Chemical Composition of *Arachis hypogaea* L. Subsp. *hypogaea* Var. *hirsuta* Peanuts[1]

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ABSTRACT

The biochemical composition of seed collected from six landrace accessions of *Arachis hypogaea* L. subsp. *hypogaea* var. *hirsuta* was investigated. Florida-grown runner- (cv. Florunner) and virginia-type (cv. NC 7) seed were used as comparative controls. Fatty acid methyl esters were prepared from hexane-extracted oil and analyzed by gas-liquid chromatography. Oil stability was determined using oxidative stability instrumentation. Tocopherols, free amino acids, and free sugars were analyzed by high-performance liquid chromatography. Total oil content ranged from 36-45% for var. *hirsuta* seed as compared to 46 and 45% for Florunner and NC 7, respectively. Oleic acid/linoleic acid ratios ranged from 0.76-0.95 for the var. *hirsuta* peanuts as compared to runner (1.71) and virginia (2.1) controls. Tocopherol levels for var. *hirsuta* (295-377 ppm in oil) were similar to NC 7 (300 ppm) and lower than Florunner (425 ppm). Oil quality characteristics were reflected in much shorter oil stability index times for var. *hirsuta* seed (5.9-8.0 hr) compared to Florunner (11.6 hr) and NC 7 (12.9 hr). In general, var. *hirsuta* peanuts contained more free sugars (141-178 μmol/g defatted meal) and free amino acids (18.5-37.2 μmol/g defatted meal) than Florunner (127 and 20.2 μmol/g defatted meal free sugars and free amino acids, respectively) or NC 7 (122 and 20.3 μmol/g defatted meal).

Key Words: Tocopherols, oil content, oleic/linoleic acid ratio, free amino acids, free sugars, oil stability index.

The roasted flavor of peanuts is unique and investigation of heritability of the roasted peanut component of this complex flavor has been conducted by Pattee and coworkers (Pattee and Giesbrecht, 1990; Pattee *et al.*, 1993). They found that parental lines influence flavor and that the identification of peanut germplasm with high roast flavor quality will be important to improve roast peanut flavor in future cultivars.

Arachis hypogaea L. subsp. *hypogaea* var. *hirsuta* Köhler, the "hairy" peanut, is grown in Mexico and local consumers have a distinct preference for its flavor (Becker, 1993). The *hirsuta* peanut is not a germplasm source found in peanut varieties presently cultivated in the U.S. Sanchez-Dominguez and Williams (1993) collected 12 *hirsuta* landrace samples from Mexico in a 1992 exploration. Because of the potential for high roasted peanut flavor, Pattee *et al.* (1995) examined the flavor characteristics of six landraces of the *hirsuta* peanut which were collected in Mexico in 1993. They found that the intensity of the sensory descriptor 'roasted peanut' in the six *hirsuta* samples was not higher than the widely grown cultivars Florunner and NC 7, but the intensity of sweet taste was significantly higher in some landraces.

The relationship of peanut composition to flavor and shelf life has been established directly in several studies and indirectly in studies relating flavor and shelf life to maturity (Sanders *et al.*, 1982; Sanders *et al.*, 1989; Bett and Boylston, 1992). Oil components, free amino acids, sugars, pro- and antioxidants, and other components have been implicated in flavor or shelf life of peanuts. Oil composition, especially oleic acid/linoleic acid ratio (Fore *et al.*, 1953; Worthington *et al.*, 1972; Young *et al.*, 1972; Brown *et al.*, 1975), influences shelf life as it relates to development of off-flavors from oil degradation. Free amino acids and free sugars are important precursors of roasted peanut flavor (Newell *et al.*, 1967).

The documentation of potentials for both flavor and shelf life improvement, as well as associated chemical characteristics, are important to the use of new germplasm in development of future peanut cultivars. Because very few *hirsuta* samples are found in germplasm collections, no documentation of their composition presently exists in the literature on peanuts. This study was conducted to provide a chemical composition evaluation of six landraces of *A. hypogaea* subsp. *hypogaea* var. *hirsuta* relative to previously determined flavor characteristics (Pattee *et al.*, 1995) and to determine their potential value in development of other improved peanut characteristics. This report details the results of an evaluation of oil content, tocopherols, oleic acid/linoleic acid ratio, oxidative stability, free sugars, and free amino acids in var. *hirsuta* seed.

Materials and Methods

Genotype Resources and Handling. As previously described by Pattee *et al.* (1995), six landrace accessions of *A. hypogaea* var. *hirsuta* (PI numbers 576633 through 576638) were collected from farms located in the states of Puebla and Guanajuato, Mexico during Nov. 1993 (Table 1). Approximately 25 kg in-shell lots of each accession were air-expressed to Raleigh, NC and were held at 4-5 C and 55-60% R.H. until shelled and analyzed. The large single lot of each accession was divided into three subsamples for analysis. Comparative controls were Florunner and NC 7 grown the same year in Gainesville, FL. Duplicate analyses were

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Table 1. Origin in Mexico of var. *hirsuta* peanuts.

Plant introduction no.	Origin (state)
PI 576633	Puebla
PI 576634	Puebla
PI 576635	Puebla
PI 576636	Guanajuato
PI 576637	Guanajuato
PI 576638	Guanajuato

performed on subsamples unless otherwise indicated. Because the intent of the study was to survey var. *hirsuta* composition and only one sample was available, statistical comparisons of the data were not justified.

Fractionation of Seed Components. Peanut seed oil was extracted using a modification of the methods described by Young *et al.* (1974) and Oupadissakoon *et al.* (1980). Approximately 35 g dried seed from each replicate was ground to a homogeneous texture in a Krupps coffee mill. For each sample, 2.0 g ground seed was placed in a large polypropylene, screw-cap tube (50 mL) containing 30 mL HPLC-grade hexane. The tube was transferred to a platform shaker (Barnstead/Thermolyne, Dubuque, IA) and shaken at moderate speed for 30 min. The sample was centrifuged at 1600 × g for 30 min in a clinical centrifuge. The supernatant was decanted into a 100-mL pyrex beaker and placed under a fume hood. The pellet was extracted two more times with hexane, shaken 30 min with each extraction, and the resulting supernatants were added to the beaker. Pooled supernatants were evaporated to dryness (*ca.* 16 hr) under the hood, producing the oil for total oil analysis. The percentage oil in the meal was determined gravimetrically.

The defatted pellet was extracted with the addition of 25 mL methanol:chloroform:water (60:25:15 by vol.) followed by high-speed blending in a Tekmar Tissuemizer (Tekmar Co., Cincinnati, OH) for 1 min. The homogenate was centrifuged for 30 min at 1600 × g. A 1-mL aliquot of the supernatant was transferred to a 2-mL polypropylene microcentrifuge tube and evaporated to dryness in a vacuum desiccator. The dried residue, containing free amino acids and free sugars, was stored at -20 C until analysis.

For tocopherol and oil stability measurements, seed oil was collected by pressing ground peanut meal in a Carver laboratory press (Fred S. Carver Equipment Co., Menomonee Falls, WI) at 16,000 psi for 15 min. The expressed oil was filtered through several layers of cheesecloth prior to analyses.

Fatty Acid Analysis. Fatty acid methyl esters were prepared and analyzed by gas chromatography as described by Sanders (1980). Toluene was used in place of benzene in the benzene/BF_3-methanol reagent. Approximately 50 mg of the hexane-extracted seed oil was transferred to a small (9 mL) pyrex screw-cap tube containing 2 mL of the toluene/BF_3-methanol solution. The tube was transferred to a heating block and heated for 30 min at 100 C. Five mL hexane was added to the cooled tube and the sample was mixed by vortex. The hexane layer, containing methyl esters of fatty acids, was transferred to a screw-cap vial. The extraction procedure was repeated and the hexane extracts were pooled. The hexane was removed by drying under a stream of N_2. The dried residue from the hexane extracts was resuspended in chloroform and analyzed.

Fatty acid ester analysis was performed on a Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector and stainless steel (3.17 mm × 1.83 m) column packed with 5% DEGS-PS on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA). The carrier gas was helium at 30 mL/min and the column was operated isothermally at 200 C. Fatty acid percentages were determined by digital integration and normalization of peak area. Qualitative accuracy was verified by daily analysis of a standard sample (Std. 21A) (NuChek Prep Inc., Elysian, MN).

Tocopherols. The tocopherol content of *hirsuta* seed oil was determined by measuring the concentrations of α-, β-, and γ-tocopherols by high-performance liquid chromatography (HPLC). One mL of the pressed oil was placed in a 10-mL screw-cap pyrex tube containing 5 mL 0.25% isopropanol in hexane. The contents were mixed by vortex and *ca.* 1 mL of the resulting solution was transferred to a 2 mL-polypropylene microcentrifuge tube. The solution was centrifuged at 14,000 × g for 5 min. The supernatant was transferred to a UV-safe capped glass vial and stored at -20 C until analysis. Tocopherols were detected by absorbance at 305 nm after separation on a LC-SI 5 μ pore size, 15-cm × 4.6-mm column with the same solvent at 1.5 mL/min. Quantitation was accomplished with standard curves prepared with known standards of tocopherols found in peanuts.

Oxidative Stability Index. The relative stability of oil from *hirsuta* and control peanuts was estimated using the Oxidative Stability Instrument (Omnion, Inc., Rockland, MA) and is expressed as the oxidative stability index (OSI), the units of which are given in hours (AOCS, 1992). OSI is a measure of the induction period or length of time until rapid oxidation of the oil begins. For each sample, pressed, filtered oil (*ca.* 5 mL) was placed in a 15-mL glass test tube and held at 110 C while air was sparged through the oil. Volatiles produced from lipid oxidation were collected in 50-mL ultra-pure water. Change in conductivity of the water was monitored to determine the time until the maximum rate of volatile production was reached. The OSI time was automatically calculated from a conductivity/time plot generated by software supplied with the oxidative stability instrument.

Analysis of Free Sugars. To determine free sugar content, the residue from the chloroform:methanol:water extraction of defatted seed of each sample was resuspended in 100 μL of 18 megaohm deionized water, mixed by vortex, and centrifuged for 10 min at 14,000 × g. The untreated supernatant was loaded directly into the sample loop of a Dionex model DX300 liquid chromatography system (Dionex Corp., Sunnyvale, CA). Sugars were separated on a Dionex Carbopac™ anion exchange column (4 x 250 mm) and held at 30 C using a dilute NaOH gradient and detected by pulsed amperometry on a Dionex Ionchrom™ pulsed amperometric detector. Elution of sugars was accomplished with 90% water:10% 0.2N NaOH for 2 min followed by a linear gradient to 20% water:80% 0.2N NaOH over 18 min. At 20 min, 100% 0.2N NaOH was used for 10 min. The column was washed with 90% water:10% 0.2N NaOH for 6 min before sample injection. A flow rate of 1 mL/min was maintained throughout the analysis.

Free Amino Acid Analysis. Free amino acid analysis

followed the procedure of Bidlingmeyer *et al.* (1984) and Cohen *et al.* (1984). This method used pre-column derivatization of primary and secondary amines to the phenylthiocarbamyl (PTC) derivative of the amino acid and separation by reverse-phase HPLC. The limit of detection for amino acid derivatives analyzed by this method is 1 pmol. Phenylisothiocyanate (PITC) triethylamine, and amino acid standards were obtained from Pierce Chemical Co., Rockford, IL. All samples were analyzed in triplicate.

The dried sample from the methanol:chloroform extraction of oil-free seed was resuspended in 5 mL of a diluent solution. Diluent was prepared by adding 0.71 g Na_2HPO_4 to 1 L of water and titrating to pH 7.40 with 10% (w/v) H_3PO_4; the resulting solution was mixed with acetonitrile so that acetonitrile equaled 5% by volume. An aliquot of the sample (*ca.* 1 mL) was transferred to a 2-mL microcentrifuge tube and centrifuged at 14,000 ×g for 10 min. Duplicate 20-µL aliquots of supernatant were collected and transferred, along with deionized water controls, to new microcentrifuge tubes and dried completely in a vacuum desiccator. The samples were removed from the vacuum, and 20 µL redrying solution (2:2:1, by vol. absolute ethanol:water:triethylamine) was added to each tube. The tubes were mixed by vortex and vacuum-dried.

PTC amino acids were formed by adding 20 µL freshly prepared derivatization reagent to the tubes. The reagent consisted of absolute ethanol:triethylamine:water:PITC (7:1:1:1, by vol). The contents of the tubes were mixed by vortex and left at room temp (20-25 C) for 20 min. The samples were then vacuum-dried and reconstituted in 50-200 µL sample diluent. The reconstituted samples were centrifuged at 2000 × g to remove any precipitate. The supernatant was decanted and saved for HPLC analysis.

Derivatized amino acids were separated using the Dionex model DX300 liquid chromatography system consisting of a solvent delivery system, a variable-wavelength absorbance detector (254 nm), and automated gradient controller. A C-18 reversed-phase PICOTAG™ column, 3.9 x 150 mm, was used to separate PTC amino acids. The temperature was maintained at 40 C with an Eppendorf column heater-controller (Brinkman Instruments, Westbury, NY). The mobile phase consisted of two eluents: (A) an aqueous buffer of 0.14 M sodium acetate containing 0.06% triethylamine and titrated to pH 6.39 with glacial acetic acid and (B) 60% acetonitrile in water, pH 8.0. A linear gradient was run from 100% A to 54% A:46% B over a 10-min period at a flow rate of 1 mL/min. This was followed by a column wash with 100% B before equilibration with eluent A. Signals from the absorbance detector were integrated and compared to amino acid standards with a Dionex AI 450 software package.

Results and Discussion

Oil Composition and Quality. Most of the *hirsuta* peanuts contained slightly less oil than the controls, ranging from 35-45% (by wt) compared to 46 and 45% for Florunner and NC 7, respectively (Fig. 1). The two landraces designated PI 576634 (37.8%) and PI 576635 (35.9%) had unusually low oil content compared to controls. The normal range for oil content in peanuts is 44 to 56% with a mean of 50% (Cobb and Johnson, 1973). One explanation for the low oil content in these two samples is that they contained higher percentages of immature seeds, which have lower oil content (Sanders *et al.*, 1982). We believe this is not the case since the levels of arginine, another indicator of maturity (Young and Mason, 1972), found in these samples compared favorably with the levels in the control samples as discussed below. Given the recent consumer concern for fat content in foods, the finding of low oil content may prove to be of significance in breeding for low oil-acceptable flavor cultivars. Low oil content in these landraces has been verified in other studies although segregation for high and low oil has been observed (H. E. Pattee, unpubl. data, 1995).

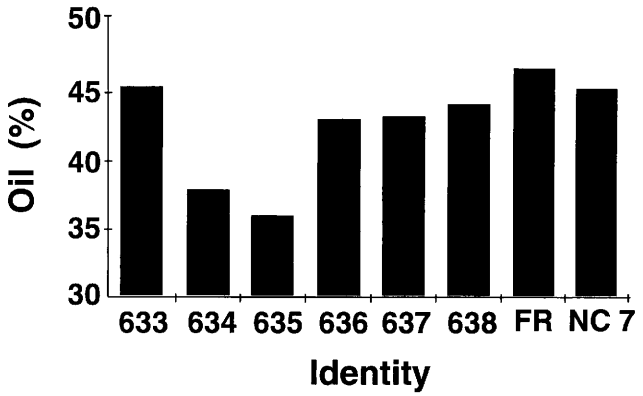

Fig. 1. Total oil content of six *hirsuta* landrace accessions, Florunner (FR) and NC 7 peanuts. Identity numbers are the last three digits of PIs 576633 through 576638.

O/L Ratio. Variation in oleic acid/linoleic acid (O/L) ratios of oil extracted from *hirsuta* peanuts was observed (Table 2). The O/L ratios for *hirsuta* landrace accessions ranged from 0.76 to 0.95 as compared to 1.7 for Florunner and 2.1 for NC 7. The O/L ratio is commonly considered a good indicator of oil stability (Worthington *et al.*, 1972; Young *et al.*, 1972; Brown *et al.*, 1975). Higher O/L ratios generally relate to increased oil stability and increased shelf life of peanut products (Fore *et al.*, 1953). The O/L ratio increases with peanut maturity (Young *et al.*, 1972; Sanders *et al.*, 1982) and is influenced by cultivar and environmental factors. Higher O/L ratios are pro-

Table 2. Oleic/linoleic acid (O/L) ratio, tocopherol content and oxidative stability index (OSI) of six *hirsuta* landrace accessions, Florunner, and NC 7.

Seed identity	O/L ratio	Tocopherol	OSI
		ppm in oil	hr
PI 576633	0.86	295.1	8
PI 576634	0.95	376.6	8
PI 576635	0.86	339.5	7.8
PI 576636	0.76	341.8	5.9
PI 576637	0.76	300.7	7.7
PI 576638	0.77	297.1	7.5
Florunner	1.71	656.0	11.6
NC 7	2.10	486.7	12.9

duced at higher growth temperatures (Holaday and Pearson, 1974; Brown *et al.*, 1975). The *hirsuta* landraces were collected at an altitude of *ca.* 1800 m, suggesting a cool growing environment and possibly resulting in O/L values which may be at the low end of their genetic potential. The O/L ratio genetic potential of *hirsuta* has not been evaluated when grown in warmer environments. Because of the strong relationship between shelf life and O/L ratio, values greater than 1 are preferred in general commerce even though ratios less than 1 are often found in world trade (Sanders *et al.*, 1992).

Tocopherols. The total tocopherol content of oil extracted from *hirsuta* seed ranged from 295 to 376.6 ppm. Tocopherols from all landrace accessions were less than that of the Florunner and NC 7 controls (Table 2). Florunner seed contained 656 ppm tocopherols in oil which was much higher than *hirsuta* or NC 7 peanuts. Tocopherol contents of peanuts from China, Argentina, and the U.S. in a 3-yr period ranged from 102.9-183.9, 149.4-219, 210-243.8 ppm, respectively, in whole peanuts (Sanders *et al.*, 1992). Sanders *et al.* (1994) reported tocopherol values from 419 to 644 ppm in oil from six peanut cultivars grown in various regions of the U.S. The full effect of tocopherols as antioxidants in peanuts has not been explored although they are potent antioxidants in some systems. Activity varies with structure and the alpha and gamma forms are the most abundant in peanuts.

Oxidative Stability Index. The oxidative stability index (OSI) is a measure of relative stability of oil under strictly defined conditions. Oil stability is dependent upon a number of factors including O/L ratio, antioxidant concentration, and concentrations of copper and iron. The lower O/L ratios for *hirsuta* seed compared to controls correlate to the shorter oil stability times as measured by OSI (Table 2). The OSI range for *hirsuta* seed oils was 5.9 to 8.0 hr under the stated conditions. By contrast, OSI times for the reference samples of Florunner and NC 7 oils were 11.6 and 12.9 hr, respectively.

Free Sugar Content. All seed samples contained detectable levels of seven sugars as measured by pulsed amperometry. Seed sugars provide a source of carbon for the production of flavor compounds (Koehler *et al.*, 1969). Glucose and fructose, products of the hydrolysis of sucrose, are reducing sugars and can, upon heating, react with specific amino acids to produce flavor components. In general, the free sugar content of var. *hirsuta* seed was higher than control peanuts, ranging from *ca.* 141 to 179 µmol/g defatted seed for *hirsuta* seed compared to *ca.* 125 µmol/g for Florunner and NC 7 (Fig. 2). The major sugar in all samples was sucrose (93-96% of the total), ranging in concentration from 134 to 167 µmol/g defatted seed for *hirsuta* samples and 114 and 118 µmol/g defatted seed for Florunner and NC 7 peanuts, respectively. Other sugars present were (in descending order of concentration) stachyose, raffinose, inositol, fructose, galactose, and glucose. These data compare favorably with the sensory analysis of these *hirsuta* landraces by Pattee *et al.* (1995). They found that the sweet taste intensity ranged from 2.8 to 3.8 in the six landraces of *hirsuta* while Florunner and NC 7 were 2.7

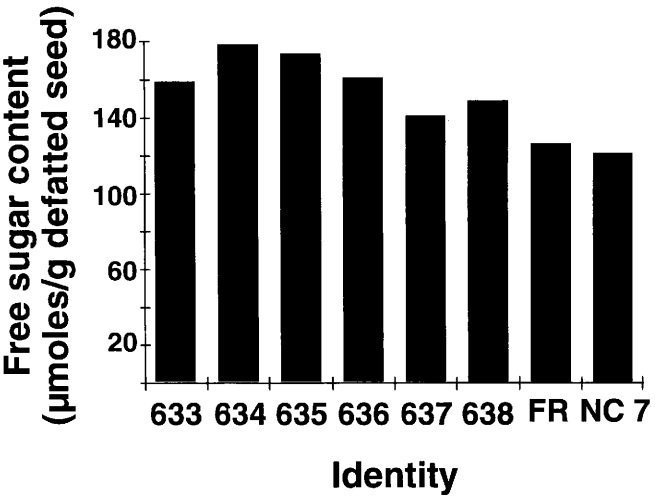

Fig. 2. Free sugar content of peanuts from six *hirsuta* landrace accessions, Florunner (FR) and NC 7 peanuts. Identity numbers are the last three digits of PIs 576633 through 576638.

and 2.5, respectively. PIs 576634 and 576635 had the highest sweet taste (3.8 and 3.6, respectively) and the highest free sugar content. Although studies to correlate sweet taste intensity and total sugar content are not presently found in the literature, these data provide a very limited suggestion of the potential of such a relationship.

Free Amino Acid Composition. The major free amino acids of *hirsuta* seed were glutamic acid, phenylalanine, alanine, and asparagine. Their relative concentrations in seed varied widely depending on the landrace accession. The total free amino acid content of *hirsuta* samples ranged from 18.5 to 37.2 µmol/g defatted seed (Fig. 3). Control peanuts averaged 20.1 µmol/g defatted seed. Some of these amino acids have been linked to the development of roasted peanut flavor. Aspartic acid, glutamic acid, asparagine, glutamine, histidine, and phe-

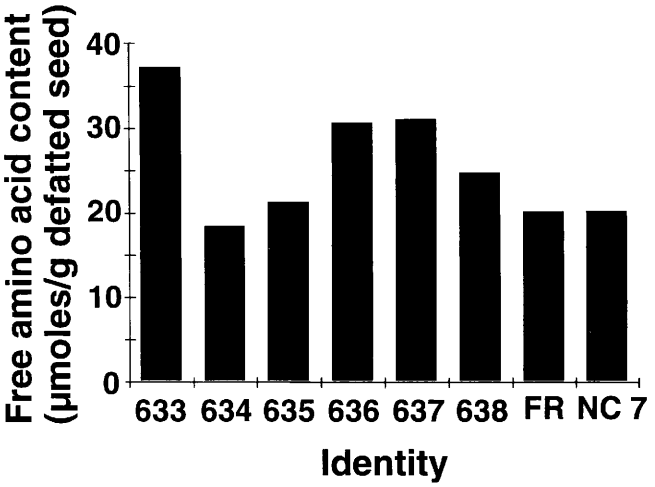

Fig. 3. Free amino acid content of six *hirsuta* landrace accessions, Florunner (FR) and NC 7 peanuts. Identity numbers are the last three digits of PIs 576633 through 576638.

nylalanine have been associated with 'typical' roasted peanut flavor (Newell *et al.*, 1967). Arginine, threonine, tyrosine, and lysine have been linked to 'atypical' roasted peanut flavor (Newell *et al.*, 1967; Cobb and Johnson, 1973). Differences in concentrations of selected free amino acids averaged for all var. *hirsuta* compared to those of control peanuts were greatest in phenylalanine, glutamic acid, and tyrosine (Fig. 4). Arginine was lower in *hirsuta* samples and indicates an acceptable level of maturity (Young and Mason, 1972). Free amino acids and free sugars are important precursors of roasted peanut flavor (Newell *et al.*, 1967). The reactions between sugars and amino acids which influence peanut flavor are not well understood, but a study by Oupadissakoon and Young (1984) showed a strong positive correlation (r^2 = 0.928) between roasted peanut flavor and the amino acid and sugar content of raw seed.

Because the *hirsuta* accessions contained much more phenalanine and glutamic acid (Fig. 4), amino acids identified with typical flavor (Newell *et al.*, 1967), it might be expected that the *hirsuta* seed had higher roasted peanut flavor than the controls. However, Pattee *et al.* (1995) did not find any significantly higher roast peanut flavor intensity in any of the *hirsuta* samples.

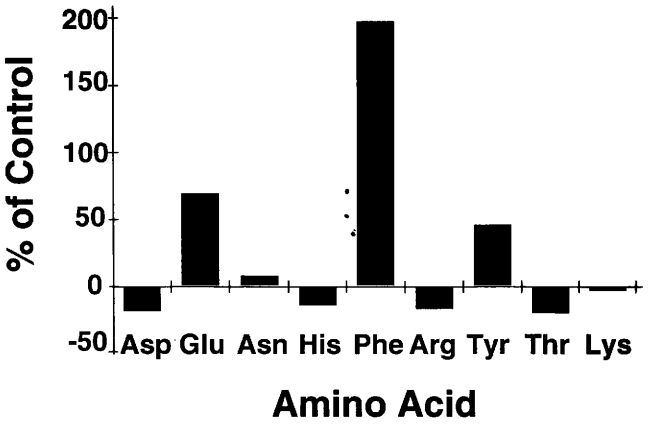

Fig. 4. Difference in the mean seed content of selected free amino acids from six *hirsuta* landrace accessions versus mean value of Florunner and NC 7 control peanuts.

Conclusions

The six landraces of *hirsuta* peanuts examined in this study were consistently compositionally different from Florunner and NC 7. Oil content, O/L ratio, tocopherols, and oxidative stability were lower than Florunner and NC 7, while free sugar content was higher. The high sugar content may be associated with preference for *hirsuta* in local situations. High sugar content may also be related to the sensory data on *hirsuta* peanuts which indicated a higher intensity of sweet taste than that found in Florunner or NC 7. The differences in means for total tocopherols, O/L ratio, and OSI among var. *hirsuta* samples were not of sufficient size to be meaningful. However, in the case of oil and free amino acid content among var. *hirsuta* samples, variability among subsamples was very low and differences among samples were large.

Acknowledgments

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