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Evaluation of Free Amino Acid and Free Sugar Contents in Five Lines of Virginia-Type Peanuts at Four Locations[1]

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ABSTRACT

Five lines of Virginia-type peanuts, Florigiant (FG), NC 6, NC 17921 (FG x Florunner), NC 17922 (FG x Valencia), NC 17976 (FG x Spanhoma), were selected from the advanced breeding lines and the standard variety test. They were grown at 4 county locations, 2 in North Carolina and 2 in Virginia, with 3 replications (blocks) in a randomized complete block design; adjacent plots were used for each digging. Free amino acid and free sugars were determined on sound mature kernels. Statistical analysis showed significant differences for varieties and locations. The variety effects were larger in the case of the free sugar contents. NC 6 had the largest quantity of glucose (0.12 mg/g), sucrose (37.45 mg/g), and stachyose (4.24 mg/g), while FG was highest in inositol (0.15 mg/g). In the case of free amino acids (μmoles/g), significant variety effects were observed for threonine-serine (1.55-1.92), alanine (0.95-1.53), peptide-cystine (1.28-1.50), valine (0.42-0.62), and histidine (0.58-0.66). Peanuts grown at Northampton County (NC) had the highest quantities of inositol (0.16 mg/g), sucrose (32.39 mg/g), stachyose (4.63 mg/g), aspartic acid (1.48 μmoles/g), threonine-serine (2.55), glutamic acid (7.49), alanine (1.89), valine (0.76), isoleucine (0.23) unknown 4-tyrosine (0.37), and histidine (0.78). The arginine maturity index value, calcium content, % sound mature kernels, % extra large kernels, and yield data are discussed.

Key Words: Free Amino Acids, Peanuts, *Arachis hypogaea* L., Sugars.

The United States leads the world in direct consumption of peanuts, and seventy percent of its peanut production is consumed as food (1). Roasted peanuts are the most desirable peanut product because of their pleasant and unique flavor. This is utilized to advantage in the manufacture of a variety of roasted peanut products; e. g., peanut butter accounts for about 50% of the domestic food use (13, 15). There has been an attempt among peanut breeders and other researchers to produce raw peanuts of the best quality in order to have good roasted peanuts.

A suggestion was made in 1952 that amino acids and carbohydrates were roasted peanut flavor precursors (23); this was confirmed in 1967 after experimentation with a non-aqueous model system (20). Model systems of amino acids and sugars have been studied by many investigators (7-12, 16, 20, 25, 26). In peanuts, the majority of free amino acids were thought to be released from a large peptide (14), whereas glucose and fructose, were partially released from sucrose (20) during roasting. The peptide contained high amounts of glutamic acid, phenylalanine, glycine, aspartic acid (14) and was associated with the production of typical roasted peanut flavor (20).

Genotypes and environments (planting locations, irrigation, disease infection and maturation) were reported to cause variation in free amino acid and sugar composition of peanuts (2-6, 21, 28, 33-35). The ability to quantitate these free amino acid and sugar variations among peanuts was necessary in order to exploit their natural differences. In this study, peanuts grown in the North Carolina and Virginia areas were examined for significant differences in the contents of free amino acids and free sugars among selected varieties, and planting locations.

Materials and Methods

Five lines of peauts were obtained through the variety and quality evaluation program at Tidewater Research and Continuing Education Center (TRACEC), Suffolk, VA. Criteria used for their selection was as follows: Florigiant (FG), at present the most commonly grown Virginia-type variety; NC 6, a variety which has a level of resistance to the southern corn rootworm; NC 17921 (a cross of FG and Florunner) was selected because it contains a high % SMK content, good yield, and matures early; NC 17922 (FG x Valencia) was chosen because it might have a better flavor than FG; and NC 17976 (FG x Spanhoma) carries identity of the Spanhoma variety peanut which is known for good yield, excellent flavor and ease of blanching.

The four locations used for this test were Martin and Northampton Counties in North Carolina and Sussex County and City of Suffolk in Virginia. All experiments, except the one at Suffolk, were conducted on privately owned farms. Two adjacent plots at each location (L) contained five varieties (V) planted in a randomized complete block design where 3 replications (R) were assigned as blocks. The plots were used for the two diggings (D). The time between diggings at each location was 8, 11, 12 and 14 days for Northampton, Martin, Suffolk, and Sussex, respectively. Peanuts were cured, shelled, and graded at TRACEC and a sample of sound mature kernels was used for laboratory analysis. Peanuts (50 g) were weighed and then stored in air-tight plastic bags for the arginine maturity index (AMI) and calcium analysis. A second sample (25 g) was ground thoroughly using a KRUP KM-45 grinder (Robert Krups, Germany) and placed in a vial for the sugar and free amino acid analysis. The remainder of the peanut samples and these subsamples were then stored at -4°C until analyzed.

Extraction

Free amino acids and sugars were extracted by the improved method of Young *et al.* (34). Ground peanuts (2 g) were weighed into a 50 ml glass culture tube. Oil was extracted 3 times by using a 30 ml solution of hexane: diethyl ether (60:40; v:v) each time, agitation on a platform shaker at moderate speed for 30 min, and centrifugation at 2000 x g for 15 min (clinical Model Centrifuge - International Equipment Co.). Two ml of

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the first solvent extraction for oil was removed, evaporated, and the residue stored in an 8 ml glass culture tube (1,3 x 8.5 cm) at -4°C for future fatty acid analysis.

Following removal of the oil, the peanut residue was extracted with 30 ml of methanol: chloroform: water (60:25:15; v:v:v) using a Teckmar Tissumizer at full speed (10,000 rpm) for 1 min, then centrifuged as above. A 10 ml aliquot was transferred to a 100 ml beaker, placed under a hood overnight for evaporation of the solvent, and utilized for the free amino acid determination. A 6 ml aliquot was pipetted into a 10 ml glass vial (2 x 4.5 cm), placed under the hood to remove methanol and chloroform, lyophilized, and analyzed for sugars.

Free Amino Acid Determination

The free amio acid residue was taken up in 2.5 ml of pH 2.2 citrate buffer which contained 1.2N NaCl and was centrifuged twice in a Beckman Microfuge B. The supernatant was stored at 4°C (up to 30 days) until anlayzed by a Durrum D-500 amino acid analyzer with the data acquisition accessary. The column (1.75 mm, ID, x 48 cm) was packed with Durrum DC-4A, 8 ± 1 μ bead dia, high resolution cation-exchange resin.

Sugar Determination

The method using trimethylsilyl-sugar-oxime derivative formation was applied (24). One ml of "STOX" (oxime-internal standard reagent prepared in the laboratory from 5 mg/ml phenyl-β-glucopyranoside as internal standard, 25 mg/ml hydroxylamine hydrochloride and 50 ml pyridine) was added to the lyophilized sugar sample. The mixture was heated at 73 °C for 30 min and then cooled to room temp. One ml of hexamethyldisilazane was added while shaking, followed by 0.1 ml of trifluoroacetic acid. The mixture was shaken by hand for 20 sec and then allowed to stand for at least 2 hr. This allowed complete settling of the white precipitate which can interfere with the quantitation of the high boiling point sugars derivatives.

The clear solution was injected (0.9 μl) into an F&M model 810 gas chromatograph equipped with 2 mm ID x 1.8 m glass column packed with 2% OV-17 on Anakrom ABS (110-120 mesh). The temperature was programmed from 165°C (hold 2 min) at 15°C/min to 325°C (hold 4 min). The flame ionization detector temperature was 330°C and the injector temperature was 295°C. Carrier gas (helium) flow rate was 60 ml/min. Attenuation at the start was 3.2 x 10^{-10} amp F. S. and was changed to 5.12 x 10^{-9} amp F. S. after 5 min and to 1.28 x 10^{-9} after 9.4 min. The integration of the area under the peaks was performed by a computer data acquisiton system; by comparing the data to standards, the peak areas were converted to sugar content (mg/g) of peanuts. The sugar standards contained 0.05 mg/ml each of ribose, fructose, glucose, and inositol, 0.5 mg/ml raffinose, 1.0 mg/ml stachyose, and 7.5 mg/ml sucrose.

Statistical Analysis

The analysis of variance for diggings 1 and 2 followed the randomized complete block design where replication was treated within location. Location was the only effect to be tested against the replication within location. A combined analysis of variance over location and digging was conducted to observe the digging effect. Variety, location, and digging were considered fixed. The effects of location, digging, and their interaction were tested by replication within interaction (27). The mean comparison of the significant effects were calculated using Waller and Duncan's new multiple comparison system (32).

Results and Discussion

Free Amino Acids

Table 1 lists the average values of free amino components of Virginia peanuts by variety and location. When the results are compared with those of Spanish peanuts published by Young *et al.* (34), the Virginia peanuts are noted to have lower isoleucine (ILE) (0.09-0.24 vs 0.30-0.42) and phenylalanine (PHE) (0.30-160 vs 1.04-4.85), and higher histidine (HIS) (0.42-0.86 vs 0.30-0.48) contents. Some of the amino acids (proline, glycine, methionine, and leucine) were not measured due to poor separation and quantitation problems. These problems were not observed when analyzing peanuts from the southeast (5, 6). Often a single peak occurred in the threonine-serine area of the chromatogram and is referred to as the threonine - serine peak (TSER). Increasing the concentration of ethanol in buffer 1 made it possible to detect 2 additional components in the TSER peak which were suspected to be asparagine and glutamine as previously reported (35). There were 4 unknown peaks on the chromatogram. Unknowns 1(U1), 2(U2), and 3(U3) eluted before aspartic acid (ASP) and unknown 4 (U4) eluted with the tyrosine peak (U4T). These unknowns were reported by Young *et al.* (35). The peptide, previously referred to as peptide 2 (14), eluted with cysteine (PC).

Peanuts of different varieties showed highly significant differences in TSER, alanine (ALA), PC, valine (VAL) and significant difference in U1,

Table 1. Mean comparison of free amino acids (μmoles/g) of Virginia-type peanuts among varieties and locations.[1]

Free Amino Acids	Varieties					Locations			
	Florigiant	NC6	NC17921	NC17922	NC17976	Martin	Northampton	Sussex	Suffolk
U1	0.091ab	0.070c	0.082b	0.087ab	0.097a	0.098a	0.082bc	0.073c	0.089ab
	0.071	0.070	0.070	0.067	0.069	0.073	0.072	0.074	0.059
	0.081a	0.070b	0.076ab	0.077ab	0.083a	0.085a	0.077b	0.073b	0.074b
U2	0.084a	0.082a	0.085a	0.073a	0.048b	0.057c	0.093a	0.076b	0.072b
	0.083b	0.092ab	0.092ab	0.103a	0.095ab	0.101a	0.084b	0.105a	0.082b
	0.084a	0.087a	0.088a	0.088a	0.072b	0.079b	0.089a	0.091a	0.077b
U3	0.197	0.224	0.237	0.237	0.263	0.165c	0.207b	0.236b	0.319a
	0.167	0.191	0.174	0.185	0.182	0.154	0.172	0.198	0.195
	0.182b	0.208ab	0.205ab	0.211ab	0.223a	0.159d	0.190c	0.217b	0.257a
ASP	1.510	1.547	1.466	1.370	1.375	1.144b	1.576a	1.496a	1.598a
	1.330	1.305	1.400	1.374	1.235	1.158	1.384	1.453	1.320
	1.420	1.426	1.433	1.372	1.305	1.151b	1.480a	1.475a	1.459a
TSER	1.913a	1.488b	1.891a	1.700ab	1.846a	0.971c	2.607a	1.866b	1.626b
	1.850	1.606	1.940	1.964	1.858	1.154c	2.502a	1.951b	1.766b
	1.881a	1.547b	1.915a	1.832a	1.852a	1.063d	2.554a	1.909b	1.696c
GLU	6.858	6.304	6.450	6.210	6.335	5.231d	7.864a	6.571b	6.060c
	5.950	6.188	6.244	6.540	6.053	6.642ab	7.121a	6.303b	4.714c
	6.404	6.246	6.347	6.375	6.194	5.937c	7.492a	6.437b	5.387d
ALA	1.385ab	0.865c	1.461a	1.160b	1.323ab	0.728c	1.995a	1.287b	0.945c
	1.491ab	1.040c	1.591a	1.317b	1.386b	0.970c	1.780a	1.336b	1.375b
	1.438ab	0.953d	1.526a	1.239c	1.354bc	0.849c	1.887a	1.312b	1.160b
PC	1.354	1.505	1.269	1.311	1.249	1.698a	1.433b	1.049c	1.171c
	1.221c	1.488a	1.346abc	1.457ab	1.314bc	1.920a	1.258b	1.280b	1.003c
	1.287b	1.496a	1.308b	1.384ab	1.281b	1.809a	1.345b	1.164c	1.087c
VAL	0.639a	0.438b	0.668a	0.639a	0.657a	0.387d	0.870a	0.666b	0.510c
	0.589a	0.403b	0.613a	0.597a	0.577a	0.329b	0.655a	0.642a	0.597a
	0.614a	0.421b	0.641a	0.618a	0.617a	0.358d	0.763a	0.654b	0.553c
ILE	0.172	0.158	0.162	0.168	0.168	0.114c	0.278a	0.143b	0.128bc
	0.129	0.133	0.137	0.152	0.125	0.072c	0.180a	0.139b	0.150b
	0.151	0.146	0.149	0.160	0.147	0.093c	0.229a	0.141b	0.139b
U4T	0.345	0.345	0.328	0.334	0.325	0.267d	0.411a	0.350b	0.313c
	0.293	0.322	0.311	0.301	0.284	0.265	0.330	0.310	0.304
	0.319	0.333	0.320	0.317	0.305	0.266c	0.371a	0.330b	0.308b
PHE	0.675	0.950	0.805	0.779	0.894	0.588bc	1.202a	0.973ab	0.519c
	0.789b	0.766b	0.895ab	1.178a	0.936ab	0.249c	1.290a	1.552a	0.562b
	0.732	0.858	0.850	0.979	0.915	0.418b	1.246a	1.263a	0.540b
HIS	0.600	0.556	0.536	0.583	0.587	0.393d	0.763a	0.662b	0.472c
	0.718ab	0.610c	0.757a	0.657bc	0.694ab	0.488c	0.803a	0.776ab	0.682b
	0.659a	0.583b	0.647a	0.620ab	0.641ab	0.441d	0.783a	0.719b	0.577c
LYS	0.156	0.142	0.147	0.131	0.140	0.064c	0.165b	0.198a	0.146b
	0.134	0.117	0.136	0.125	0.116	0.063c	0.165a	0.162a	0.113b
	0.145	0.130	0.142	0.128	0.128	0.063c	0.165a	0.180a	0.130b
NH4	1.691	1.998	1.709	1.766	1.579	1.573	1.788	1.766	1.867
	1.508	1.449	1.570	1.578	1.468	1.316c	1.676a	1.486b	1.581ab
	1.599	1.724	1.639	1.672	1.524	1.444b	1.732a	1.626a	1.724a
ARG	1.829	1.585	1.657	1.508	1.555	0.625c	1.726b	2.489a	1.667b
	1.307	1.129	1.361	1.177	1.149	0.626c	1.625a	1.593a	1.055b
	1.568	1.357	1.509	1.343	1.352	0.625d	1.676b	2.041a	1.361c

[1]The comparison is made across the row. Means which have the same letter show non-significant differences according to the Waller-Duncan multiple range test. Means which are down the column represent digging 1, digging 2, and combined results respectively.

Table 2. Mean squares and components of variance from the analysis of variance of free amino acid composition in Virginia-type peanuts.

Source	df	U1	U2	U3	ASP	TSER	GLU	ALA	PC	VAL	ILE	U4T	PHE	HIS	LYS	NH4	ARG
								Digging 1									
V	4	0.0013**	0.0028**	0.0068NS	0.0757NS	0.3757**	0.7694NS	0.6705**	0.1236NS	0.1103**	0.0004NS	0.0011NS	0.1358NS	0.0081NS	0.0011NS	0.2883NS	0.1890NS
L	3	0.0017**	0.0033**	0.0636**	0.6680**	6.8387**	18.2505**	4.6066**	1.2489**	0.6510**	0.0860**	0.0558**	1.5681*	0.4327**	0.0487**	0.2332NS	8.7979**
VXL	12	0.0002NS	0.0010**	0.0027NS	0.0765NS	0.1682NS	0.8060NS	0.3022**	0.0769NS	0.0246**	0.0005NS	0.0015NS	0.1362NS	0.0092NS	0.0021NS	0.1912NS	0.3904NS
R/L	8	0.0002NS	0.0002NS	0.0026NS	0.0211NS	0.1216NS	0.1597NS	0.1621NS	0.0509NS	0.0051NS	0.0005NS	0.0008NS	0.2635*	0.0088NS	0.0015NS	0.0593NS	0.3602NS
Error	32	0.0002	0.0003	0.0029	0.0689	0.0925	0.4791	0.1060	0.0480	0.0076	0.0009	0.0011	0.1138	0.0076	0.0016	0.1255	0.3337
CV(%)		17.13	24.08	23.39	18.05	17.21	10.76	26.29	16.37	14.34	18.37	10.08	41.13	15.23	27.81	20.26	35.52
G.M.		0.085	0.075	0.232	1.454	1.768	6.432	1.239	1.338	0.6084	0.166	0.335	0.820	0.573	0.143	1.748	1.627
								Digging 2									
V	4	0.00003NS	0.0006*	0.0010NS	0.4931NS	0.2413NS	0.6031NS	0.5249**	0.1427*	0.0894**	0.0012NS	0.0026NS	0.3245*	0.0382**	0.0010NS	0.0404NS	0.1275NS
L	3	0.0008NS	0.0021*	0.0065NS	0.2389NS	4.6315**	16.3102**	1.6461**	2.2878**	0.3531**	0.0315NS	0.0112NS	5.5754**	0.3046**	0.0347**	0.3541**	3.4185**
VXL	12	0.0002NS	0.0002NS	0.0007NS	0.0756NS	0.1372NS	0.5164NS	0.1495*	0.0693NS	0.0191NS	0.0016*	0.0006NS	0.2259*	0.0157NS	0.0006NS	0.0776NS	0.1482NS
R/L	8	0.0002NS	0.0003NS	0.0025NS	0.1484**	0.1259NS	0.3785NS	0.1331NS	0.0435NS	0.0139NS	0.0009NS	0.0038*	0.1137NS	0.0174NS	0.0017*	0.0311NS	0.2171NS
Error	32	0.0002	0.0002	0.0012	0.0405	0.1465	0.3900	0.0654	0.0373	0.0095	0.0007	0.0014	0.1062	0.0090	0.0008	0.0828	0.1232
CV(%)		20.71	16.22	19.23	15.15	20.76	10.08	18.73	14.15	17.50	18.88	12.34	35.70	13.81	22.22	18.996	28.67
G.M.		0.069	0.093	0.180	1.329	1.843	6.195	1.365	1.365	0.556	0.135	0.302	0.913	0.687	0.126	1.515	1.225
								Combined Digging 1 and 2									
V	4	0.0006*	0.0012*	0.0052*	0.0693NS	0.5255**	0.1908NS	1.1840**	0.1982**	0.1979**	0.0008NS	0.0025NS	0.2000NS	0.0213*	0.0014NS	0.1370NS	0.2661NS
L	3	0.0009*	0.0014**	0.0514**	0.7711**	11.350**	24.050**	5.6833**	3.1411**	0.8884**	0.0973**	0.0573**	6.0814**	0.6999**	0.0806**	0.5364**	10.861**
VXL	12	0.0002NS	0.0005NS	0.0019NS	0.1097*	0.2021NS	0.7580NS	0.3066**	0.1030*	0.0292NS	0.0011NS	0.0012NS	0.2069NS	0.0101NS	0.0015NS	0.0179NS	0.3639NS
D	1	0.0076**	0.0104**	0.0805**	0.4687*	0.1726NS	1.6776*	0.4791NS	0.0227NS	0.0830**	0.0275**	0.0328**	0.2572NS	0.3961**	0.0094**	1.6398**	4.8504**
VXD	4	0.0007*	0.0022NS	0.0026NS	0.0558NS	0.0917NS	1.1817*	0.0117NS	0.0683NS	0.0018NS	0.0009NS	0.0011NS	0.2603NS	0.0250*	0.0004NS	0.1918NS	0.0504NS
LXD	3	0.0015**	0.0040**	0.0183**	0.1358NS	0.1204NS	10.510**	0.5694*	0.3957**	0.1157**	0.0202**	0.0097**	1.0622**	0.0374NS	0.0029NS	0.0508NS	1.3550*
VXLXD	12	0.0002NS	0.0006NS	0.0015NS	0.0425NS	0.1035NS	0.5641NS	0.1422NS	0.0433NS	0.0146NS	0.0009NS	0.0009NS	0.1551NS	0.0148NS	0.0012NS	0.0889NS	0.1747NS
R/LXD	16	0.0002NS	0.0003NS	0.0026NS	0.0223NS	0.1238NS	0.2691NS	0.1476NS	0.0472NS	0.0095NS	0.0007NS	0.0022NS	0.1886NS	0.0131NS	0.0017NS	0.0452NS	0.2886NS
Error	64	0.0002	0.0003	0.0021	0.0547	0.1195	0.4346	0.0857	0.0426	0.0085	0.0008	0.0013	0.1100	0.0083	0.0012	0.1041	0.2885
CV(%)		18.73	19.78	22.09	16.81	19.15	10.44	22.4855	15.28	15.87	18.67	11.17	38.27	14.47	25.59	19.78	33.53
G.M.		0.077	0.084	0.206	1.391	1.806	6.313	1.3019	1.351	0.562	0.150	0.319	0.867	0.630	0.134	1.134	1.426

NS, *, ** Mean square is non-significant and significant at 5% and 1% level of probability, respectively.

U2, U3, and PHE (Table 2). Location effects on the free amino acid composition were large and were all highly significant. Location effects were consistent enough to have no influence on the V x L interactions. The V x L interactions were large because of the influence of the variety effects.

The free amino acid content showed large significant differences due to digging effect, except for TSER, ALA, PC and PHE. Changes in composition between diggings was observed. Free amino acids which increased at the second diggings were U2, U3, TSER, ALA, PC, PHE, and HIS, while U1, ASP, GLU (glutamic acid), VAL, ILE, U4T, LYS (lysine), NH4 (ammonia), and ARG (arginine) decreased. Most of the amino acids which increased were reported to be associated with typical peanut flavor whereas most of the amino acids which decreased (except for GLU) were associated with atypical peanut flavor (20). A decrease of glutamic acid indicated that the peauts were not yet fully mature at the second digging (19). The digging effect and location effect produced a large significant difference in the L x D first order interaction. The V x D interactions were mostly non-significant. There was no significance in the second order interaction or in variation within replications.

When the means of all free amino acids were compared among varieties and locations, varying patterns could be observed (Table 1). FG had the highest amount of GLU, LYS, and ARG. NC 6 had the highest amount of PC and U4T. NC 17921 was the highest in ASP, TSER, ALA, and VAL. NC 17922 was the highest in U2, ILE, and PHE. Peanuts grown at Martin County had the highest amount of U1 and PC. Those grown at Northampton were significantly high in TSER, GLU, ALA, HIS, VAL, ILE, and U4T. Peanuts grown at Sussex were highest in PHE, LYS, and ARG.

Free Sugars

Eight sugars, which were detected in all peanut samples, eluted in the following order: unknown, ribose, fructose, glucose, inositol, sucrose, raffinose and stachyose. Sucrose was found to predominate as previously stated (22, 29, 30), and accounted for about 30 mg/g of peanuts which was about 85% of the total sugar (Table 3). Means for stachyose, raffinose, fructose, inositol, glucose and an unknown in descending order were 3.98, 0.35, 0.21, 0.13, 0.10 and 0.06 mg/g, respectively. The small amounts of ribose measured were variable and were less than 0.1%; therefore, it was dropped from the statistical analysis. Trace amounts of ribose were also reported by Amaya-Farfan, et al. (3). The unknown sugar might be deoxyribose since it is an integral part of DNA. Variety and location produced highly significant differences in most of the free sugars as shown in the analysis of variance of combined digging data (Table 3). Glucose did not vary with location and was consistently non-significant in both diggings. Fructose, stachyose, and raffinose varied significantly (0.01, 0.01, and 0.05 respectively) between the two diggings. Differences between grand means of these variables in each digging were noted. Stachyose had the largest variations among varieties, locations, and digging, which resulted in highly significant first and second order interactions. An effect of replications within location was not observed. Significant differences for other sugars within replications were observed in digging 2, which resulted in a significant difference of the second order interactions for sucrose. Location had an influence

Table 3. Mean squares and components of variance from the analysis of variance of free sugars, AMI value, and calcium in Virginia-type peanuts.

Source	d.f.	Unknown	Fructose	Glucose	Inositol	Sucrose	Raffinose	Stachyose	AMI value	Calcium
					Digging 1					
Variety(V)	4	0.0038NS	0.0074*	0.0007NS	0.0026NS	176.97**	0.0108**	1.239**	46.81NS	0.013**
Location(L)	3	0.0082NS	0.0030NS	0.0005NS	0.0377**	60.50*	0.0166*	14.67**	1505.66**	0.027**
VXL	12	0.0025NS	0.0024NS	0.0007NS	0.0009NS	8.76NS	0.0038NS	0.98**	76.79NS	0.001NS
Rep/L	8	0.0031NS	0.0014NS	0.0007NS	0.0005NS	8.19NS	0.0022NS	0.22NS	93.90NS	0.001NS
Error	32	0.0015	0.0020	0.0006	0.0012	4.80	0.0027	0.27	60.63	0.001
C.V.(%)		62.22	19.15	24.30	27.46	7.11	14.21	12.77	21.82	9.47
Grand Mean		0.0618	0.2308	0.0979	0.1259	30.80	0.3656	4.10	35.68	0.311
					Digging 2					
Variety(V)	4	0.0028**	0.0010NS	0.0021**	0.0039**	214.89**	0.0029NS	0.44	50.96NS	0.017**
Location(L)	3	0.0431**	0.0139*	0.0016NS	0.0677**	53.76**	0.0600**	8.73**	746.99**	0.023**
VXL	12	0.0017NS	0.0015NS	0.0005NS	0.0013**	3.41NS	0.0026NS	0.48**	17.48NS	0.001NS
Rep/L	8	0.0040**	0.0025*	0.0008*	0.0018**	9.64*	0.0064**	0.19NS	31.23NS	0.001NS
Error	32	0.0009	0.0011	0.0003	0.0003	3.14	0.0015	0.16	26.28	0.001
C.V.(%)		54.82	16.72	16.76	12.16	5.90	11.88	10.26	14.97	9.97
Grand Mean		0.0536	0.1956	0.1032	0.1370	30.04	0.3306	3.86	34.25	0.332
					Combined Digging 1 and 2					
Variety(V)	4	0.0065**	0.0057**	0.0021**	0.0061**	380.50**	0.0105**	1.08**	69.20NS	0.028**
Location(L)	3	0.0432**	0.0069*	0.0016NS	0.0936**	64.47**	0.0387**	24.58**	1926.67**	0.047**
VXL	12	0.0026*	0.0021NS	0.0007NS	0.0014*	4.13NS	0.0029NS	0.86**	61.59NS	0.001NS
Digging(D)	1	0.0020NS	0.0371**	0.0008NS	0.0035NS	17.19NS	0.0368*	1.77**	61.63NS	0.013**
VXD	4	0.0001NS	0.0027NS	0.0007NS	0.0003NS	11.36*	0.0032NS	0.61*	25.57NS	0.002NS
LXD	3	0.0080NS	0.0099*	0.0004NS	0.0118**	49.79**	0.0378**	2.16**	325.99*	0.003NS
VXLXD	12	0.0016NS	0.0019NS	0.0005NS	0.0007NS	8.04*	0.0036NS	0.60**	32.68NS	0.001NS
Rep/(LXD)	16	0.0035**	0.0020NS	0.0007NS	0.0012NS	8.92*	0.0043*	0.20NS	62.57NS	0.001NS
Error	64	0.0012	0.0015	0.0004	0.0007	3.97	0.0021	0.21	43.45	001
C.V.(%)		59.31	18.23	20.68	20.66	6.55	13.23	11.63	18.85	9.74
Grand Mean		0.0577	0.2132	0.1005	0.1313	30.42	0.3481	3.98	34.97	0.322

NS, *, ** Mean square is non-significant and significant at 5% and 1% level of probability, respectively.

on inositol from variety and digging, producing significant first order interaction between L x V and L x D.

Table 4 shows means of free sugars among varieties and locations with their significant differences. NC 6 had significantly higher amounts of glucose, sucrose, and stachyose than the other varieties but was significantly lower in amounts of unknown and raffinose. FG had significantly higher amounts of unknown, fructose, inositol, and raffinose, but was significantly lower in stachyose. Peanuts grown at Martin County showed signficantly higher amounts of unknown and fructose, and significantly lower amounts of inositol, sucrose, and stachyose than at other locations. Northampton County showed significantly higher amounts of inositol, sucrose, and stachyose, and significantly lower amounts of unknown, fructose, glucose, and raffinose. Suffolk, VA. showed high amounts of glucose and raffinose. When the amount of free sugars was summed to represent total free sugar content, the NC 6 variety had the highest amount (42.49 mg/g) among varieties, and peanuts grown at Northampton County had the highest total free sugar content (37.82 mg/g) among counties.

Table 4. Mean comparison of free sugars (mg/g of peanuts), AMI value and calcium content (mg/g) of Virginia-type peanuts among varieties and locations.[1]

Sugars	Varieties					Location			
	Florigiant	NC6	NC17921	NC17922	NC17976	Martin	Northampton	Sussex	Suffolk
Unknown	0.080	0.034	0.069	0.071	0.055	0.094	0.038	0.061	0.054
	0.067a	0.029b	0.065a	0.057a	0.049ab	0.134a	0.021b	0.029b	0.031b
	0.074a	0.032c	0.067ab	0.064ab	0.052b	0.114a	0.030b	0.045b	0.042b
Fructose	0.247a	0.231a	0.257a	0.226ab	0.192b	0.226	0.216	0.232	0.249
	0.198	0.207	0.194	0.196	0.182	0.241a	0.185b	0.173b	0.184b
	0.222a	0.219a	0.226a	0.211a	0.187b	0.233a	0.200b	0.202b	0.217ab
Glucose	0.102	0.102	0.103	0.098	0.085	0.098	0.090	0.104	0.101
	0.105b	0.125a	0.099b	0.096b	0.092b	0.096	0.095	0.106	0.117
	0.103ab	0.114a	0.101b	0.097bc	0.088c	0.097	0.092	0.105	0.109
Inositol	0.143	0.107	0.135	0.116	0.129	0.057c	0.178a	0.138b	0.131b
	0.157a	0.121b	0.155a	0.120b	0.131b	0.038b	0.151a	0.174a	0.183a
	0.150a	0.114c	0.145a	0.118bc	0.130b	0.048b	0.164a	0.156a	0.157a
Sucrose	30.742b	37.435a	29.141bc	28.170c	28.499c	27.984b	32.582a	30.828a	31.796a
	28.331bc	37.470a	28.051bc	29.312b	27.038c	30.023ab	32.199a	30.347	27.592b
	29.536b	37.453a	28.596bc	28.741bc	27.769c	29.003b	32.391a	30.588b	29.694b
Raffinose	0.376a	0.313b	0.369a	0.384a	0.387a	0.326c	0.359bc	0.406a	0.374ab
	0.344	0.312	0.334	0.317	0.346	0.319b	0.282b	0.299b	0.423a
	0.360a	0.313b	0.352a	0.350a	0.366a	0.322b	0.320b	0.353b	0.397a
Stachyose	3.548b	4.339a	4.298a	4.108a	4.208a	2.649c	5.086a	3.878b	4.788a
	3.837ab	4.147a	3.895ab	3.617b	3.788ab	2.723b	4.166a	4.160a	4.379a
	3.692c	4.243a	4.097ab	3.863bc	3.998ab	2.686c	4.626a	4.019b	4.584a
AMI value	37.50	34.92	38.08	34.00	33.92	22.00c	39.93ab	45.47a	39.33b
	36.58	30.92	34.67	34.33	34.75	24.47c	40.80a	34.20b	37.53ab
	37.04	32.92	36.38	34.17	34.33	23.23b	30.37a	39.83a	36.43a
Calcium	0.303b	0.259c	0.324ab	0.328a	0.343a	0.293b	0.301b	0.373a	0.279b
	0.356a	0.267b	0.340a	0.338a	0.359a	0.293c	0.326b	0.386a	0.323b
	0.329b	0.263c	0.332b	0.333b	0.351a	0.293c	0.313b	0.380a	0.301bc

[1]The comparison is made across the row. Means which have the same letter show non-significant differences according to the Waller-Duncan multiple range test. Means which are down the column represent digging 1, digging 2, and combined results respectively.

Differences of AMI values were highly significant among locations but not among varieties (Table 3). Comparison of means in Table 4 showed that peanuts grown at Martin County were the most mature with an AMI value of 23. Peanuts grown at Northampton and Sussex were immature at harvest time as indicated by AMI values of 40 and 39. Peanuts grown in Martin County received the highest CLER (Critical Laboratory Evaluation Roast) score and contained the highest oil percentage (18). Highly significant differences were observed for calcium content among varieties and location (Table 3). The means (from high to low) varied for varieties (NC 17976, NC 17922, NC 17921, FG, NC 6) and locations (Sussex, Northampton, Suffolk, and Martin) (Table 4). Further research is needed to determine if a correlation exists between calcium content and flavor of the roasted peanut. Calcium was reported to be necessary for peanut seed germination, increasing fruit fill, and fruit yield (31).

Rather large coefficients of variation for the amino acids were obtained due to the variability within the sound mature kernel peanut grade as previously found by Young *et al.* (34). The percentage sound mature kernels in all varieties and locations were in the range of 63-72% from a yield of 3458-4534 kg/ha (17). Even though peanuts grown at Martin County were the most mature peanuts, they did not give the highest yield. They gave the highest percentage of extra large kernels in the range of 36-44% compared to 21-53% at Northampton, 18-41% at Sussex, and 15-43% at Suffolk.

Using these results showing significant variations in free amino acids and free sugars, a portion of these samples has been selected for roasting. This study, which is now in progress, will enable us to establish the mathematical relationship between content of flavor precursors and roasted peanut flavor in Virginia-type peanuts.

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