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Free and Total Amino Acid Composition of Maturing Seed from Six Peanut (*Arachis hypogaea* L.) Cultivars

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ABSTRACT

Maturing seeds of six peanut cultivars (*Arachis hypogaea* L.) varying in protein content at maturity showed differences in rate of change of dry weight, crude protein, and free and total amino acids. Seeds of the high-protein cultivars increased in dry weight and deposited protein at a more rapid rate between immature and low intermediate stages of maturation than did those of the low- and intermediate-protein cultivars. Free amino acid content in seeds classified as low-intermediate maturity from all cultivars was significantly less than was that of those at the immature stage. The greatest change was in seed of the high-protein group. The rate of change of content of selected free amino acids among seed was different for the three groups of cultivars. Similar observations were noted as the content of select total amino acids increased in maturing seeds. Variations in quantities of free amino acids in immature seeds and differences in the rate at which they are incorporated into proteins of seeds from various cultivars suggest that there is genetic variability in the mechanism for synthesis of selected proteins. These differences also indicate the potential for the development of peanut cultivars having seed with nutritionally desirable protein.

Key Words: Amino acids, proteins, peanut seeds, *Arachis hypogaea* L., seed maturation, peanut cultivars.

Morphological alterations and embryonic development in the maturing peanut (*Arachis hypogaea* L.) seed have been described (7, 30-32). However, there are only a few studies about the biochemical bases for these changes (4, 9, 24, 25, 28, 29). In general, studies have shown that during 14 weeks after pegging, immature peanut seeds increase in weight, synthesize genetic material including deoxyribonucleic acids, ribonucleic acids, and ribonucleases, and incorporate free amino acids into proteins (1, 4).

The proteins of peanut seeds have high levels of the acidic amino acids, aspartic acid and glutamic acid, and low amounts of the sulfur-containing amino acids, cysteine and methionine (6, 16, 17, 23). Peanut seeds are also low in lysine, tryptophan, and threonine. High levels of free amino acids, such as arginine, have been correlated with off-flavor of immature peanut seeds. Other amino acids, including aspartic acid, glutamic acid, phenylalanine, and histidine, react with sugars to contribute to flavor quality of roasted peanut seeds (12, 22). Arginine promotes shoot growth in isolated oat embryos (18). It is effective in increasing the growth response of oat coleoptiles to growth regulators (5). Noting that free arginine decreases rapidly as peanut seeds mature, Young and coworkers (35, 36) developed the arginine maturity index.

The protein content of peanut seeds depends on the genetic make-up of the cultivar and location where they are grown (4, 9-12, 15, 19, 27, 34). Peanut seeds having high levels of free amino acids during the early stages of maturation have a high protein content at maturity (4). In another plant system, rice cultivars producing grain high in protein content translocated more nitrogen from the leaves to the maturing seed, accumulated high levels of free leucine, and incorporated these substances into storage protein more rapidly than did low protein plants (8, 13, 26).

Because amino acids influence nutritional value and flavor properties of peanut seeds, additional information is needed on their quantitative changes during seed maturation. This study examines the changes in free and total amino acid content of maturing peanut seeds from six cultivars that vary in protein content.

Materials and Methods

The six peanut cultivars were: 'NC5', 'F-334-A-B-14', 'Virginia Bunch 67', 'Argentine', 'Tennessee Red', and 'Florida Jumbo'. Plants were grown during the 1975 crop year at the South-west Georgia Station, Plains, by recommended cultural practices and with irrigation as needed; results of a similar experiment with these cultivars grown at the same location during the 1974 crop year was reported by Basha *et al.* (4). Peanut fruits were harvested at 17 (digging 1) and 20 (digging 2) weeks after planting.

At harvesting, peanut fruits were water-washed, packed in ice, and separated according to maturity level as mature, high intermediate, low intermediate, or immature according to pericarp and testa color as described by Basha *et al.* (4). During the first digging, no peanut fruits of cultivars Argentine and Virginia Bunch 67 contained seed that could be classified as mature. In the second digging, no immature seeds were classified in harvested fruits of all cultivars. Testa-free seeds were freeze dried, weighed for dry-weight determination, ground into meal, defatted with diethyl ether, and stored at -22°C until analyzed. Crude protein of defatted meals was determined by multiplying the nitrogen value of macro-Kjeldahl analysis by 5.46 (2).

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Content of 16 free amino acids (NH_4, and a number of unknown components summed and labeded as others) in defatted meals of maturing seeds from six peanut cultivars was determined by the method of Young *et al.* (36). In summary, meals (0.5 g) were blended with a 50-ml mixture of methanol: chloroform:water (60:25:15; v:v:v) in a Polytron[4] homogenizer run at full speed for 1 min. The extracts were centrifuged at 20,000 x g for 15 min at 23°C. The resulting pellet was re-extracted with an additional 10 ml and centrifuged. The two supernatants were combined, evaporated to dryness in a low humidity refrigerator, and resuspended in pH 2.2 citrate buffer. The profile of free amino acids was determined by an ion-exchange chromatography technique that used a Durrum D-500 amino acid analyzer (33, 36).

For analysis of 17 total amino acids (free and protein-containing amino acids) and NH_4, defatted meals (125 mg) were hydrolyzed with 20 ml of 6N HCl at 145°C for 2 hr as described by Young *et al.* (37). The hydrolyzed samples were then cooled, neutralized with 10 ml of 12N NaOH, adjusted to pH 2.2, and made up to a final volume of 50 ml with pH 2.2 citrate buffer. The profile of total amino acids and NH_4 was determined by an ion exchange chromatography technique that used a Durrum D-500 amino acid analyzer (33, 37).

Results and Discussion

Dry Weight

Between 17 and 20 weeks after planting, peanut plants of the six cultivars had seed ranging from immature to mature. Peanut seeds of the six cultivars at immature, low intermediate, and high intermediate stages of maturity showed increasing levels of dry weight, then a small change between the latter stage and maturity (Fig. 1). The increase in dry weight between the immature and high intermediate stages was very rapid for Florida Jumbo (high protein cultivar) peanut seeds. Dry weights of immature seed from all cultivars were similar (ca 2 to 5 g/5.0 seed). Differences in dry weight among seeds from the various cultivars occurred between the low and high intermediate stages. Comparable values were noted for dry weights of similar maturation levels from the two diggings.

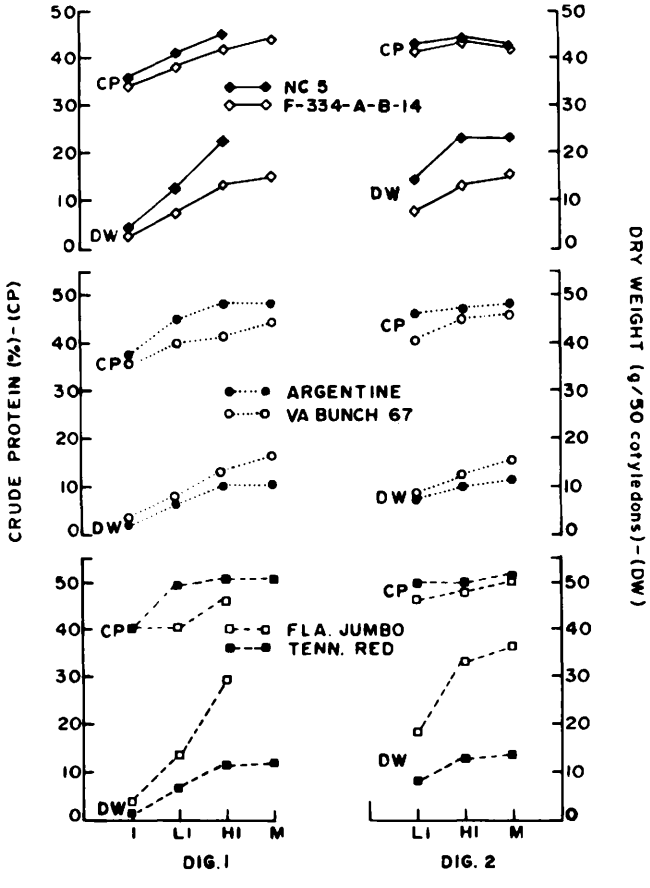

Fig. 1. Changes in the total protein and dry weights of maturing seeds from six peanut cultivars. Dig. = digging, I = immature, LI = low intermediate, HI = high intermediate, M = mature.

Crude Protein

The crude protein content of defatted meal from maturing seeds increased between immature and mature stages. The greatest changes were between seeds at immature and high intermediate stages (Fig. 1). Defatted meals of immature seeds from cultivars classified as low and intermediate protein content had similar amounts of protein of approximately 35%. Those of the high-protein group contained 40% protein. The protein content of defatted meal indicates that seeds of the high-protein cultivars deposited protein at a more rapid rate between immature and low intermediate stages of maturation than did those of the low- and intermediate-protein cultivars. In general, the protein content of peanut seeds at equivalent stages of maturity from the two diggings were similar.

The low-protein group included NC5 and F-334-A-B-14 (approximately 40% protein); intermediate, Argentine, and Virginia Bunch 67 (43% to 47% protein); and high, Florida Jumbo and Tennessee Red (50% protein). These values coincide with data of Young and Hammons (34). On the other hand, Basha *et al.* (4) showed that mature seeds of cultivars classified as intermediate-protein levels contained amounts of these components similar to either of those grouped as storing high (Argentine) or low (Virginia Bunch 67) quantities of protein.

Differences in protein values may be attributed to the following factors: (a) the use of trichloroacetic acid to separate protein from free amino acids followed by quantitation of the protein with the method of Lowry *et al.* (20) versus the Kjeldahl technique (cf the method used in this paper versus that of Basha *et al.*): (b) fluctuations in seasonal and agronomic conditions that affect seed composition between the 1974 and 1975 crop years; and (c) variation among cultivars in the morphological characteristics used for classifying the four maturity levels of seeds. Because the quantity of free amino acids in the samples analyzed by the Kjeldahl method was too low to have a major effect on the quantity of protein and because values from the two publications for the other cultivars were comparable, the variation is most probably related to (b) or (c), or both.

Free Amino Acids

Free amino acid content in defatted meals of seeds at a low intermediate stage of maturity from all cultivars was significantly less than that of those at the immature stage (Figs. 2 and 3). This difference was not as great for arginine, valine, and theonine in seeds of cultivars having intermediate amounts of protein at maturity (Argentine, Virginia Bunch 67). The change, between stages of seed maturity, in glutamic acid, serine, and aspartic acid were more gradual for cultivars classified as low and intermediate in protein content. Samples from seeds having low protein at maturity had more histidine and threonine in immature seeds than did those of the intermediate- and high-protein cultivars. Immature seeds of Tennessee Red (high-protein cultivar) had the highest content of phenylalanine, leucine, and glutamic acid, and lowest quantity of threonine. Except for glutamic acid, seeds of Florida Jumbo had similar levels of these amino acids as Tennessee Red, within the high protein group. Methionine was highest in seeds of cultivars classified as intermediate protein content. Contents of alanine, glycine, serine, aspartic acid, tryptophan, lysine, and tyrosine were similar among all immature seeds of the six cultivars. Between the low intermediate and mature stages of peanut seed maturation, the changes in amino acid content of seeds from all cultivars were not as great as those developing between immature and low intermediate stages. Only small differences were noted in free amino acids of seeds from the two diggings.

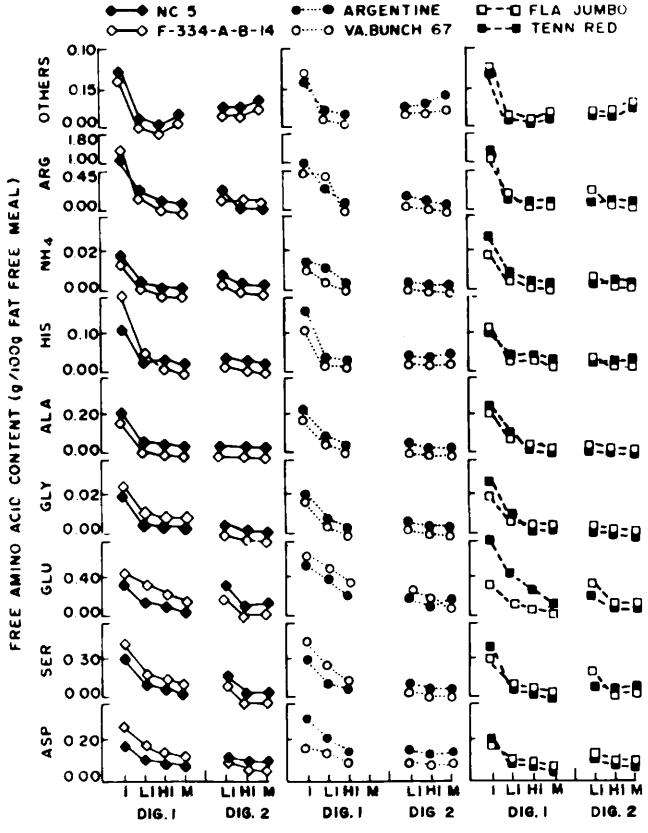

Fig. 2. Changes in free amino acids of maturing seeds from six peanut cultivars. See Figure 1 for description of labels.

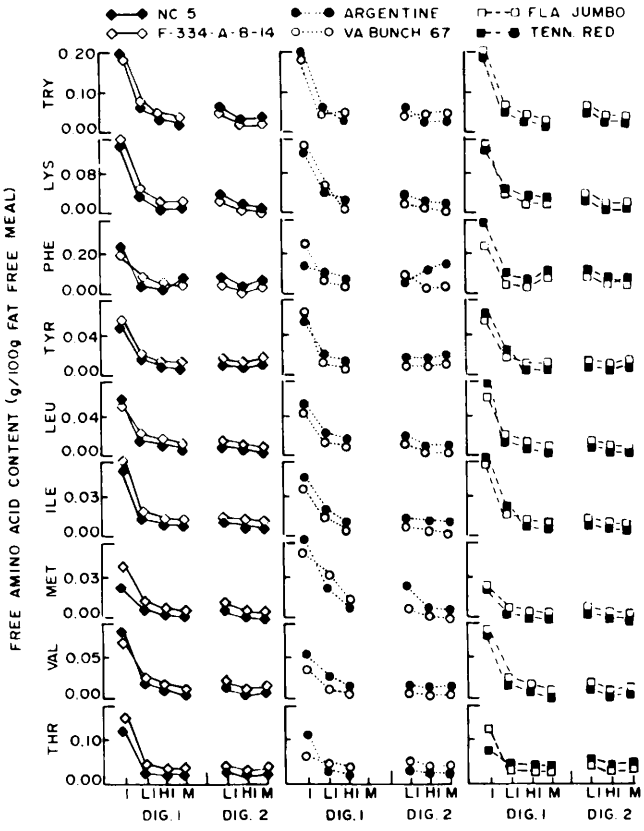

Fig. 3. Changes in free amino acids of maturing seeds from six peanut cultivars. See Figure 1 for description of labels.

An analysis of variance for levels of free amino acids showed that the observed changes in quantities of these seed constituents during various maturity levels are highly significant. The changes in levels of glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, and arginine are also significant for the variable, cultivar. These data suggest that genetic (cultivar) variation exists for the rate of incorporation of select free amino acids into protein and nonprotein components. Only methionine had a significant interaction term of cultivar times maturity.

These data show that maximum incorporation of free amino acids into proteins occurs during the early stages of seed maturation. These changes coincided with the greatest increase in dry weight and crude protein content of maturing seeds. High levels of free amino acids in maturing seeds contribute to a faster and greater accumulation of proteins in seeds (1, 4, 13, 21). Variations in quantities of free amino acids and differences in the rate at which they are incorporated into proteins suggests genetic variability in the mechanism for synthesis of many proteins stored in the seeds.

In addition, the environment (or agronomic practices) may be affecting the reactions incorporating the amino acids into proteins. Such information is useful to geneticists interested in applying selection pressures for increasing the content of certain amino acids into a specific protein, or in

increasing the quantity of a select protein that is high in essential amino acids (for example, methionine). Additionally, the data suggest that as similarly shown with free arginine, the rapid decreases in free alanine, phenylalanine, threonine, lysine, and histidine may also be used as criteria to determine peanut maturity and optimum flavor quality (35, 38).

Total Amino Acids

Quantities of total amino acids varied in defatted meals of maturing seeds from different cultivars (Figs. 4 and 5). Between the immature and mature stages, seeds from all three groups of cultivars showed decreases in alanine, phenylalanine, isoleucine, and threonine. Arginine decreased continually in maturing seeds of cultivars in the low- and high-protein groups. In the intermediate-protein group, arginine decreased rapidly between the immature and low intermediate stages of seed maturation, then leveled off to maturity. Serine showed little change quantitatively among maturing seeds from all cultivars.

Except for seed of Argentine (intermediate-protein group) and Tennessee Red (high-protein group) which showed an increase in histidine and lysine during the later stages of maturity, little change or a decline was noted in these amino acids during seed maturation. The cultivars of the high-protein group showed an increase in tyrosine and leucine

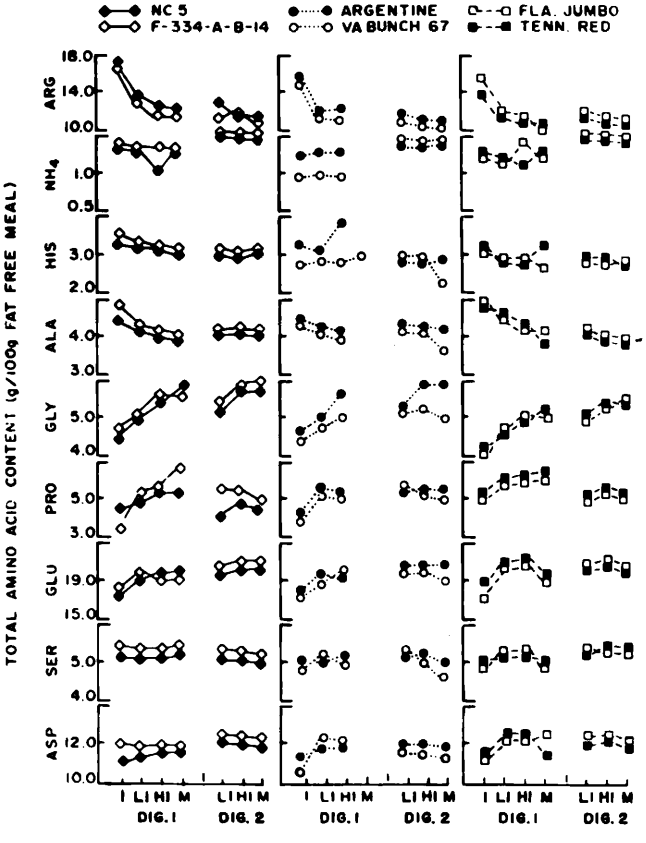

Fig. 4. Changes in total amino acids of maturing seeds from six peanut cultivars. See Figure 1 for description of labels.

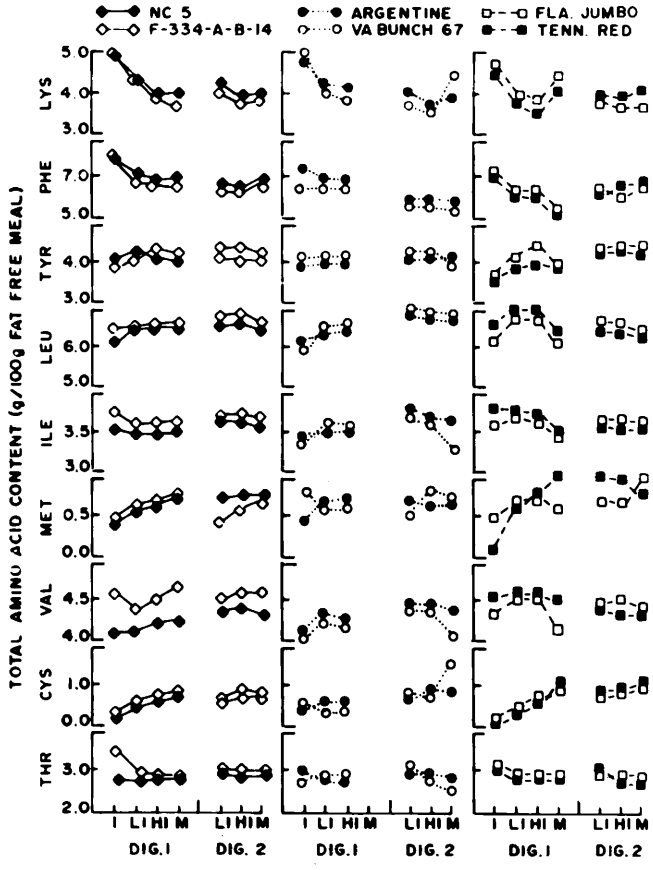

Fig. 5. Changes in total amino acids of maturing seeds from six peanut cultivars. See Figure 1 for description of labels.

during the early stages of maturation, then a decrease as the seed matured; little change was noted in seeds of the other two cultivars. Valine content of seeds increased between the immature and high intermediate stages, then decreased at maturity of both cultivars in the intermediate-protein group, and Florida Jumbo (high-protein group). Little change, or a gradual increase was noted in valine of maturing seed from NC5 and Tennessee Red. Valine content of seeds from F-334-A-B-14 decreased between the immature and low intermediate stages of seed maturation, then increased to maturity. Defatted meals of maturing seeds from all cultivars showed increases in glycine, proline, glutamic acid, aspartic acid, methionine, and half-cystine between the immature and mature stages.

Analysis of variance showed that variation in maturity was significant for the total amino acids. Glutamic acid, methionine, phenylalanine, and histidine were significant for the variable cultivar. No total amino acid had a significant interaction term.

Correlation coefficients for content of select free and total amino acids of seeds from different cultivars showed the following: (a) in most cases, the free amino acids glutamic acid, methionine, and arginine are negatively correlated with the total amino acids glutamic acid, isoleucine, phenylalanine,

lysine, and arginine in seeds of Tennessee Red, Argentine, NC5, and Virginia Bunch 67; (b) free lysine is negatively correlated with the total amino acids, glutamic acid, methionine, isoleucine, phenylalanine, lysine, and arginine in seeds of Florida Jumbo, Virginia Bunch 67, and F-334-A-B-14; and (c) except for seed of Florida Jumbo and F-334-A-B-14, the changes in free methionine were negatively correlated at a significant level with the total amino acids glutamic acid, methionine, isoleucine, phenylalanine, lysine, and arginine in seed of all other cultivars.

These data suggest that the decreases in free amino acids can indicate increased incorporation of select amino acids into proteins of seeds from select cultivars. Because seed protein quality and quantity are affected by genetic and agronomic factors and maturity status, the type and amount of amino acids incorporated into the protein is also influenced by these factors. The decrease in select amino acids of both the free and total amino acid pools suggests that in addition to their function as precursors in protein synthesis, these amino acids may also serve as intermediates in the synthesis of other cellular constituents.

Conclusions

The relative amounts of free amino acids in various cultivars early during seed maturation may be used as indicators of select cultivars that efficiently store high quantities of quality proteins. Basha *et al.* (4) and Cherry (9), using gel electrophoretic techniques, have shown that the nonarachin proteins that are high in essential amino acids (3) are deposited early in seed maturation. During the later stages of seed maturation, arachin, or the storage globulins that are low in select essential amino acids are deposited in protein bodies (14, 15).

Figures 4 and 5 show that select total amino acids changed quantitatively at varying rates during seed maturation. Information presented in this paper can assist in determining the pattern of synthesis of essential amino acids and their incorporation into proteins during peanut seed maturation.

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