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Changes in the Polypeptide Composition of Maturing Seeds from Four Peanut (*Arachis hypogaea* L.) Cultivars[1].

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ABSTRACT

Developing seeds from four peanut (*Arachis hypogaea* L.) cultivars were obtained and classified into Immature, Low-Intermediate, Intermediate, High-Intermediate and Mature groups. Seed proteins were extracted from the defatted meals using either 2 M NaCl, 0.01 M Tris HCl (pH 8.2) or 9.3 M urea, 5 mM K_2CO_3, 2% Nonidet P-40 and 0.5% dithiothreitol and examined by one and two-dimensional polyacrylamide gel electrophoresis. Two dimensional gel electrophoresis showed major qualitative and quantitative changes in seed protein composition during maturation. Several major protein components gradually disappeared while others increased in their content. Major changes in the polypeptide composition were observed between the Intermediate and High-Intermediate maturity stages. In addition, the arachin (major storage globulin of peanut) components were present from the very early stages of seed development. Variation in the amount of protein components indicate selective synthesis and modification of certain seed proteins during seed development.

Key Words: Cotyledons, Maturity, Peanut, Polypeptides, Two-dimensional gel electrophoresis, Protein.

Developing peanut seeds have been subjected to numerous studies involving morphological (6, 17, 18), physiological, and biochemical (1, 14, 15, 16) changes. In peanut, the major storage globulin, arachin, constitutes more than 70% of the seed protein (11, 12). Using gel electrophoresis, Cherry (7), found that the deposition of large molecular-weight storage globulins was rapid between 9 and 12 weeks after pegging and varied quantitatively among mature seeds grown in different environments. Recently, Basha *et al.*, (5) separated maturing peanuts into four groups based on their maturity level and examined differences in their protein composition. They found that arachin was present in lower quantity early in the cotyledon development, but rapidly became the predominant component during the High-Intermediate and Mature stages. Because of arachin domination in seed proteins and incomplete resolution of the proteins by one-dimensional gel electrophoresis, the previous studies have failed to show detailed qualitative and quantitative changes in the protein composition of maturing peanut seed. Recently, Basha (3), using two-dimensional gel electrophoresis (2-D PAGE) resolved peanut proteins into more than 200 polypeptides based on their isoelectric point and molecular weight and found cultivar differences in seed polypeptide composition. Because of its resolving power 2-D PAGE was employed to determine the compositional changes in the proteins of maturing peanut seed.

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Materials and Methods

Seed Material.

Two peanut (*Arachis hypogaea* L.) cultivars (Early Bunch and Florunner) and two breeding lines (Jenkins Jumbo and Altika) from the breeding stock of the University of Florida were grown during the 1978 season in experimental plots at the University of Florida Agricultural Research Station, Gainesville. Randomly selected plants were collected at 18 weeks after planting, fruits were harvested, and seeds were separated into different maturity groups based on the testa and pericarp color as follows: Mature-seeds having black splotches throughout the pericarp and a brown, tightly bound testae; High-Intermediate-seeds having brown splotches on the inner pericarp, and a dark pink seed coat; Intermediate-seeds with a semi-dry and cracked pericarp and a pink testae; Low-Intermediate-seeds having a soft and white inner pericarp and a white to light pink testae; immature-seeds with watery, soft and spongy pericarp and a very thick white testae (5). These maturity groups will approximately correspond to the maturity stages of Pattee *et al.*, (14) as follows: Immature - stage 4; Low-Intermediate - stage 6; Intermediate - stage 8; High-Intermediate - stage 11; Mature - stage 15. After classification, the testae and embryonic axes were removed from the seeds and the cotyledons were ground into a meal and defatted as follows: A 10 g quantity of full-fat meal was placed in a scintillation vial and 15 ml of cold diethylether was added. The contents were mixed throughly by shaking and then allowed to stand overnight. The ether layer was removed with a Pasteur Pippette and the residue was again suspended in 15 ml of ether. This procedure was repeated three more times and the final residue was air dried and stored at -20 C.

∝-Amino Nitrogen.

Defatted peanut meals (100 mg) were extracted with 30 ml of a mixture of methanol:chloroform:water (60:25:15, v/v/v) according to the method of Young *et al.*, (21). The insoluble material was removed by centrifugation at 20,000 g for 20 min. The resulting pellet was reextracted with 15 ml of methanol:chloroform:water mixture and centrifuged. The resulting supernatants were mixed and made up to 50 ml. Three replicate 100 µl aliquots were used to determine ∝-amino nitrogen content of this extract by the method of Yemm and Cocking (19).

Total Protein.

Proteins from the defatted meals (50 mg) were extracted with 5 ml of hot (37 C) 1 M NaOH by grinding in a mortar with a pestle. The material was then incubated at 37 C for 16 hours in a shaker bath. This suspension was then centrifuged at 20,000 g for 20 min. The pellet was reextracted with 3 ml of 1 M NaOH and centrifuged. The two supernatants were pooled and made up to 10 ml. This procedure extracted more than 95% of the seed proteins (5). Protein content of this extract was assayed by the method of Lowry *et al.*, (13) using bovine serum albumin as the standard.

One-dimensional Gel Electrophoresis.

Deffated meal (100 mg) was extracted with 5 ml of 2 M NaCl, 0.01 M Tris-HCl (pH 8.2) by grinding in a mortar with a pestle. The homogenate was stirred for 15 min and centrifuged at 20,000 g for 30 min. The supernatant was diluted (1:20) and electrophoresed in 7.5% (w/v) polyacrylamide gels under non-denaturing conditions (8). About 50 to 100 µg of protein were applied per gel. After electrophoresis the proteins were stained with coomassie blue R-250 and destained with 7% acetic acid and 10% methanol. Destained gels were scanned in a Beckman Model 25 Spectrophotometer equipped with a gel scanner using a 0.05 mm slit. The position of arachin in the gels was identified using purified arachin (4).

Two-dimensional Gel Electrophoresis.

Defatted meals of the maturing peanut seed were homogenized with a solution containing 9.3 urea, 0.5% (w/v) dithiothreitol, 0.005 M K_2CO_3 and 2% (v/v) Nonidet P-40 (BDH Chemicals Ltd., Poole, UK). The non-

ionic detergent Nonidet P-40, improved the protein solubility and enabled direct examination of seed protein extracts by two-dimensional gel electrophoresis. The homogenate was centrifuged at 20,000 g for 30 min and the clear supernatant (300 μg protein/gel) was subjected to two-dimensional gel electrophoresis (2-D PAGE) by the method of Basha (3), and Horst and Roberts (10). The isoelectricfocusing dimension (first) was performed in 4% (w/v) acrylamide gels containing 9.3 M urea, 2% (v/v) ampholine mixture (pH 3.5 to 10, pH 5 to 7 and pH 9 to 11; 10:7:3) and 2% (v/v) Nonidet P-40. Isoelectricfocusing was carried out for 18 hours at 18 C. The second dimension was in 10% (w/v) acrylamide slabs containing 0.1% (w/v) sodium dodecyl sulfate (SDS). After electrophoresis the gels were stained with coomassie blue R-250 and destained with 7% acetic acid and 10% methanol.

Molecular Weight Estimation.

The molecular weight (MW) of the dissociated seed proteins in the SDS-electrophoresis dimension was determined using the following molecular weight markers: Thyroglobulin (330,000), 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).

Results

Total α-amino Nitrogen and Protein.

The peanut cotyledons were examined for total α-amino nitrogen and protein content, which are known to exhibit an inverse relationship during seed maturation. The α-amino nitrogen content decreased rapidly between the Immature and High-Intermediate stages of the seed development (Fig. 1). Further, the amount of α-amino nitrogen at each maturity stage appeared to be different for each peanut genotype. For example, at the Immature stage Jenkins Jumbo and Early Bunch contained higher amounts (9.22% and 8.6% respectively) of α-amino nitrogen than Altika (5.34%) and Florunner (5.49%). However, at the Mature stage all of them contained similar amounts (0.47 to 0.72%) of α-amino nitrogen. Unlike α-amino nitrogen, seed protein content increased slowly until the Low-Intermediate stage and then there was a rapid increase until maturity. The amount of protein found at various stages of seed maturity (especially between Immature and Low-Intermediate stages) varied among the four peanut genotypes. Although the seeds were grown at the same location and year, the observed differences in the α-amino nitrogen and protein content may also have been influenced somewhat by localized environmental conditions.

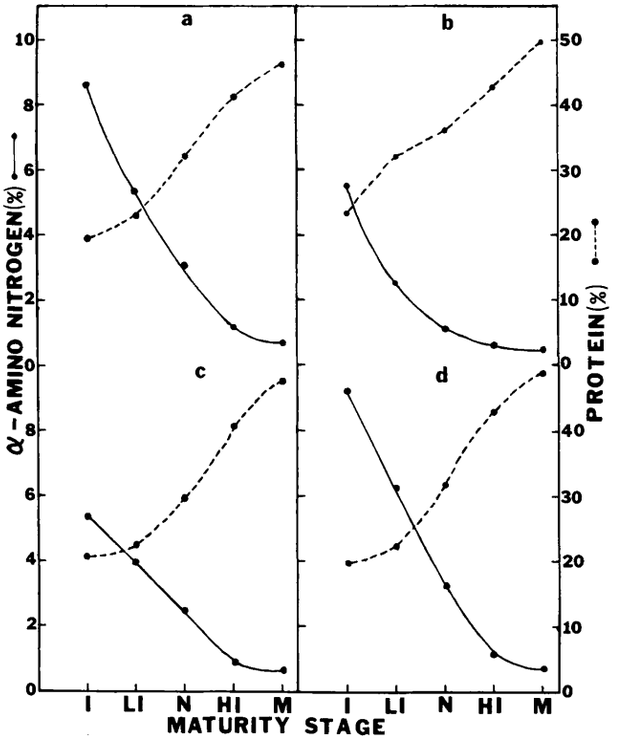

Fig. 1. Changes in the α-amino nitrogen and protein content of developing peanut seeds. The values are expressed as g/100 g of defatted peanut meal. a=Early Bunch, b=Florunner, c=Altika, d=Jenkins Jumbo. The maturity stages are: I = Immature, LI = Low-Intermediate, N = Intermediate, HI = High-Intermediate, M =Mature.

One-dimensional Gel Electrophoresis.

The relative amounts of protein deposited during seed maturation are shown in Fig. 2. Electrophoresis of peanut proteins under non-denaturing conditions showed the presence of three major and one or two minor components. The general profile of proteins showed that the ratio of the two major seed components (A,B) remained unchanged during the period of seed maturation. However,

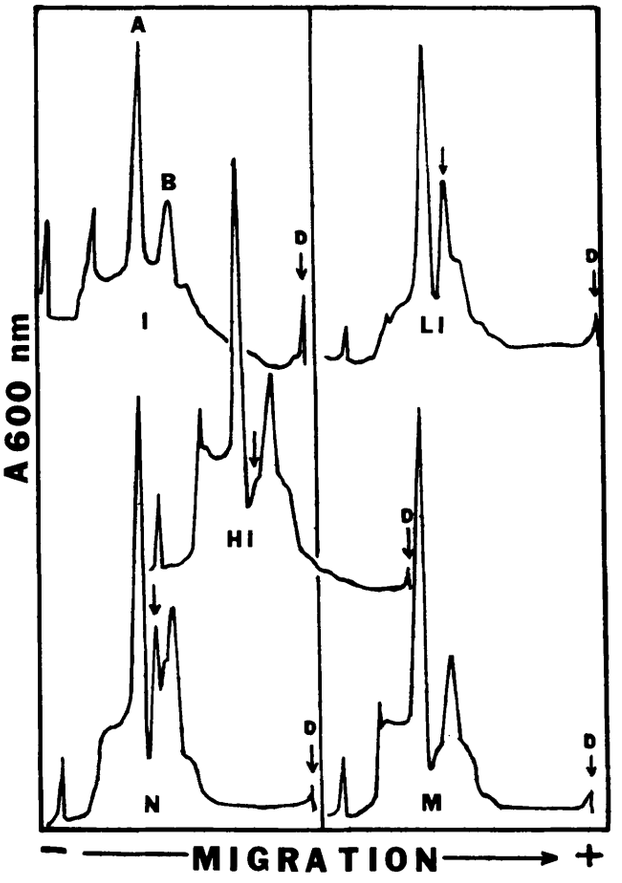

Fig. 2. One-dimensional polyacrylamide gel electrophoretic patterns of changes in the Florunner seed proteins during seed maturation. Maturity stages include: Immature (I), Low-Intermediate (LI), Intermediate (N), High-Intermediate (HI), and Mature (M). D = Dye front. A component (Rm 0.39) shown with an unmarked arrow, decreased between Low-Intermediate and High-Intermediate stages. The major protein peak (A) in the gels is arachin. About 50 μg of protein was applied to each gel.

a few changes were detected in the faster migrating components between the Immature and High-Intermediate stages. For example, a component with an Rm value of 0.39 (arrow) decreased considerably by the High-Intermediate stage, and almost disappeared in the mature peanut. Since the rate of protein migration (as determined in relation with the mobility of tracking dye bromophenol blue) was similar in all the gels, it is unlikely that the above differences are artifacts due to variations in the rates of protein migration.

Two-dimensional Gel Electrophoresis.

Two-dimensional gel electrophoresis resolved peanut proteins into more than 200 components having different isoelectric points and molecular weights. Many of these were not shown by 1-D PAGE because of the high arachin content (70%) and presence of proteins having similar mobility, especially in the non-arachin components of maturing peanut cotyledons.

Figures 3, 4, 5 and 6 show the major changes (indicated by arrows) in the polypeptide composition of maturing seeds from Early Bunch, Jenkins Jumbo, Altika and Florunner. Overall, the component number decreased with maturation accompanied by an enrichment in certain other components. Although the polypeptide pattern remained unchanged between Immature and Low-Intermediate stages, there were major changes in the polypeptide composition between the Intermediate and High-Intermediate stages. For example, several polypeptides with molecular weights between 30,000 and 80,000 that were present at the Immature stage either disappeared or decreased by maturity. Additionally, some polypeptides (one in Early Bunch and two in Jenkins Jumbo) with a molecular weight around 35,000 (arrows) disappeared by maturity. Similarly, several high molecular weight (100,000 to 200,00) components that were seen in Immature seeds were absent in Intermediate seeds. The majority of the components found in mature seeds were present in Immature seeds. Thus, except for intensification of certain major polypeptides no new major components appeared by the Mature stage. It should be noted that since arachin dominates the peanut seed proteins most of the major polypeptides seen in the 2-D maps belong to arachin.

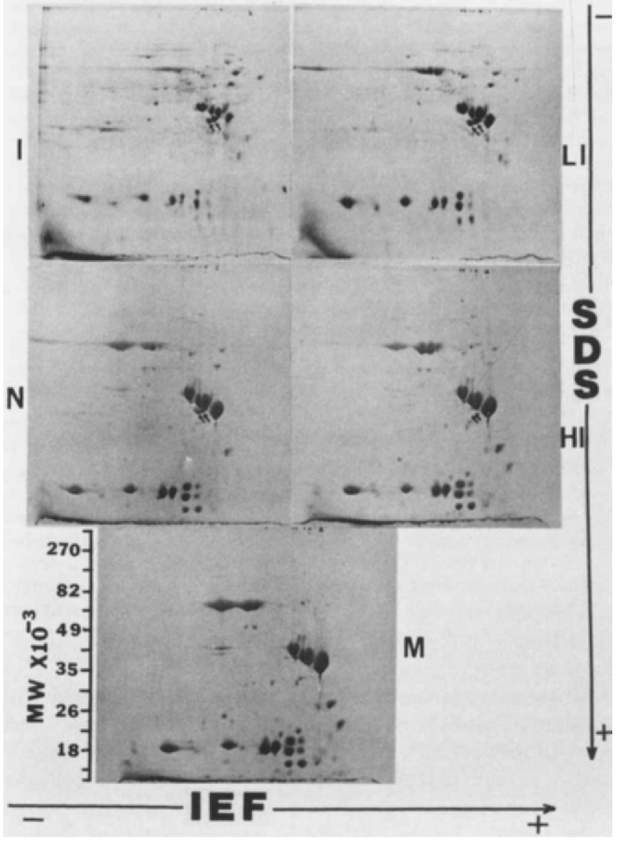

Fig. 3. Two-dimensional polypeptide profiles of maturing seed from peanut cultivar Early Bunch. Maturity stages include: Immature (I), Low-Intermediate (LI), Intermediate (N), High-Intermediate (HI), and Mature (M). About 300 μg of protein was spotted on the gel. The arrow shows the major components that undergo changes during maturation. Notice, intensification of a component with a MW between 49,000 and 82,000 and a decrease in the total component number between Immature and Intermediate stages. IEF = Isoelectricfocusing, SDS = Sodium dodecyl sulfate gel electrophoresis.

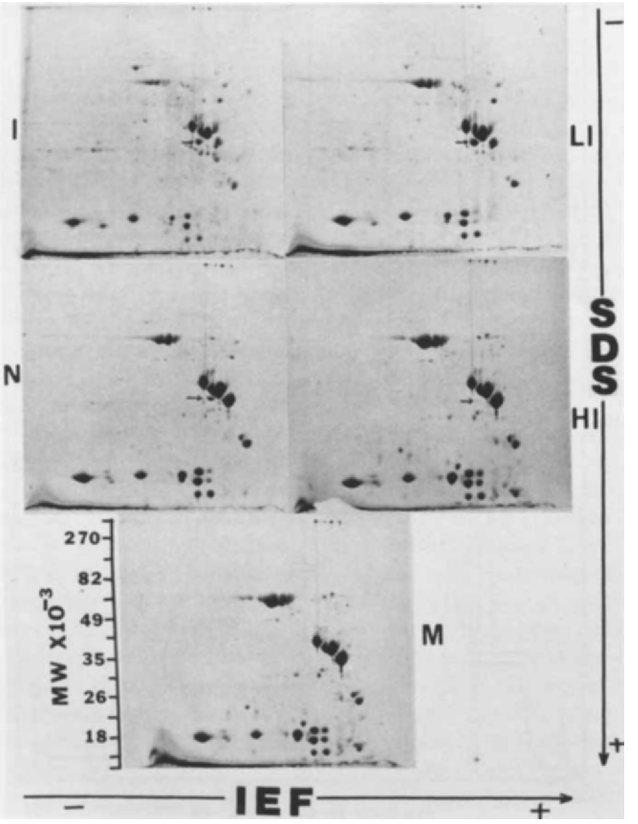

Fig. 4. Two-dimensional gel electrophoretic profiles of proteins from maturing peanut seed of Jenkins Jumbo. Maturity stages include: Immature (I), Low-Intermediate (LI), Intermediate (N), High-Intermediate (HI), and Mature (M). About 300 μg of protein was spotted on the gel. The arrow shows the major components that undergo changes during maturation. Notice, intensification of a component with a MW between 49,000 and 82,000 and a decrease in the total component number between Immature and Intermediate stages. IEF = Isoelectricfocusing, SDS = Sodium dodecyl sulfate gel electrophoresis.

Discussion

In peanut, arachin (the major storage globulin) consti-

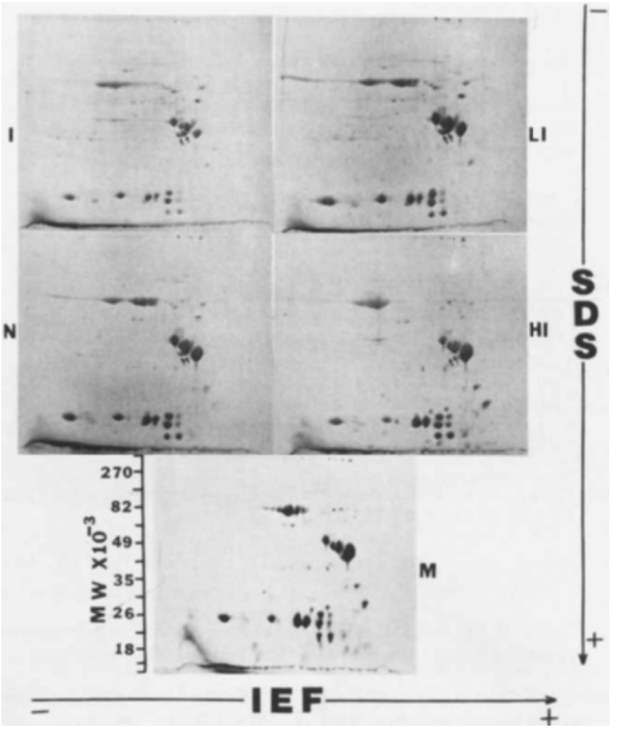

Fig. 5. Two-dimensional gel electrophoretic profiles of proteins from maturing peanut seed of Altika. Maturity stages; Immature (I), Low-Intermediate (LI), Intermediate (N), High-Intermediate (HI), Mature (M). About 300 μg of protein was spotted on the gel. The arrow shows the major components that undergo changes during maturation. Notice, intensification of a component with a MW between 49,000 and 82,000 and a decrease in the total component number between Immature and Intermediate stages. IEF = Isoelectricfocusing, SDS = Sodium dodecyl sulfate gel electrophoresis.

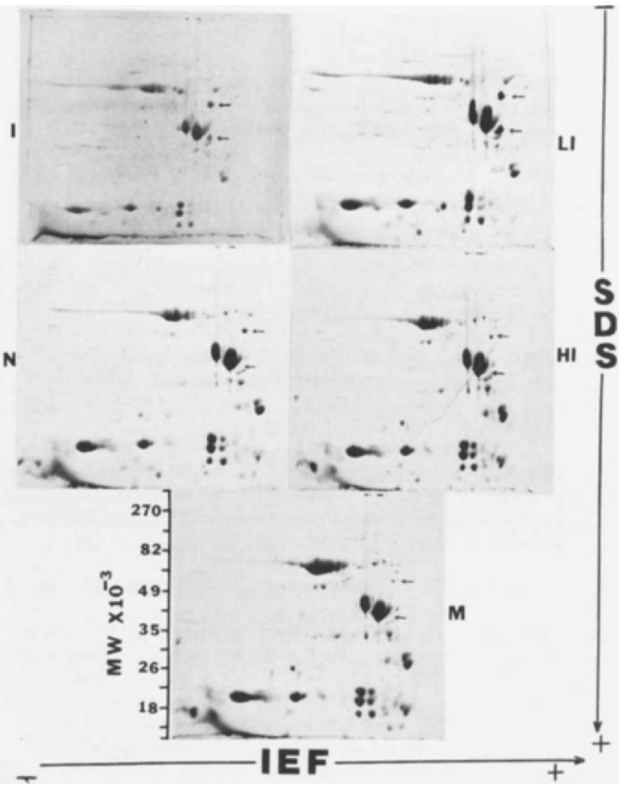

Fig. 6. Two-dimensional gel electrophoretic profiles of proteins from peanut seed of cultivar Florunner. Maturity stages include: Immature (I), Low-Intermediate (LI), Intermediate (N), High-Intermediate (HI), and Mature (M). About 300 μg of protein was spotted on the gel. The arrow shows the major components that undergo changes during maturation. Notice, intensification of a component with a MW between 49,000 and 82,000 and a decrease in the total component number between Immature and Intermediate stages. IEF = Isoelectricfocusing, SDS = Sodium dodecyl sulfate gel electrophoresis.

tutes more than 70% of the seed protein. Consequently, it tends to mask the non-arachin components when proteins are examined by conventional separation techniques. Although 1-D PAGE has been used to study the peanut protein deposition (5, 7), no significant differences were found in protein composition of maturing seeds. Since, non-arachin proteins are nutritionally superior (4, 9), it is essential to determine their synthetic patterns in studies aimed at improving the quality of peanut seed protein. Hence, in this study, an attempt has been made to study the peanut seed protein deposition during seed maturation using 2-D PAGE, which effectively resolves both arachin and non-arachin components (3) based on their isoelectric points and molecular weights.

Peanut plants are indeterminate in growth habit and hence at a given stage the plant produces seeds of different maturities. Although the maturity classification commonly used in peanuts is based on morphological characteristics (1, 5, 14, 20), our results demonstrate concomitant changes in the proteins of the seed. Thus total free amino acid and protein content of different maturity groups indicated that the morphological classification used in this study are consistent with developmental patterns of the peanuts seed (5).

Since most of the major polypeptides found in the peanut seed are reserve protein constituents (2, 3, 11) a gradual enrichment in these components during maturation is to be expected. Presence of most of the arachin components at the Immature stage indicate their synthesis from the very beginning of seed development. In addition, the gradual enrichment of certain components suggests that these polypeptides were being selectively synthesized and proteins were being modified during seed maturation. Similarities in the polypeptide deposition patterns of the four peanut cultivars indicate the existence of common pathways of protein accumulation among the cultivars. Once the nutritionally rich polypeptides of the peanut seed are identified, the observed differences in the deposition pattern of various polypeptides would be of great use in studies aimed at manipulating the seed protein composition using a range of genetic, agronomical and physiological factors to improve the nutritional value of peanut proteins.

Acknowledgements

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Literature Cited

1. Aldana, A. B., R. C. Fites and H. E. Pattee. 1972. Changes in nucleic acids, protein and ribonuclease activity during maturation of peanut seeds. Plant Cell Physiol. 13:515-521.

2. Altschul, A. M., L. Y. Yatsu, R. L. Ory and E. M. Engleman. 1966. Seed proteins. Ann. Rev. Plant Physiol. 17:113-136.

3. Basha, Shaik-M, M. 1979. Identification of cultivar differences in seed polypeptide composition of peanuts (*Arachis hypogaea* L.) by two-dimensional polyacrylamide gel electrophoresis. Plant Physiol. 63:301-306.

4. Basha, S. M. M. and J. P. Cherry. 1976. Composition, solubility, and gel eletrophoretic properties of proteins isolated from Florunner (*Arachis hypogaea* L.) peanut seeds. J. Agric. Food Chem. 24:359-365.

5. Basha, S. M. M., J. P. Cherry and C. T. Young. 1976. Changes in free amino acids, carbohydrates, and proteins of maturing seeds from various peanut (*Arachis hypogaea* L.) cultivars. Cereal Chem. 53:586-597.

6. Brennan, J. R. 1969. The peanut gynophore. Biologist 51:71-82.

7. Cherry, J. P. 1974. Electrophoretic observation on protein changes and variability during development of the peanut. J. Agric. Food Chem. 22:723-725.

8. Davis, B. J., 1964., Disc electrophoresis II. Method and application to human serum proteins. Ann NY Acad. Sci. 121:404-427.

9. Dawson, R. 1968. The nutritive value of groundnut protein. 2. The correlation between electrophoretic pattern and nutritive value. Br. J. Nutr., 22:601-607.

10. Horst, M. N. and R. M. Roberts. 1979. Analysis of polypeptide turnover rates in Chinese Hamster ovary cell plasma membrane using two-dimensional gel electrophoresis. J. Biol. Chem. 254:5000-5007.

11. Irving, G. W., T. D. Fontaine and R. C. Warner. 1945. Electrophoretic investigation of peanut proteins. 1. peanut meal extract, arachin and conarachin. Arch. Biochem. 7:475-489.

12. Jones, D. B. and M. J. Horn. 1930. The properties of arachin and conarachin and the proportionate occurence in peanut. J. Agr. Res. 40:672-682.

13. Lowry, O. H., N. Rosebrough, A. L. Farr and R. L. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

14. Pattee, H. E., E. B. Johns, J. A. Singleton and T. H. Sanders. 1974. Composition changes of peanut fruit parts during maturation. Peanut Sci. 1:57-62.

15. Pattee, H. E., J. A. Singleton, E. B. Johns and B. C. Mullin. 1970. Changes in the volatile profile of peanuts and their relationship to enzyme activity levels during maturation. J. Agr. Food Chem. 18:353-356.

16. Pickett, T. A. 1950. Composition of developing peanut seed. Plant Physiol. 25:210-224.

17. Schenk, R. U. 1961. Development of the peanut fruit. Ga. Agr. Exp. Sta. Tech. Bull., N. S. 22.

18. Smith, B. W., 1956. Embryogeny and the effect of peg elongation upon embryo and endosperm growth. Amer. J. Bot. 43:233-240.

19. Yemm, E. W. and E. C. Cocking. 1955. The determination of amino acids with ninhydrin. Analyst 80:209-213.

20. Young, C. T., M. E. Mason, R. S. Matlock and G. R. Waller. 1972. Effect of maturity on the fatty acid composition of eight varieties of peanuts grown at Perkins, Oklahoma in 1968. J. Amer. Oil Chem. Soc. 49:314-317.

21. Young, C. T., R. S. Matlock, M. S. Mason and G. R. Waller. 1974. Effect of harvest date and maturity upon free amino acid levels in three varieties of peanuts. J. Amer. Oil Chem. Soc. 51:269-273.

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