PEANUT SCIENCE

VOLUME 11 JANUARY – JUNE, 1984 NUMBER 1

Variations in the Methionine-rich Protein Composition of the Genus *Arachis*[1]

Sheikh M. Basha* and Sunil K. Pancholy[2]

ABSTRACT

Methionine-rich proteins (MRP) from seeds of different species of the Genus *Arachis* were isolated and analyzed by gel electrophoresis to detect possible compositional differences. One-dimensional gel electrophoretic analysis showed presence of quantitative and qualitative variations among the MRP-fractions. Following two-dimensional gel electrophoresis, the MRP-fractions were found to contain three groups of polypeptides with apparent molecular weights of approximately 21,000; 19,000 and 16,000, and isoelectric points between 5.1 and 5.8. Within each molecular weight group the number of polypeptides varied between 1 and 3.

Key Words: Methionine-rich proteins; Molecular weights; Isoelectric points; Peanut; Polypeptides; Two-dimensional gel electrophoresis.

Peanuts, like other legume seeds, are low in sulfur-containing amino acids such as methionine and cystine. Many studies have found considerable variation in the levels of methionine, lysine and cystine among the various protein fractions of peanut seed (2,7,10,11). Basha and Pancholy (3,4) have isolated a methionine-rich protein (MRP) fraction from peanut seed (cv. Early Bunch) having 2.9% methionine and 10.77% cystine. They have also found it to be composed of at least six polypeptides with molecular weights between 15,500 and 20,000, and isoelectric points between 5.6 and 6.2. In addition, based on the ^{35}S-methionine incorporation data (4), they have found varying amounts of ^{35}S-methionine radioactivity among the polypeptides, which indicate that they are composed of varying amounts of methionine. Previous studies (1) on the polypeptide composition of several peanut cultivars of the species, *hypogaea* showed lack of variation in the polypeptide make up of the methionine-rich proteins. Recently, Basha and Pancholy (5) analyzed several wild species of peanuts and found wide variation in their amino acid and total protein composition. In view of this finding, the present study was carried out to identify possible compositional differences in the polypeptides of the MRP-fractions from various species of the Genus *Arachis*.

[1]This research was supported by a grant from the USDA-SEA/CSRS, Washington, D.C.

[2]Associate Professor and Professor, respectively. Peanut Protein Laboratory, Division of Agricultural Sciences, Florida A&M University, Tallahassee, Florida 32307.

Materials and Methods

Seed Material: Sound mature kernels of the following accessions were a gift from Dr. Ray O. Hammons of USDA-ARS, Crops Research Unit, Tifton, Georgia. The entries used in this study were *Arachis batizogaea* (PI 405082), *A. villosulicarpa* (PI 336985), *A. stenosperma* (PI 338279), *A. monticola* (PI 405933), *A. chacoense* (10602 GKP), and *A. hypogaea* L. cv. florunner. Seed coat and embryonic axes were removed and the cotyledons were ground into a meal using a mortar and pestle, and defatted by repeated extractions with cold diethyl ether. The fat-free meal was stored at -20 C until used.

Protein Extraction: Defatted meal (125mg) was extracted with 4 mL of 0.01 M Tris-HCl buffer (pH 8.2) containing 2 M NaCl, 0.002% (w/v) NaN_3 and 0.002 M phenylmethanesulfonylflouride (PMSF) using a glass homogenizer. The homogenate was centrifuged at 20,000 g for 20 min at 10 C. The resulting supernatant was used for the purification of methionine-rich protein fraction.

Isolation of Methionine-rich Protein (MRP) Fraction: The MRP fraction was isolated following the method of Basha and Pancholy (4), by gel filtration on Sephacryl S-300 column (2.5 x 125 cm). The MRP fraction recovered from the gel filtration step was used in the subsequent analyses.

One-dimensional Gel Electrophoresis: The MRP fraction (50-100 μg protein) was subjected to one-dimensional gel electrophoresis, both under non-denaturing (6) and denaturing conditions (8) in 7.5% and 10% polyacrylamide gels, respectively. Following gel electrophoresis, the proteins were stained with Coomassie blue and scanned in a Beckman model 25 dual beam spectrophotometer equipped with a gel scanner, using a 0.05 mm slit.

Two-dimensional Polyacrylamide Gel Electrophoresis (2-D PAGE): The 2-D PAGE of MRP-fraction consisted of isoelectrofocusing (IEF) in the first dimension and SDS-gel electrophoresis in the second (1). IEF was performed in 4% acrylamide gels using pH 3.5-10; 5-7 and 9-11 ampholines (50 : 35 : 15, respectively). SDS gel electrophoresis was conducted in the 10% acrylamide slab gels. Following electrophoresis the proteins were fixed in 7% acetic acid and 40% ethanol and then stained with Coomassie blue R-250.

Molecular Weight Estimation: The molecular weight of the methionine-rich polypeptides in SDS gels were estimated using proteins of known molecular weights. Protein standards used for calibration in 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 tryspin inhibitor (20,100) and lysozyme (14,300).

Results and Discussion

In order to determine quantitative and qualitative differences in the MRP-fraction, purified MRP-fraction was subjected to one-dimensional gel electrophoresis both under non-denaturing and denaturing conditions. Figure 1 shows the protein pattern of MRP-fraction from four different species of *Arachis*. In general, the

MRP-fraction showed three to four major protein bands. The amounts and ratios of these bands varied among the various species of *Arachis*. For example, the ratios of protein bands I and II were 0.48, 0.22 and 0.33 in *A. batizogaea* (a), *A. villosulicarpa* (b), and *A. hypogaea* (d), respectively. Likewise, the ratios of protein bands II and III were 0.83, 0.88, 1.10 and 0.80 in *A. batizogaea* (a), *A. villosulicarpa* (b), *A. stenosperma* (c) and *A. hypogaea* (d), respectively.

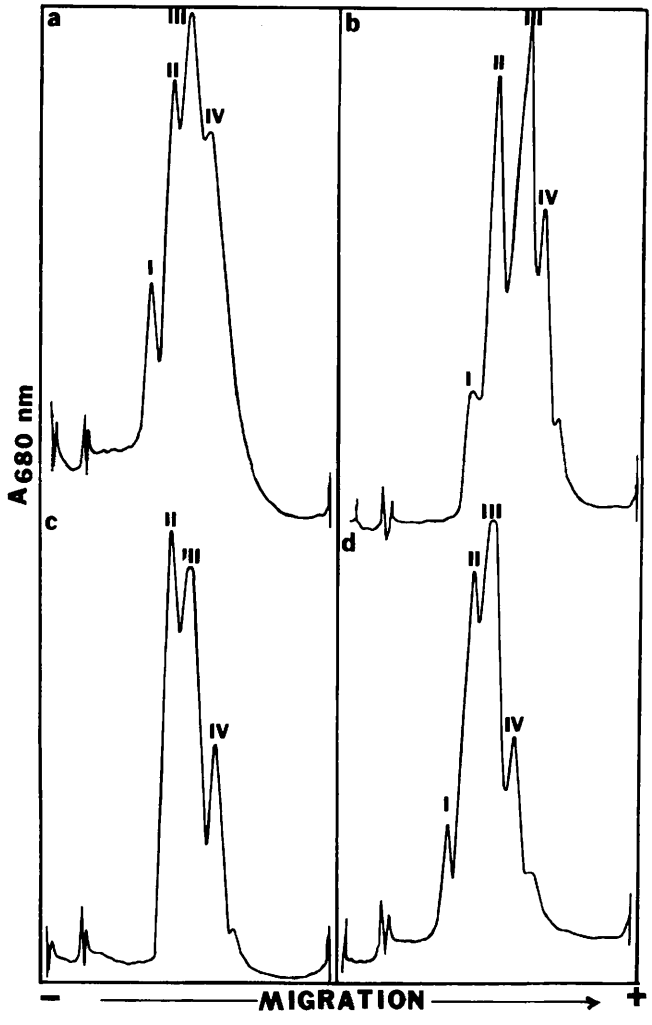

Fig. 1. One-dimensional polyacrylamide gel electrophoretic patterns of methionine-rich protein fractions from a = *A. batizogaea*, b = *A. villosulicarpa*, c = *A. stenosperma* and d = *A. hypogaea* cv. florunner. About 100 μg of protein was applied on each gel and electrophoresed toward the anode.

Major variations were also observed in the polypeptide composition of MRP fraction when examined by SDS-gel electrophoresis (Fig. 2). Following electrophoresis, the MRP-fraction resolved into three to four components with apparent molecular weights between 16,000 and 21,000. *A. batizogaea* (a) and *A. hypogaea* (d) contained all the four (I through IV) components while, component I was absent in *A. Villosulicarpa* (b), and *A. stenosperma* (c). Furthermore, these components also showed variation in their proportions among the different species. For example, the ratio of protein bands II and III were 1.06, 0.87, 1.50, and 1.05 in *A. batizogaea, A. villosulicarpa, A. stenosperma* and *A. hypogaea*, respectively. Similarily, the ratio of protein bands III and IV were 2.22, 1.24, 2.60 and 1.40 in *A. batizogaea, A. villosulicarpa, A. stenosperma* and *A. hypogaea*, respectively. The ratio of bands II and IV were 2.35, 1.08, 4.0, and 1.50 in *A. batizogaea, A. villosulicarpa, A. stenosperma* and *A. hypogaea*, respectively. Although one-dimensional gel electrophoresis facilitated detection of quantitative and qualitative variations in the protein composition, however, it failed to resolve proteins having similar molecular weights and electrophoretic mobility (3,4). Hence, the MRP-fractions were also examined by two-dimensional polyacrylamide gel electrophoresis to detect qualitative differences in their composition.

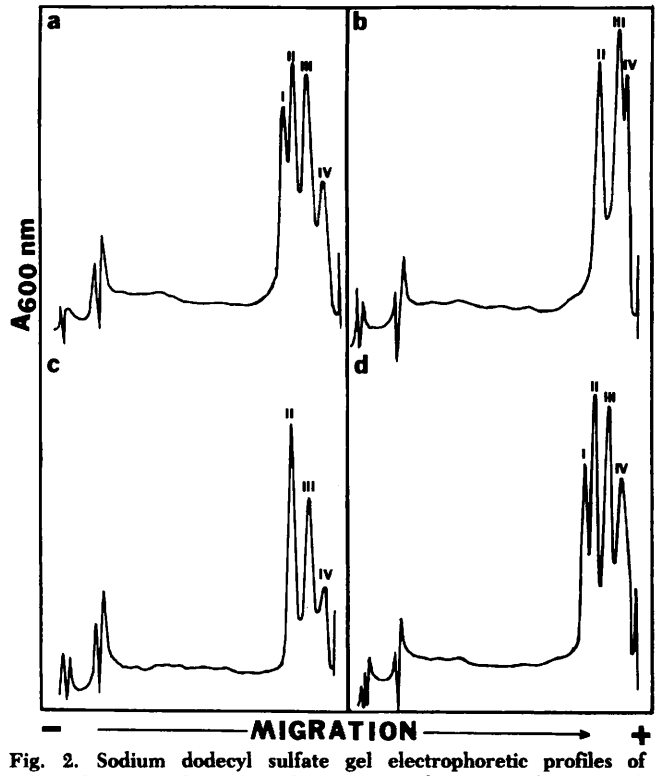

Fig. 2. Sodium dodecyl sulfate gel electrophoretic profiles of methionine-rich proteins from a = *A. batizogaea*, b = *A. villosulicarpa*, c = *A. stenosperma* and d = *A. hypogaea* cv. florunner. About 100 μg of protein was applied on each gel and electrophoresed toward the anode.

Two-dimensional gel electrophoresis of MRP-fractions from different species of *Arachis* showed the presence of 3 to 9 components. Several preparations of the MRP-fractions from each species were used to obtain the 2-D PAGE patterns and the most representative pattern of each species is shown in Figure 3. For comparison, two-dimensional polypeptide patterns of total seed proteins from *A. monticola* (a) and *A. hypogaea* cv. florunner (b) are also included. The molecular weight of the polypeptide components ranged between 16,000 and 21,000, and isoelectric-points between 5.1 and 5.8. Based on these data the methionine-rich proteins can be grouped into 21,000(A); 19,000(B); and 16,000(C) molecular weight class polypeptides. Within each molecular weight class, the number of polypeptides varied (due to differences in their isoelectric points) between 1 and 3. For example, *A. monticola* (c), *A. batizogaea* (e) and *A. hypogaea* (b) contained 9 polypep-

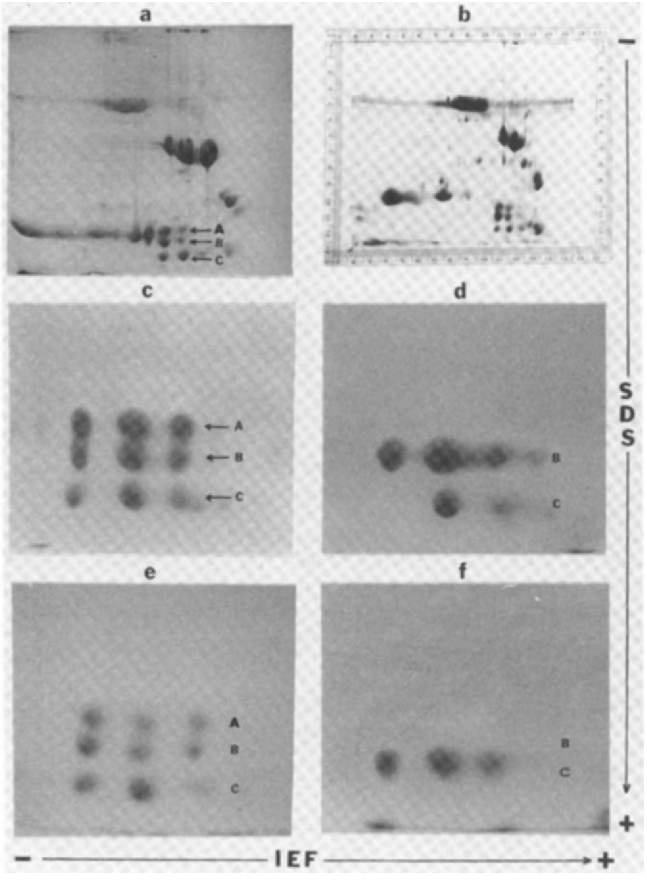

Fig. 3. Two-dimensional gel electrophoretic profiles of peanut proteins from the Genus *Arachis*. 'a' and 'b' show total protein patterns of *A. monticola* and *A. hypogaea* cv. florunner, respectively. Photographs c, (*A. monticola*), d (*A stenosperma*), e (*A. batizogaea*) and f (*A. villosulicarpa*) show the polypeptide composition of methionine-rich proteins. About 200 to 300 μg of protein was applied on each gel and subjected to isoelectricfocusing in the first dimension and sodium dodecyl sulfate gel electrophoresis in the second dimension. Polypeptides A, B, C represent the 21,000; 19,000 and 16,000 molecular weight group proteins, respectively.

tides in their MRP-fraction. However, *A. villosulicarpa* (f) contained only the 16,000 MW class proteins, while *A. stenosperma* (d) and *A. chacoense* (not shown) contained 19,000 and 16,000 MW class polypeptides and lacked the 21,000 MW class polypeptides. The total seed proteins (not shown for all the species) and the 2-D PAGE data of purified MRP-fractions suggest that its composition greatly varied among the wild species. The previous report by Basha (1) indicated lack of such variations in the methionine-rich polypeptides among the various cultivars of *A. hypogaea*. However, existence of variation in the methionine levels within the *A. hypogaea* (9,12) suggest that the polypeptides of MRP-fraction may differ in their methionine content and the methionine content of a peanut line or cultivar may depend on the polypeptide make up of the MRP-fraction. This hypothesis is consistent with the previous report by Basha and Pancholy (4) who found varying amounts of ^{35}S-methionine distribution among the polypeptides of the MRP-fraction from *A. hypogaea* cv. Early Bunch.

Additional studies involving fractionation and analysis of methionine-rich polypeptides are necessary to obtain quantitative data on the methionine content of these polypeptides. Such information can then be incorporated in the breeding programs aimed at improving the nutritional status of peanut seed.

Literature Cited

1. Basha, S.M.M. 1979. Identification of cultivar differences in seed polypeptide composition of peanuts (*Arachis hypogaea* L.) by two-dimensional gel electrophoresis. Plant Physiol. 63:301-306.
2. Basha, S.M.M. and J.P. Cherry. 1976. Composition, solubility and gel electrophoretic properties of proteins isolated from florunner (*Arachis hypogaea* L.) seed. J. Agric. Food Chem. 24:359-365.
3. Basha, S.M.M. and S.K. Pancholy. 1981a. Polypeptide composition of arachin and non-arachin proteins from Early Bunch peanut (*Arachis hypogaea* L.) seed. Peanut Sci. 8:82-88.
4. Basha, S.M.M. and S.K. Pancholy. 1981b. Identification of methionine-rich polypeptides in peanut (*Arachis hypogaea* L.) seed. J. Agric. Food Chem. 29:331-335.
5. Basha, S.M. and S.K. Pancholy. 1984. Differences in the seed protein composition of Genus *Arachis*. Can. J. Bot. 62:105-108.
6. Davis, B.J. 1964. Disc electrophoresis II. Method and application to human serum proteins. Ann. NY. Acad. Sci. 121:404-427.
7. Horn, M.J. and A.E. Blum. 1956. A microbiological method for determination of cystine in feeds. Cereal Chem. 31:18-28.
8. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680-685.
9. Pancholy, S.K., R. Supelveda and S.M.M. Basha. 1980. Oil, total protein and amino acid composition of 77 peanut lines and cultivars. Proc. Amer. Peanut Res. Ed. Assn. 12:13-22.
10. Sauberlich, H.E., E.L. Pearce and C.J. Baumann. 1948. Excretion of amino acids and mice fed proteins of different biological values. J. Biol. Chem. 175:29-38.
11. Woodham, A.A. and R. Dawson. 1968. The nutritional value of groundnut protein. (1) Some effects of heat upon nutritive value, protein composition and enzyme activity. Br. J. Nutr. 22:589-600.
12. Young, C.T. 1979. Amino acid composition of peanut (*Arachis hypogaea* L.) samples from the 1973 and 1974 uniform peanut performance tests. Proc. Amer. Peanut Res. Ed. Assn. 10:30-37.

Accepted February 25, 1984