Effect of Location and Season on Peanut Seed Protein and Polypeptide Composition

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Peanut (*Arachis hypogaea* L.) seeds from four different cultivars grown at Georgia, Oklahoma, Virginia, and Texas during the 1987 and 1988 seasons were analyzed for protein, polypeptides, free amino acids, and sugars to determine the effect of location and season on seed composition. The data showed that peanuts grown in Virginia during the 1987 season contained the highest amount of free amino acids followed by those from Oklahoma, Texas, and Georgia. Examination of protein profiles of the peanuts grown at different locations showed that the location and the season had a major effect on the arachin monomer and polymer contents of the seed. In addition, comparison of polypeptide profiles of these seeds also showed small variation in the amount of 19 000 and 40 000 molecular weight polypeptides. These data suggest that although location and season had an effect on the arachin monomer and polymer proportions, they had no effect on the total protein content and polypeptide composition of the peanut seed.

INTRODUCTION

Seed components of peanut such as free amino acids and sugars are very important roasted peanut flavor precursors (Newell et al., 1967). Genotypes and environments have been reported (Young et al., 1974a,b; Young, 1979; Hsi et al., 1981; Oupadissakoon et al., 1980a,b) to cause variation in free amino acids, protein, and sugar composition of peanuts (Dawson and McIntosh, 1973; Amaya et al., 1977, 1978; Basha et al., 1976; Young et al., 1974a,b; Young, 1979, 1980). In peanuts the majority of the free amino acids were thought to be released from a large peptide (Mason et al., 1969), whereas glucose and fructose were partially released from sucrose (Newell et al., 1967) during roasting. The peptide contained high amounts of glutamic acid, phenylalanine, glycine, and aspartic acid (Mason et al., 1969) and was associated with the production of typical roasted flavor (Newell et al., 1967). Evaluation of four peanut cultivars grown at Texas and North Carolina showed variations in the protein efficiency ratio (PER) values of Texas-grown peanuts, while no significant differences existed in PER among the four cultivars grown in North Carolina (Miller and Sanders, 1981). In view of the importance of protein as a free amino acid source during roasting, and also as a dietary protein source, this study was undertaken to determine the influence of growing season, planting locations, and genotypes upon the protein composition of peanut seed.

MATERIALS AND METHODS

Materials. Four peanut (*Arachis hypogaea* L.) cultivars (Florunner, Florigiant, GA T-2524, and TP 107-11) grown at Suffolk, VA (T. A. Coffelt); Tifton, GA (W. D. Branch and C. C. Holbrook); Stephenville, TX (C. E. Simpson); and Fort Cobb, OK (J. S. Kirby) were obtained from the 1987 and 1988 Uniform Peanut Performance Tests. Upon arrival the skins were removed, and the cotyledons were ground into powders and defatted with hexane as described earlier (Basha et al., 1976). The defatted meals were stored at −20 °C until analysis.

Soluble Sugars, α-Amino Nitrogen, and Protein. A portion of defatted peanut flour (100 mg) was extracted with methanol/chloroform/water (60:25:15 v/v/v) (Young et al., 1974), and an aliquot of the sample was analyzed for α-amino nitrogen (Yemm and Cocking, 1955) and soluble sugars (Yemm and Willis, 1954). Another portion of defatted flour (100 mg) was extracted with 1 M NaOH, and the extract was analyzed for total protein (Lowry et al., 1951) using bovine serum albumin as the standard.

High-Performance Liquid Chromatography (HPLC). Defatted peanut meal (50 mg) was extracted with 2 mL of 0.01 M sodium phosphate buffer, pH 7.0, containing 0.5 M NaCl using a Polytron homogenizer. The extract was centrifuged at 20000*g* for 20 min, and a 20-μL aliquot was injected into the HPLC column. The HPLC system consisted of a Model 510 pump, a UV–vis detector, a Model 820 data station, and a Protein-Pak 300 SW column (Waters). The run conditions were the same as described earlier (Basha, 1988).

Two-Dimensional Polyacrylamide Gel Electrophoresis (2-D PAGE). Protein from the defatted meal (200 mg) was extracted with a solution containing 9.3 M urea, 0.5% dithiothreitol, 0.005 M K_2CO_3, and 2% Nonidet P-40 (nonionic detergent). An aliquot (50 μL) of the protein extract was subjected to 2-D PAGE as described earlier (Basha, 1979). The first dimension was isoelectric focusing, and the second dimension was 10% SDS slab gel electrophoresis. The proteins were stained with Coomassie Blue R-250.

RESULTS AND DISCUSSION

α-Amino Nitrogen, Protein, and Soluble Sugars. Peanuts grown at different locations contained varying amounts of free amino acids. Of the four locations, Virginia-grown peanuts from the 1987 season contained the highest amount of free amino acids, followed by those from Oklahoma, Texas, and Georgia (Figure 1a). All four cultivars grown in Georgia contained the lowest amount of free amino acids compared to cultivars from other locations. Among the cultivars, Florunner from Georgia, Texas, and Oklahoma contained the lowest amount of free amino acids. Unlike 1987, in 1988 Oklahoma-grown peanuts (GA T-2524, Florunner, Florigiant) contained a relatively higher amount of free amino acids than other locations. Except for some minor variations, no major differences were observed (Figure 1b) in the protein content of the cultivars among the locations. In general, the 1988 crop contained more protein (53–61%) than the 1987 crop (43–55%). Unlike protein, soluble sugar composition of seed varied with the location (Figure 1c). GA

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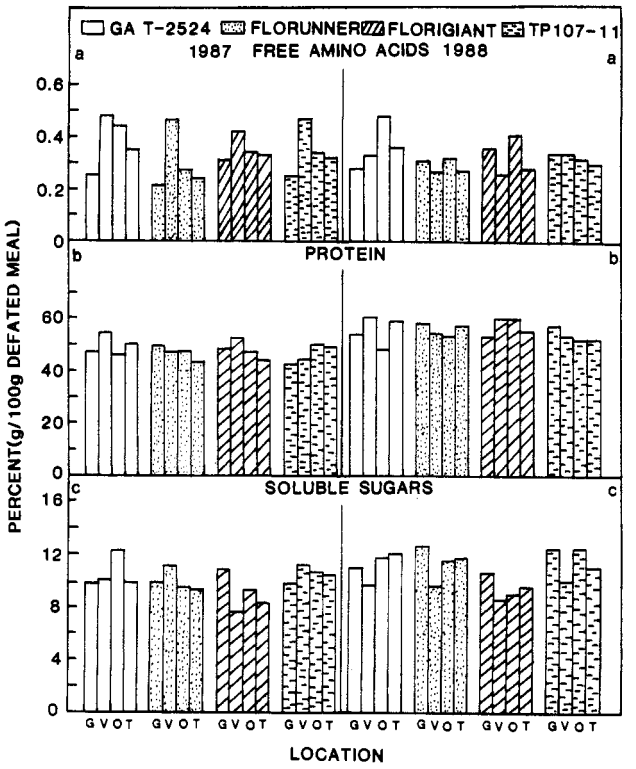

Figure 1. Effect of location and season on free amino acids (a), protein (b), and soluble sugar content of four peanut cultivars. Location: G, Georgia; V, Virginia; O, Oklahoma; T, Texas.

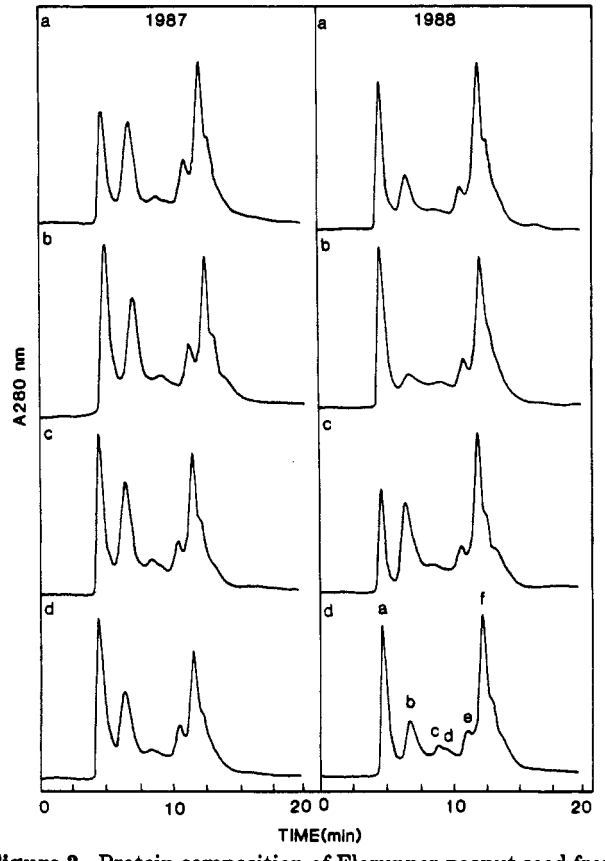

Figure 2. Protein composition of Florunner peanut seed from 1987 and 1988 seasons grown at four locations. (a) Georgia; (b) Virginia; (c) Oklahoma; (d) Texas.

T-2524 grown in Oklahoma and Florunner and TP 107-11 grown in Virginia from the 1987 crop contained higher amounts of soluble sugars compared to those from other locations. Interestingly, soluble sugar composition of

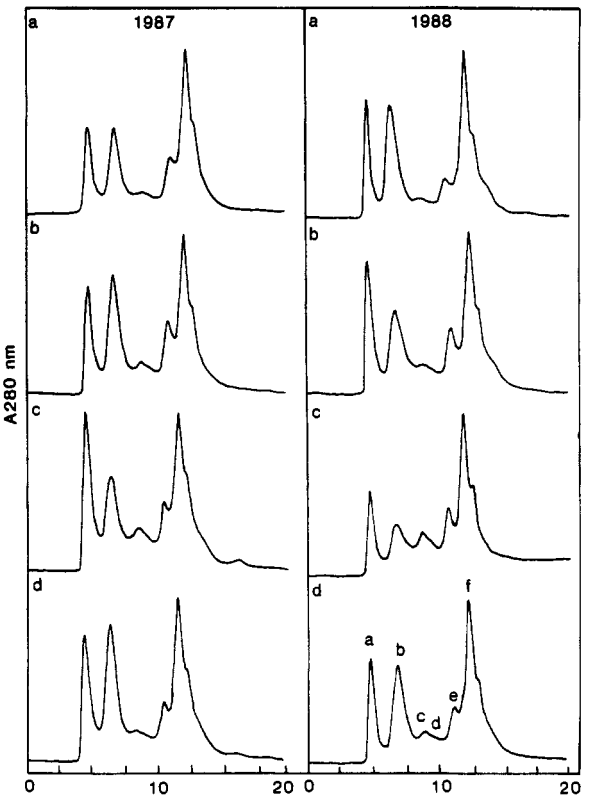

Figure 3. Protein profiles of GA T-2524 peanut seed grown at four locations during the 1987 and 1988 seasons. (a) Georgia; (b) Virginia; (c) Oklahoma; (d) Texas.

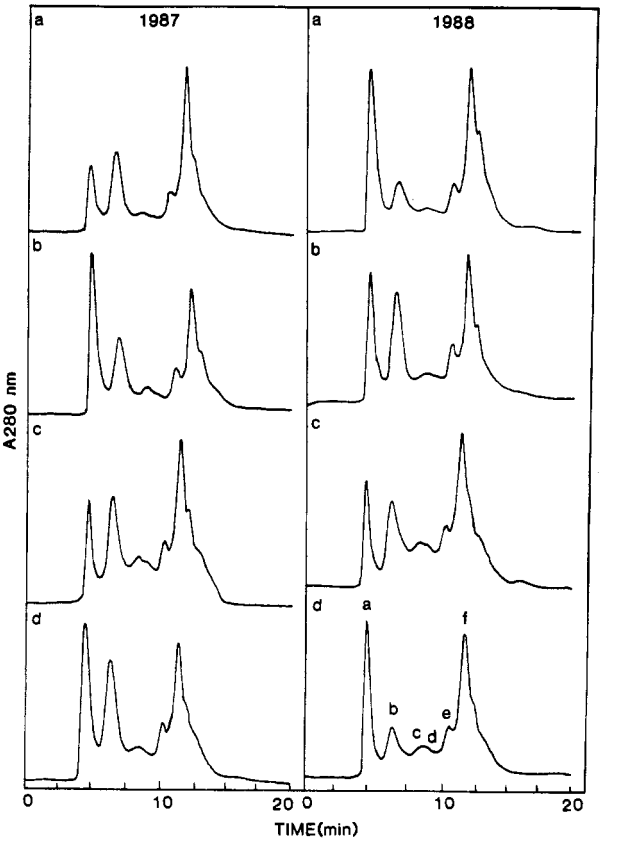

Figure 4. Protein pattern of Florigiant peanut seed grown at four locations during the 1987 and 1988 seasons. (a) Georgia; (b) Virginia; (c) Oklahoma; (d) Texas.

Virginia-grown peanuts was lowest in all of the cultivars from the 1988 crop. These observations are consistent with the previous studies (Oupadissakoon et al., 1980a,b;

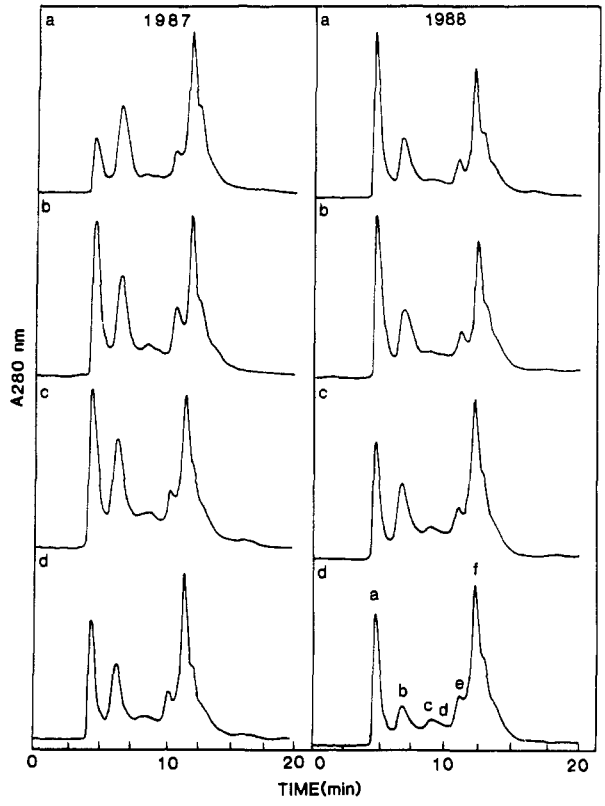

Figure 5. Protein composition of TP 107-11 peanut seed from 1987 and 1988 seasons grown at Georgia (a), Virginia (b), Oklahoma (c), and Texas (d).

Hsi et al., 1981), which reported variation in soluble sugars and free amino acid content of peanuts grown at different locations.

Protein Composition. Examination of seed protein composition of peanut cultivars grown at different locations showed major variation. Thus, comparison of protein profiles of Florunner (Figure 2), GA T-2524 (Figure 3), Florigiant (Figure 4), and TP 107-11 (Figure 5) grown at four different locations during the 1987 and 1988 seasons showed major differences in the proportion of various protein peaks. For example, in the 1987 Florunner samples (Figure 2a) the ratios of peaks a/f and b/f, respectively, were 0.68 and 0.61 for Georgia-grown Florunner, while they were 1.27 and 0.67 for Texas-grown Florunner peanuts. Likewise, in the case of TP 107-11 (Figure 5a) the ratios for peaks a/f and b/f were 0.34 and 0.53, respectively, for Georgia-grown seed, while they were 1.04 and 0.71 for the Oklahoma-grown seed. Similar variations in these peak ratios were observed in GA T-2524 (Figure 3) and Florigiant (Figure 4) seeds. Peaks a and b represent the arachin polymer and monomer proteins (Basha and Pancholy, 1981; Basha, 1988), peak c represents the maturin protein (a maturity indicator protein) (Basha, 1990), and peak f (Basha and Pancholy, 1981; Basha, 1988) contains mainly the low molecular weight (<80 000) proteins. As seen in the figures, proportion of arachin monomer and polymer and peak f appear to be greatly influenced by the location. The effect of varying proportions of arachin monomer and polymer on flavor quality is unknown. In addition to arachin, the amount of maturin protein (peak c) varied among the samples. As reported in our previous study (Basha, 1990) the maturin protein peak decreases with increasing maturity, and by mature (black) stage only trace amounts of peaks c are found in the seed. Examination of peak c in the protein profiles (Figures 1–4) revealed that, in general, peanuts grown in Virginia, Oklahoma, and Texas contained relatively higher amounts of maturin (peak c) than peanuts grown in Georgia, indicating that peanuts grown in Virginia, Oklahoma, and Texas were less mature compared to the peanuts grown in Georgia. This may be because of the shorter growing season and cooler climate toward the later stages of plant growth in Virginia, Oklahoma, and Texas locations.

Comparison of protein profiles of peanut cultivars from the 1987 and 1988 season also showed variations in seed protein profiles of cultivars between the seasons (Figures 2–5). For example, in the case of Virginia-grown Florunner (Figure 1) peanuts, the ratios of peaks a/f and b/f were, respectively, 1.11 and 0.75 for the 1987 season and 1.09 and 0.23 for the 1988 season. Likewise, the a/f and b/f ratios for Georgia-grown TP 107-11 (Figure 5), respectively, were 0.34 and 0.53 for the 1987 season and 1.3 and 0.45 for the 1988 season. These data suggested that growth season had a major effect on the arachin monomer and polymer content of the seed. Comparison of maturin peak from the 1987 and 1988 crops again showed that Georgia-grown peanuts were more mature than the peanuts grown at the other three locations. In addition, the 1988 Virginia peanuts appeared to be relatively more mature than the 1987 Virginia peanuts, but the 1988 Texas peanuts appeared to be less mature than the 1987 Texas peanuts. No flavor data are available to determine the effect of observed differences in the maturity levels of peanuts grown at these locations and between the seasons on the flavor characteristics of these seeds.

Polypeptide Composition. Effect of location and season on peanut seed protein content was further studied by examining the seed polypeptide composition. The data showed that polypeptide composition of peanut cultivars varied slightly depending upon the location. Comparison of polypeptide profiles of Florunner peanuts from the 1987 season showed that growth location had an effect on the content of 19 000 and 40 000 molecular weight (MW) polypeptides. The 19 000 (A) and 40 000 (B) MW polypeptides were present (Figure 6c) only in Oklahoma-grown Florunner peanuts; they were absent in Georgia-, Texas-, and Virginia-grown Florunner peanuts (Figure 6). In the 1988 Florunner crop both the 19 000 and 40 000 MW polypeptides were present in the Oklahoma-grown peanuts but were absent in the Georgia- and Texas-grown crops (Figure 7). In the Virginia-grown peanuts, these two polypeptides were present in relatively smaller amounts (Figure 7b) compared to Oklahoma-grown peanuts (Figure 7c). Season also appeared to have had some effect on the amount of these two polypeptides. For example, Florunner peanuts grown in Virginia during the 1988 season contained the 40 000 MW polypeptide (Figure 7b), but it was absent in the 1987 crop (Figure 6b). Likewise, quantitative differences were also observed in the 40 000 MW polypeptide between the Florunner peanuts from the 1987 (Figure 6c) and 1988 (Figure 7c) crops grown in Oklahoma. Except for these, no other differences were observed in the polypeptide profiles of peanuts grown in these locations.

This is in contrast with the study of Dawson and McIntosh (1973), who observed marked variations in the electrophoretic patterns, especially on the conarachin fractions. This discrepancy may be attributed to their protein extraction procedures and prolonged exposure (24–48 h) of samples to room temperature, which might have resulted in varying levels of proteolysis. The data in this study suggested that location and season had no major impact on seed polypeptide composition and that the observed differences in arachin monomer and polymer proportions

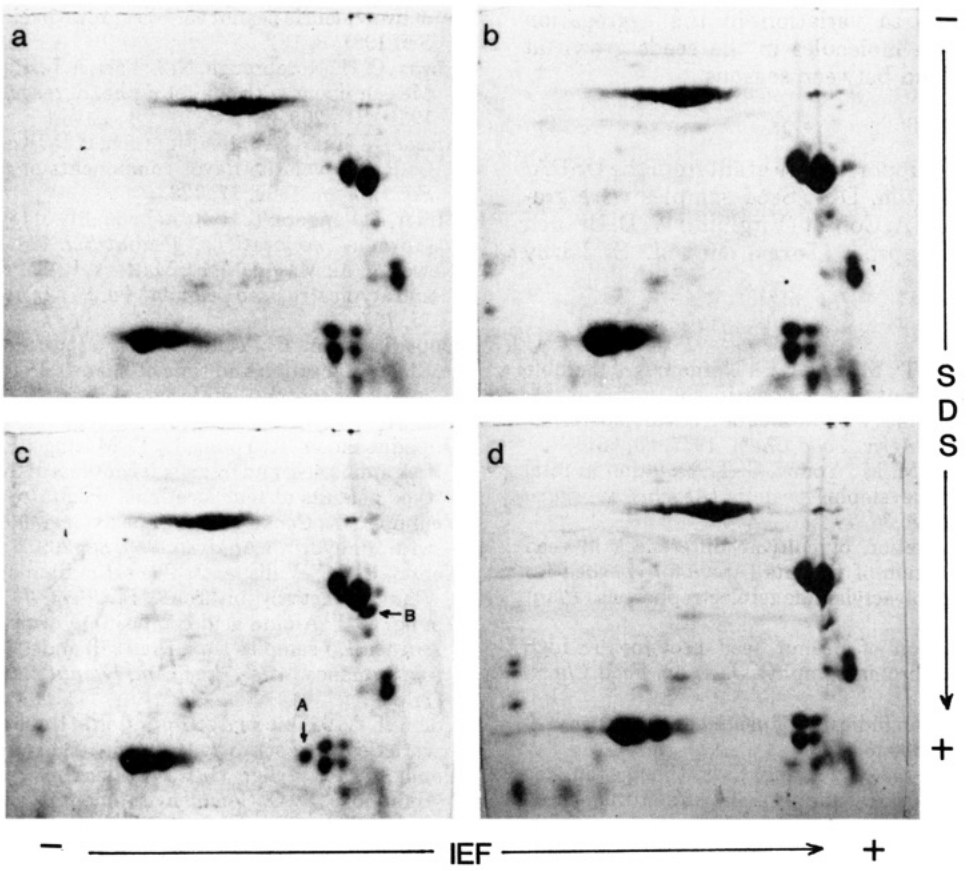

Figure 6. Two-dimensional gel electrophoretic profile of Florunner peanuts grown at Georgia (a), Virginia (b), Oklahoma (c), and Texas (d) during the 1987 season. A and B, respectively, are the 19 000 and 40 000 MW polypeptides.

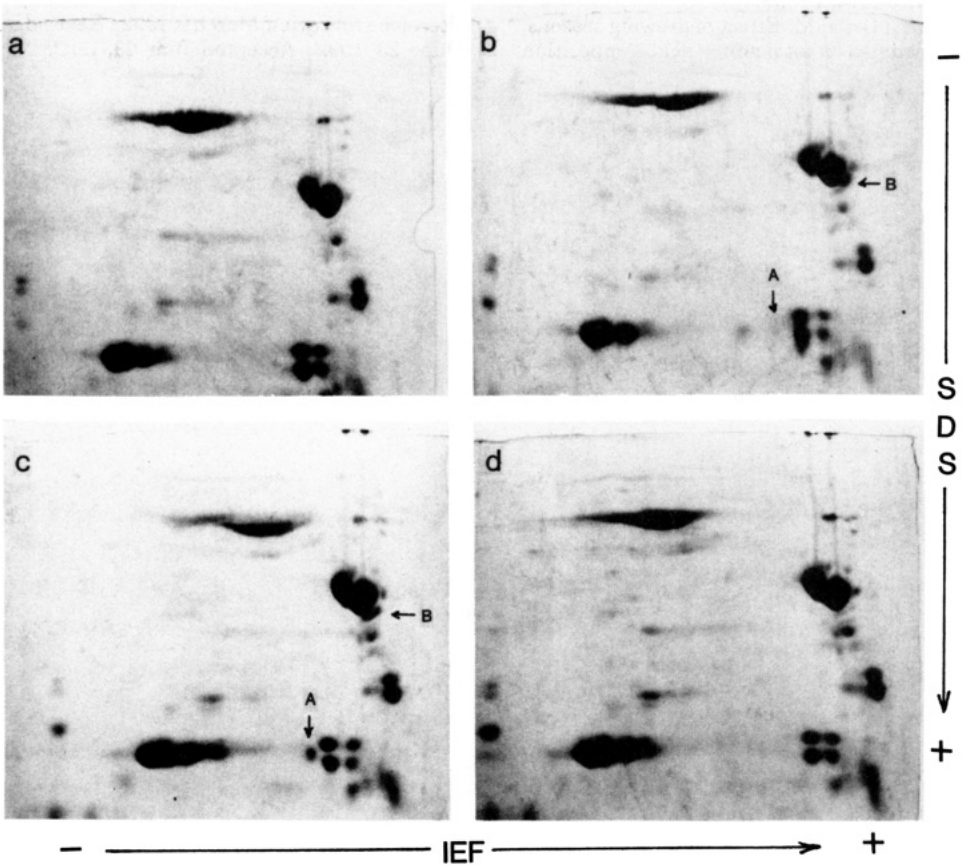

Figure 7. Two-dimensional gel electrophoretic profile of Florunner peanuts grown at Georgia (a), Virginia (b), Oklahoma (c), and Texas (d) during the 1988 season. A and B, respectively, are the 19 000 and 40 000 MW polypeptides.

may be mainly due to variation in the aggregation properties of arachin molecules in the seeds grown at different locations and between seasons.

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LITERATURE CITED

Amaya-F, J.; Young, C. T.; Mixon, A. C.; Nordon, A. J. Soluble amino and carbohydrate compounds in the testae of six experimental peanut lines with various degrees of *Aspergillus flavus* resistance. *J. Agric. Food Chem.* **1977**, *25*, 661.

Amaya-F, J.; Basha, S. M. M.; Young, C. T. Variation in total monosaccharides in developing peanuts (*Arachis hypogaea* L.). *Cienc. Cult.* **1978**, *30*, 79.

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

Basha, S. M. Resolution of peanut seed proteins by high performance liquid chromatography. *J. Agric. Food Chem.* **1988**, *36*, 778.

Basha, S. M. Protein as an indicator of peanut seed maturity. *J. Agric. Food Chem.* **1990**, *38*, 373.

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

Basha, S. M. M.; Pancholy, S. K. Polypeptide composition of arachin and non-arachin proteins from Early Bunch Peanut (*Arachis hypogaea* L.) seed. *Peanut Sci.* **1981**, *8*, 82.

Dawson, R.; McIntosh, A. D. Varietal and environmental differences in the proteins of the groundnut (*Arachis hypogaea* L.). *J. Sci. Food Agric.* **1973**, *24*, 597.

Hsi, D. C. H.; Young, C. T.; Ortiz, M. Effect of growing seasons, locations and planting dates on total amino acid composition of two Valencia peanut varieties grown in New Mexico. *Peanut Sci.* **1981**, *8*, 131.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the folin-phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265.

Mason, M. E.; Newell, J. A.; Johnson, B. R.; Koehler, P. E.; Waller, G. R. Nonvolatile flavor components of peanuts. *J. Agric. Food Chem.* **1969**, *17*, 728.

Miller, J.; Sanders, T. H. Protein quality of four peanut cultivars grown at two locations. *Peanut Sci.* **1981**, *8*, 61.

Newell, J. A.; Mason, M. E.; Matlock, R. S. Precursors of typical and atypical roasted peanut flavor. *J. Agric. Food Chem.* **1967**, *15*, 767.

Oupadissakoon, C.; Young, C. T.; Geisbrecht, F. G.; Perry, A. Effect of location and time of harvest on free amino acid and free sugar contents of Florigiant peanuts. *Peanut Sci.* **1990a**, *7*, 61.

Oupadissakoon, T.; Young, C. T.; Mozingo, R. W. Evaluation of free amino acid and free sugar contents in five lines of Virginia-type peanuts at four locations. *Peanut Sci.* **1980b**, *7*, 55.

Yemm, E. W.; Cocking, E. C. The determination of amino acids with ninhydrin. *Analyst* **1955**, *80*, 209.

Yemm, E. W.; Willis, A. J. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **1954**, *57*, 508.

Young, C. T. Amino acid composition of peanut (*Arachis hypogaea* L.) samples from the 1973 and 1974 uniform peanut performance tests. *Proc. Am. Peanut Res. Educ. Soc.* **1979**, *11*, 24.

Young, C. T. Amino acid composition of three commercial peanut varieties. *J. Food Sci.* **1980**, *45*, 1086.

Young, C. T.; Waller, G. R.; Matlock, R. S.; Morrison, R. D.; Hammons, R. O. Some environmental factors affecting free amino acid composition in six varieties of peanuts. *J. Am. Oil Chem. Soc.* **1974a**, *51*, 265.

Young, C. T.; Matlock, R. S.; Mason, M. E.; Waller, G. R. Effect of harvest date and maturity upon free amino acid levels in three varieties of peanuts. *J. Am. Oil Chem. Soc.* **1974b**, *51*, 269.

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