

Investigations into Genotypic Variations of Peanut Carbohydrates

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Carbohydrates are known to be important precursors in the development of roasted peanut quality. However, little is known about their genotypic variation. A better understanding of the role of carbohydrates in roasted peanut quality requires first an understanding of the genotypic variation in the soluble carbohydrate components. Ion exchange chromatography was used to isolate 20 different carbohydrate components in 52 genotypes grown in replicated trials at two locations. Inositol, glucose, fructose, sucrose, raffinose, and stachyose were quantitated, and 12 unknown peaks were evaluated on the basis of the peak height of the unknown relative to the cellobiose internal standard peak height. Peaks tentatively identified as verbascose and ajugose could not be properly integrated because of tailing. Of the 18 carbohydrates that were estimated, 9 exhibited significant variation between test environments, 5 among market types, 14 among genotypes within market types, and 11 exhibited some significant form of genotype \times environment interaction. Genotypes accounted for 38–78% of the total variation for the known components, suggesting that broad-sense heritability for these components is high. The observed high genotypic variation in carbohydrate components is similar to the high genotypic variation observed for the sweetness attribute in roasted peanuts, which raises the question regarding possible interrelationships. The establishment of such interrelationships could be most beneficial to peanut breeding programs to ensure the maintenance of flavor quality in future peanut varieties.

Keywords: *Arachis hypogaea* L.; sugars; genetic variation; environmental variation

INTRODUCTION

The flavor of the roasted peanut (*Arachis hypogaea* L.) seed is an important characteristic influencing consumer acceptance. Carbohydrates have been shown to be contributing precursors to the compounds imparting the roasted peanut characteristic (Newell et al., 1967; Mason et al., 1969). The peanut carbohydrates have been identified and quantitated (Tharanathan et al., 1975, 1976; Holley and Hammons, 1968; Newell et al., 1967), but their specific role in roasted peanut flavor generation has not been elucidated. Vercellotti et al. (1993) studied peanut mono-, oligo-, and polysaccharide fractions as the origin of intermediates for flavor molecules and found that, on heating, the fractions yielded products with various sensory impacts but specific roles were not identified. The individual components of the peanut carbohydrate fraction have been shown to change during maturation (Pattee et al., 1974; Ross and Mixon, 1989) as well as during curing (Vercellotti et al., 1995). They also change across seed size and over storage time (Pattee et al., 1981), decrease with higher soil temperatures (McMeans et al., 1990), and

have been shown to vary among a limited number of genotypes (Basha et al., 1976; Oupadissakoon et al., 1980; Gupta et al., 1982; Gadgil and Mitra, 1983; Basha, 1992). There is, however, little known about the genotypic relationships of this variation. Roasted peanut quality sensory attributes have been shown to be heritable traits and to be influenced by environmental conditions (Pattee and Giesbrecht, 1990; Pattee et al., 1993, 1994, 1995, 1997, 1998; Isleib et al., 1995). Knowledge about genotypic carbohydrate variation should provide a basis for further understanding of the role carbohydrates have in roasted peanut quality development. The objective of this study is to increase the understanding of carbohydrate component–genotypic relationships.

MATERIALS AND METHODS

Genotype Resources. In 1993 a total of 208 peanut samples were obtained from field trials conducted at Gainesville, FL, and Lewiston, NC. Fifty-two genotypes and two replications per trial are represented within these samples. Utilizing the findings of Pattee et al. (1994) on genotype-by-environmental interaction in roasted peanut attribute, mean values having less than four observations were not included in this paper, thus providing reasonable estimates of the experimental error in the mean values. All samples were obtained from plants grown and harvested under standard recommended procedures for the specific location.

Sample Handling. A 1000 g sample of the sound mature kernel (SMK) fraction from each replicate of each location entry was shipped to Raleigh, NC, in February following

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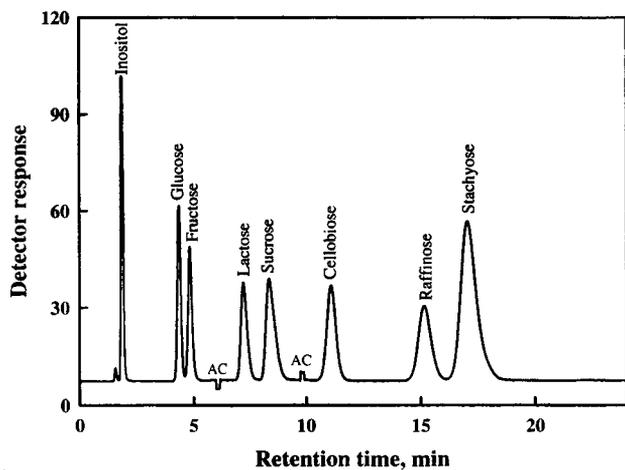


Figure 1. HPLC separation of sugar standards. Lactose and cellobiose are internal standards. Detector attenuation changes are indicated by AC.

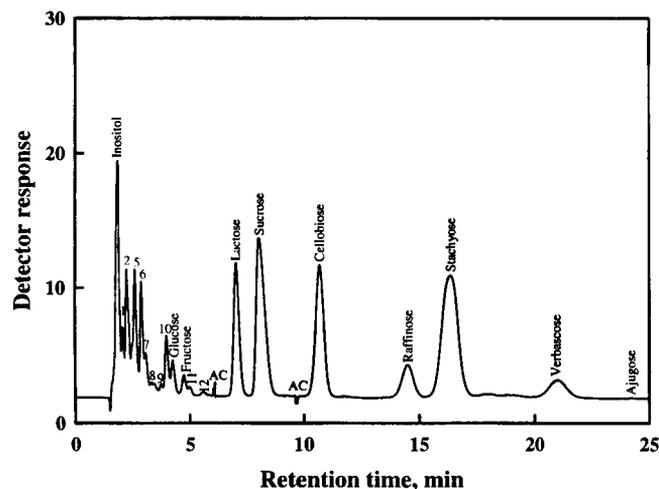


Figure 2. Separation of sugars in White's Runner peanut genotype. Lactose and cellobiose are internal standards. Detector attenuation changes are indicated by AC. Unknown components are numbered.

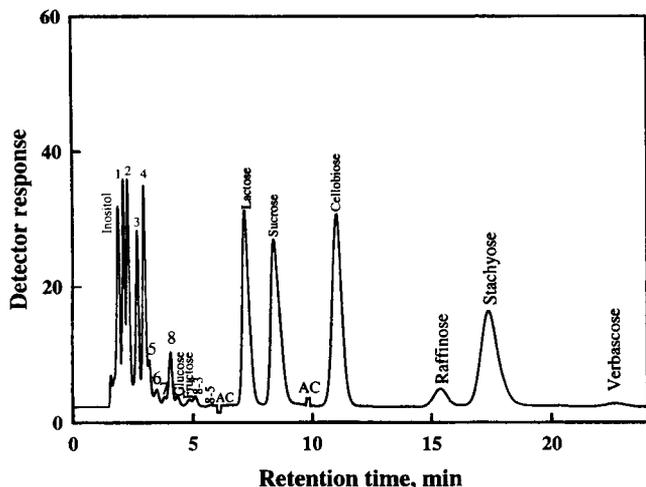


Figure 3. Separation of sugars in Improved Spanish 2B peanut genotype. Lactose and cellobiose are internal standards. Detector attenuation changes are indicated by AC. Unknown components are numbered.

harvest and placed in controlled storage at 5 °C and 60% relative humidity until analyzed in 1995.

Carbohydrate Analysis. A 35 g subsample was taken from each genotype sample and ground for 10 s into a meal using

Table 1. Partial Mean Squares from Analysis of Variance of Known Carbohydrate Components in 52 Peanut Lines Grown at Two Locations

source	df	total sugars		inositol		glucose		fructose		sucrose		raffinose		stachyose	
		MS ^a	SST ^b	MS	SSTo	MS	SSTo	MS	SSTo	MS	SSTo	MS	SSTo	MS	SSTo
total	216														
location	1	34457199	0.3	96526*	1.9	172	0.0	1709	0.2	1093839	0.0	206383*	3.4	58563239**	32.7
rep in location	2	641417	0.0	2429	0.1	273	0.1	465	0.1	3598223	0.1	9638	0.3	333889†	0.4
genotypes (G)	51	135339062**	64.0	38112	38.0	14949**	77.9	13371**	74.8	115051326**	64.9	71458**	59.9	1364683**	38.9
market type (M)	2	85739932	1.6	194379	7.6	128014†	26.1	112098†	24.6	775081461	17.2	474612†	15.6	3180860	3.6
G in M	49	106313416**	48.3	32239	30.9	11657**	58.3	10509**	56.5	88929445**	48.2	51756**	41.7	1275696**	34.9
location × genotype	51	42558584**	20.1	27669**	27.6	1765†	9.2	2059*	11.5	35725423**	20.2	24116**	20.2	556876**	15.9
location × M	2	242456698**	4.5	85143*	3.3	10315**	2.1	8875*	1.9	198800990**	4.4	27584	0.9	2050642*	2.3
location × G in M	49	36193651**	16.4	25927**	24.8	1487	7.4	1857*	10.0	30375502**	16.5	24828**	20.0	511582**	14.0
error	111	13011321	13.4	14926	32.4	1242	14.1	1244	15.1	10498937	12.9	7830	14.3	140433	8.7

^a Given in ppm. ^b Partial sum of squares for the effect expressed as a percentage of the total corrected sum of squares. †, *, **, significance at the 0.10, 0.05, and 0.01 levels of probability, respectively.

Table 3. Least-Squares Means for Known Carbohydrate Components

genotype	total sugars	inositol	glucose	fructose	sucrose	raffinose	stachyose
large-seeded Virginia market-type							
Early Bunch	39929	325	71	76	36823	619	2014
Early Bunch Component 1	32147	408	135	114	29715	482	1292
Early Bunch Component 2	31274	200	46	39	29067	461	1461
Early Bunch Component 3	30755	245	38	37	28575	495	1365
Early Bunch Component 4	28908	158	47	40	26744	442	1477
Early Bunch Component 5	26727	525	46	31	24275	416	1433
Florigiant	31429	210	62	55	28096	544	2463
GA 119-20	31125	157	33	32	28153	368	2382
Holland Virginia Jumbo	37964	242	162	157	33749	494	3072
Jenkins Jumbo	42625	322	40	49	39747	447	2020
NC 2	31308	144	29	22	28283	365	2474
NC 4	43128	301	61	105	39010	577	3073
NC 7	36014	344	65	54	33109	465	1978
NC 9	44795	497	46	85	39727	917	3525
NC Ac 17921	28776	301	54	42	25312	609	2459
NC Ac 18016	23169	199	26	24	20515	264	2142
NC Ac 18423	42322	414	68	75	39465	650	1649
NC Ac 18431	36011	293	55	51	32226	669	2704
White's Runner	47708	407	79	76	42282	687	4177
runner market-type							
Basse (NCSU Collection)	40402	434	63	58	36202	600	3045
Basse (PI 229553)	34578	262	63	42	30929	479	2803
Basse 32-15 (PI 237511)	30790	211	44	34	27672	415	2414
Bradford Runner	28922	155	37	37	27230	307	1156
Dixie Runner	31016	181	42	37	28838	363	1555
Early Runner	27467	193	17	15	24638	271	2333
Early Runner Component 1	26963	190	24	21	24178	290	2261
Early Runner Component 2	27905	177	22	17	25009	343	2337
Early Runner Component 3	27469	193	21	17	24644	288	2306
Early Runner Component 4	27605	173	18	16	24864	288	2246
Early Runner Component 5	26796	170	19	15	23816	304	2472
Florispan Component 1	24478	143	46	46	22112	347	1783
Florispan Component 2	26291	201	47	48	23599	347	2049
Florispan Component 3	23824	157	37	35	21034	361	2201
Florispan Component 4	30363	204	49	51	27301	417	2341
Florispan Component 5	27310	181	57	60	24600	385	2028
Florunner	29861	199	29	25	26814	389	2404
Florunner Component 1	29466	191	45	41	26172	403	2615
Florunner Component 2	30441	180	46	44	27249	425	2498
Florunner Component 3	29826	166	33	28	26771	374	2453
Florunner Component 4	30443	214	59	54	27110	444	2562
F439-17-2-1-1 (Florunner sister line)	29343	151	24	16	26248	253	2652
GA 207-2	25777	250	47	45	23453	315	1667
GA 207-3-4	36270	414	64	77	32452	658	2604
NC 3033	23766	211	28	21	20345	338	2824
PI 109839	31450	194	30	29	28167	419	2611
Small White Spanish (PI 264180)	25774	205	48	40	23254	344	1883
Southeastern Runner 56-15	26583	168	34	25	23692	378	2285
fastigiate market-type							
Improved Spanish 2B	22716	327	22	22	20320	320	1705
Pearl	34636	239	325	309	31163	496	2105
PI 337396	30103	374	52	49	27612	387	1628
Small White Spanish (NCSU collection)	30919	141	342	308	28136	469	1522
Spanette (Spanish 18-38-42)	25837	184	41	35	23635	291	1651
av critical value for genotype means							
mean for Virginia market-type	6827	171	42	45	6260	156	752
mean for runner market-type	35057	300	61	61	31835	525	2271
mean for fastigiate market-type	28971	206	39	35	26014	377	2300
mean for Gainesville, FL, location	28842	253	156	145	26173	393	1722
mean for Lewiston, NC, location	30588	282	93	83	28060	461	1604
	31325	224	78	78	27955	402	2592

However, no chromatogram or tabular presentation is given to indicate the number of components observed. They indicated that this group of components could constitute up to 12% of the carbohydrate weight. Visual comparison of the unknown peak heights to inositol (Figure 3) would suggest that in some genotypes these components might be similar in concentration to inositol, assuming they have similar detector response values. Retention times for arabinose, mannose, and galactose did not match any of the unknown components. The use of the cation syringe filters (Singleton et al., 1996) on the rehydrated extract before analysis

eliminated the possibility of interfering amino acids in the unknowns. Verbascose and ajugose have been reported as carbohydrate components in peanuts by Tharanathan et al. (1975, 1976) and Amaya et al. (1978). Vercellotti et al. (1994) suggested from mass spectral data on methylated products that verbascose might be present in peanuts. Our data indicate that verbascose was present in at least trace amounts in all but 2 genotypes (Dixie Runner and Early Bunch Component 5) of the 52 surveyed. Thus, the variability in consistently identifying verbascose in peanuts has several reasons: (a) it is present in only trace amounts

Table 4. Least-Squares Means for Unknown Carbohydrate Components

	ratio of unknown peak height to cellobiose peak height												
	total unk	unk1	unk 2	unk 3	unk 4	unk 5	unk 6	unk 7	unk 8	unk 9	unk 10	unk 11	unk 12
genotype													
large-seeded Virginia market-type													
Early Bunch	2.351	0.401	0.000	0.369	0.000	0.347	0.000	0.000	0.082	0.033	0.591	0.000	0.000
Early Bunch Component 1	2.585	0.532	0.000	0.323	0.000	0.294	0.000	0.000	0.155	0.103	0.575	0.093	0.000
Early Bunch Component 2	2.001	0.322	0.000	0.226	0.000	0.000	0.397	0.000	0.054	0.042	0.424	0.033	0.019
Early Bunch Component 3	1.919	0.358	0.000	0.291	0.000	0.000	0.000	0.000	0.079	0.049	0.484	0.000	0.000
Early Bunch Component 4	1.466	0.000	0.000	0.240	0.000	0.000	0.000	0.000	0.043	0.000	0.499	0.000	0.000
Early Bunch Component 5	1.676	0.322	0.000	0.255	0.000	0.000	0.000	0.000	0.057	0.000	0.413	0.000	0.000
Florigiant	2.427	0.285	0.000	0.076	0.000	0.460	0.691	0.159	0.040	0.000	0.298	0.000	0.000
GA 119-20	3.023	0.411	0.435	0.236	0.000	0.539	0.757	0.171	0.000	0.000	0.354	0.038	0.000
Holland Virginia Jumbo	3.177	0.332	0.483	0.264	0.132	0.763	0.704	0.205	0.088	0.037	0.196	0.000	0.018
Jenkins Jumbo	3.320	0.000	0.395	0.191	0.191	0.950	0.000	0.000	0.206	0.107	0.699	0.057	0.000
NC 2	1.959	0.000	0.522	0.000	0.000	0.657	0.000	0.000	0.000	0.000	0.322	0.000	0.000
NC 4	4.006	0.718	0.411	0.353	0.176	0.685	0.914	0.258	0.083	0.037	0.341	0.000	0.019
NC 7	2.737	0.000	0.401	0.188	0.000	0.511	0.551	0.131	0.067	0.056	0.384	0.072	0.000
NC 9	2.947	0.000	0.429	0.250	0.115	0.738	0.449	0.112	0.102	0.086	0.405	0.071	0.000
NC Ac 17921	1.908	0.000	0.000	0.000	0.000	0.460	0.407	0.000	0.050	0.042	0.376	0.058	0.000
NC Ac 18016	3.949	0.330	1.047	0.326	0.000	1.230	0.539	0.170	0.000	0.000	0.241	0.000	0.000
NC Ac 18423	2.019	0.465	0.000	0.373	0.000	0.000	0.000	0.000	0.099	0.066	0.304	0.000	0.000
NC Ac 18431	3.075	0.000	0.521	0.195	0.121	0.589	0.599	0.141	0.074	0.058	0.432	0.044	0.000
White's Runner	4.171	0.356	0.689	0.408	0.245	0.822	0.741	0.216	0.073	0.058	0.499	0.044	0.020
runner market-type													
Basse (NCSU collection)	5.636	0.790	0.487	0.000	0.181	0.526	2.387	0.570	0.102	0.034	0.463	0.000	0.000
Basse (PI 229553)	4.345	0.642	0.865	0.285	0.000	0.607	1.141	0.184	0.057	0.042	0.401	0.000	0.000
Basse 32-15 (PI 237511)	3.416	0.631	0.462	0.201	0.000	0.479	0.867	0.119	0.040	0.046	0.470	0.000	0.000
Bradford Runner	2.087	0.613	0.000	0.200	0.000	0.281	0.382	0.000	0.000	0.027	0.292	0.035	0.000
Dixie Runner	2.120	0.572	0.000	0.253	0.000	0.000	0.349	0.000	0.045	0.000	0.408	0.000	0.000
Early Runner	3.019	0.000	0.494	0.000	0.133	0.858	0.608	0.278	0.000	0.000	0.380	0.000	0.000
Early Runner Component 1	2.868	0.000	0.428	0.112	0.000	0.788	0.682	0.293	0.000	0.000	0.247	0.000	0.000
Early Runner Component 2	3.137	0.309	0.414	0.247	0.000	0.819	0.687	0.276	0.000	0.000	0.358	0.000	0.000
Early Runner Component 3	3.679	0.405	0.585	0.245	0.000	0.857	0.789	0.296	0.000	0.000	0.383	0.000	0.000
Early Runner Component 4	3.021	0.334	0.491	0.165	0.000	0.719	0.688	0.235	0.000	0.000	0.349	0.000	0.000
Early Runner Component 5	3.393	0.310	0.524	0.209	0.000	0.880	0.780	0.320	0.000	0.000	0.320	0.000	0.000
Florispán Component 1	1.683	0.000	0.386	0.000	0.000	0.319	0.351	0.000	0.000	0.000	0.215	0.000	0.000
Florispán Component 2	2.284	0.302	0.615	0.000	0.000	0.520	0.406	0.000	0.000	0.000	0.183	0.000	0.000
Florispán Component 3	1.821	0.000	0.490	0.000	0.000	0.353	0.325	0.000	0.000	0.000	0.271	0.000	0.000
Florispán Component 4	2.959	0.518	0.611	0.106	0.000	0.473	0.715	0.140	0.000	0.000	0.306	0.000	0.000
Florispán Component 5	2.193	0.351	0.504	0.000	0.000	0.449	0.481	0.105	0.000	0.000	0.201	0.000	0.000
Florunner	2.517	0.000	0.480	0.000	0.108	0.572	0.531	0.154	0.000	0.000	0.327	0.000	0.000
Florunner Component 1	2.500	0.000	0.502	0.000	0.000	0.645	0.530	0.173	0.000	0.000	0.260	0.000	0.000
Florunner Component 2	3.158	0.400	0.658	0.000	0.000	0.501	0.923	0.183	0.000	0.031	0.298	0.000	0.000
Florunner Component 3	2.737	0.313	0.563	0.000	0.133	0.608	0.677	0.186	0.000	0.000	0.207	0.000	0.000
Florunner Component 4	2.514	0.000	0.703	0.000	0.000	0.688	0.467	0.096	0.000	0.000	0.183	0.000	0.000
F439-17-2-1-1 (Florunner sister line)	2.739	0.000	0.782	0.000	0.000	0.560	0.695	0.189	0.000	0.000	0.359	0.000	0.000
GA 207-2	2.070	0.000	0.000	0.000	0.000	0.487	0.386	0.136	0.037	0.000	0.438	0.000	0.000
GA 207-3-4	2.428	0.000	0.385	0.234	0.120	0.560	0.000	0.000	0.073	0.044	0.481	0.042	0.000
NC 3033	3.482	0.000	1.016	0.352	0.000	1.065	0.397	0.121	0.000	0.000	0.196	0.000	0.000
PI 109839	2.738	0.000	0.375	0.106	0.000	0.393	0.836	0.215	0.000	0.033	0.388	0.000	0.000
Small White Spanish (PI 264180)	2.447	0.000	0.000	0.237	0.000	0.466	0.644	0.205	0.039	0.049	0.280	0.041	0.000
Southeastern Runner 56-15	3.358	0.395	0.375	0.190	0.121	0.523	1.031	0.283	0.000	0.000	0.433	0.000	0.000
fastigiata market-type													
Improved Spanish 2B	4.504	1.268	0.675	0.000	0.000	0.853	1.074	0.238	0.063	0.000	0.301	0.000	0.000
Pearl	3.724	0.717	0.509	0.000	0.000	0.842	1.191	0.308	0.056	0.000	0.000	0.000	0.000
PI 337396	3.743	0.552	0.354	0.000	0.124	0.423	1.529	0.399	0.000	0.000	0.317	0.000	0.000
Small White Spanish (NCSU collection)	4.824	1.013	0.000	0.000	0.000	0.302	2.555	0.498	0.042	0.000	0.000	0.000	0.000
Spanette (Spanish 18-38-42)	3.118	0.507	0.462	0.000	0.000	0.655	0.799	0.202	0.036	0.039	0.282	0.000	0.000
av critical value for genotype means													
mean for Virginia market-type	2.669	0.000	0.342	0.245	0.089	0.540	0.451	0.105	0.073	0.045	0.413	0.038	0.000
mean for runner market-type	2.870	0.000	0.502	0.135	0.062	0.579	0.677	0.181	0.232	0.019	0.325	0.000	0.000
mean for fastigiata market-type	3.983	0.811	0.464	0.000	0.000	0.615	1.430	0.329	0.042	0.000	0.211	0.000	0.000
mean for Gainesville, FL, location	2.878	0.626	0.000	0.103	0.000	0.487	0.872	0.224	0.038	0.023	0.248	0.000	0.000
mean for Lewiston, NC, location	3.469	0.000	0.678	0.159	0.073	0.670	0.833	0.186	0.053	0.030	0.385	0.036	0.000

in most genotypes analyzed, (b) it is not present in all genotypes, and (c) a verbascose standard could not be found. We did not locate a verbascose standard for this study until after the analysis had been completed. Tharanathan et al. (1975, 1976) found ajugose to occur in a single genotype. We found a carbohydrate component thought to be ajugose to occur in a few genotypes (NC 4, White's Runner, and NC Ac 18423). Lack of a standard and variability in the relative retention time of the peak make this observation tenuous.

Quantitative Variation in Carbohydrate Components. Because there were some missing values in

the analysis of variance, partial mean squares are reported. In the analysis of variance at least one source of variation was significant ($P < 0.05$) for all six of the known carbohydrate components as well as for their total (Table 1). There was variation ($P < 0.01$) among genotypes for all of the known carbohydrates except inositol. The variation was not due to consistent differences among the three market types but rather to variation among genotypes within market types. Only for glucose, fructose, and raffinose was there a trend toward significant variation ($P < 0.10$) among market types. The genotypic variation was relatively large,

accounting for 38–78% of the total sum of squares for each component, whereas location effects accounted for <5% of total variation in all known carbohydrates except stachyose, for which 33% of the total variation was due to location. Likewise, block effects accounted for only a small proportion of the total variation in any carbohydrate. Location-by-genotype interaction was significant for all known carbohydrates and accounted for 9–28% of the total variation. All components except raffinose exhibited location-by-market-type interaction. The preponderance of genotypic variation in this study suggests that the broad-sense heritability of these carbohydrates should be high and that selection among genotypes for high or low values of different components should be effective.

A similar pattern of significance of effects is seen in the unknown carbohydrates (Table 2). Genotypic effects were important ($P < 0.05$) for all unknowns except 4, 11, and 12. Again, only when genotypes accounted for at least one-third of the total variation was that variation statistically significant.

Genotype-by-Carbohydrate Component Relationships. Two factors make it difficult to compare our findings to other peanut carbohydrate literature. First, there is a wide variation in the basis of the reported data: fatfree versus full-fat meal, extraction methods used, only total carbohydrates reported, etc. Second, there is limited literature on carbohydrate variation across genotypes. Previous studies have relatively few genotypes and even fewer that are comparable to those reported here. The ranges of the known carbohydrates reported in this study were similar to those previously reported (Oupadissakoon et al., 1980; Pattee et al., 1981). Sucrose accounted for ~90% of the total carbohydrates present (Table 3). There was a 2-fold difference in the sucrose content of the genotypes with the minimum and maximum values 20320 and 42282 ppm, respectively. There were 4-fold differences in raffinose and stachyose (253 versus 917 ppm and 1156 versus 4177 ppm, respectively), whereas glucose and fructose showed 20-fold differences (17 versus 342 ppm and 15 versus 309 ppm, respectively). The range for total sugars was 24538 ppm for Virginias, 16636 ppm for runners, and 11920 ppm for fastigiates. The range order in individual components mirrored total sugars for inositol, sucrose, raffinose, and stachyose. For glucose and fructose the order was fastigiates, Virginias, and runners. The variation observed in the ranges of components is due primarily to corresponding variation in the maximum values rather than the minimum values. As mentioned previously, the means for the market-types did not differ significantly.

For several of the individual unknowns, only a few genotypes had values significantly different from zero (Table 4). White's Runner, a Virginia-type, was the only line in which the presence of all unknowns was statistically significant (Figure 2). No line was completely free of all unknowns. Across all genotypes, unknowns 6, 3, 1, and 2 had the highest average values, respectively. Only three unknowns showed no genotypic variation (3, 11, and 12). Unknowns 3 and 6–9 showed significant effects for market-types. The level of unknowns 3 and 9 was so small in fastigiate lines that the least-squares mean values for that group were not significantly different from zero.

In summary, there is high genotypic variation in carbohydrate components across different peanut geno-

types. Earlier work has shown similarly high genotypic variation for the sweetness attribute in roasted peanuts (Pattee et al., 1998). Higher values of sweetness are associated with generally superior flavor profiles, that is, lower bitter and higher roasted peanut attribute intensities. Because sensory evaluation is a costly process, establishment of interrelationships between sensory attributes and chemical composition could be most beneficial to peanut breeding programs to ensure the maintenance of flavor quality in future peanut varieties. Indirect selection for flavor based upon a simple chemical assay of a carbohydrate component(s) may be more efficient than direct selection based upon sensory panel data.

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