5. to 6.2 and 4.7. to 5.6, repsectively. Similarly, the non-arachin proteins contained nine molecular weight classes of polypeptides (Fig. 6-b). Their MW's and pI's were 170,000 (6.0 to 6.3), 95,000 (6.5 to 6.9), 66,000 (6.3 to 6.9), 43,000 (5.5 to 7.0), 34,000 (5.5 to 6.4), 24,500 (4.7 to 5.6), 19,500 (5.5 to 7.9), 18,000 (5.5 to 6.2), and 16,000 (5.5 to 7.9). When the composite maps of arachin and non-arachin proteins were superimposed, several polypeptides having similar mobilities were found between arachin and non-arachin. For example, a majority of the polypeptides of arachin and non-arachin proteins were present mainly in the quadrants, F, K, P and T. This would suggest that most of the polypeptides of arachin and non-arachin proteins have similar electrophoretic mobilities. Although we have used only one peanut cultivar to prepare the composite polypeptide maps of arachin and non-arachin proteins, this pattern should be generally applicable to most of the peanut cultivars since the protein compostion of Early Bunch was similar to the other cultivars (1). However, genetic and environmental effects on the composition of arachin and non-arachin proteins can not be precluded.

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