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Free Amino Acids in Peanuts as Affected by Seed Size and Storage Time[1]

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ABSTRACT

Peanuts from a commercial source were segregated into specific seed sizes and placed in storage conditions approximating commercial conditions to determine the amino acid changes taking place during storage within various size seeds. Concentrations of the isolated free amino acid fractions significantly decreased with seed size except for phenylalanine which significantly increased with seed size. Significant changes also occurred in the free amino acid fractions across storage periods up to nine months in duration. These results are the first to document a change in amino acids during the storage of peanuts.

Key Words: Peanuts, *Arachis hypogaea* L., Free Amino Acids, Seed Size, Storage.

Amino acids are involved in two important quality aspects of peanuts (*Arachis hypogaea* L.). These are nutritional quality (protein) and roasted flavor quality (free amino acids). Roasted flavor quality has probably received more attention because of its economic impact. It has been postulated that free aspartic acid (ASP), glutamic acid (GLU), glutamine (GLN), asparagine (ASN), histidine (HIS), and phenylalanine (PHE) are associated with typical roasted peanut flavor while threonine (THR), tyrosine (TYR), lysine (LYS) and an unidentified nitrogen-containing compound (which was referred to as a peptide) are precursors of atypical roasted peanut flavor (8). Oupadissakoon et al. (9) have studied the effect of geographical location and harvest time on the individual amino acids of peanuts. Location effects were significant for many of the precursors of typical roasted peanut flavor. At optimum harvesting time roasted flavor precursors were predominant among free amino acids, composing 64% of the total free amino acids. Other researchers have reported on the variation in free amino acids as influenced by cultivar, disease, irrigation, location, and maturation of peanut (1, 4, 5, 6, 10, 14, 16, 17). To date no work has been reported on the differences in individual amino acids related to seed sizes within the marketing grade system for virginia-type peanuts. However, the relationships between seed size and physiological maturity has recently been documented (11) and the differences in carbohydrates related to seed size determined.

Storage effects on amino acid composition seems to have received little attention in most fruits and vegetables. In a series of reports dealing with storage effects on proteins and non-protein nitrogen in sweet potatoes, Purcell and co-workers (12, 13, 15) showed percent protein to increase during the early part of the storage period and then decrease. Non-protein nitrogen, which contains the free amino acid fraction, generally showed the opposite effect. These changes were attributed to metabolic activity during storage. Molina and co-workers (7) stored black beans (*Phaseolus vulgaris*) under ambient conditions for up to 6 months. They showed methionine (MET) and available LYS contents to increase in the raw beans. However, storage, in general, had a detrimental effect on the protein qulity of processed beans. With storage of dry beans under selected temperature and relative humidity (R.H.) conditions the protein efficiency ratio was shown to drop markedly as temperature and R.H. increased (2). Biological availability of MET also dropped significantly as did cysteine (CYS) availability.

It is the objective of this study to determine the concentration of individual free amino acids of peanuts of various seed sizes and the changes that occur during storage.

Materials and Methods

Peanuts from the 1978 crop were obtained from a commercial sheller pregraded into U.S. No. 1, Medium, and Extra Large virginias. The peanuts were from North Carolina farms and considered to be the Florigiant cultivar. The peanuts were separated into four size categories with slotted screens: 5.95 mm (ride a 5.95 mm screen and pass a 6.35 mm screen), 7.14 mm (ride a 7.14 mm screen and pass a 7.54 mm screen), 7.94 mm (ride a 7.94 mm screen and pass a 8.34 mm screen), 8.73 mm (ride a 8.73 mm screen and pass a 9.13 mm screen). The 5.95, 7.14 and 7.94 mm seed sizes are the minimum seed size in each of the three market grades of virginia-type peanuts. In meeting seed count standards for marketing virginia-type peanuts it is often necessary to combine the next higher screen size peanuts into the grade. The 7.14 mm and 7.94 mm size seed allowed us to look at the largest seed size generally found in the U.S. No. 1 and Medium grades. Splits, as a seed fraction, were obtained by handpicking the U.S. No. 1 grade lot. All designated seed size fractions were also handpicked to insure only intact seeds in these fractions.

The peanuts were purchased with an 8% (wet basis) moisture content and had a final moisture content of 6.3% at time of storage. Approximately 4 weeks elapsed between purchase and initiation of storage.

Controlled environment storage rooms were used with conditions set at 4 C and 65% R.H. to produce characteristics similar to those used for commercial peanut storage. At pre-set times, designated samples, 50 g each, were withdrawn for analysis. There were three replications for all treatments.

Free amino acid extraction and analysis were carried out as described by Oupadissakoon et al. (10). A ground peanut sample was defatted and the residue extracted with a methanol:chloroform:water (60:25:15) solution. Individual aliquots of the supernant were taken for amino acid and sugar analysis. The analysis was conducted using a Durrum D-500 amino acid analyzer with the data acquisition accessory. Data analysis of variance was performed as described by the General Linear Models procedure (3).

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Results and Discussion

The role of free amino acids in the formation of roasted peanut flavor marks free amino acids for special consideration in studying quality changes related to the chemical composition of peanuts. Table 1 shows the effect of seed size and storage time on the content of 18 free amino acids in peanuts. These data were analyzed statistically and the variance results are reported in Table 2.

The most notable characteristics are the complete absence of ASN and GLN and the absence of the peptide. This phenomenon has been observed previously by Young and co-workers (16). They speculated that enzyme activity was probably responsible for the disappearance of ASN, GLN, and the peptide in peanuts.

Oupadissakoon et al. (9, 10) have determined individual free amino acid contents of peanuts which had been stored at -18 C immediately after harvest. Comparison of the Oupadissakoon data (10) to our data shows differences in ASP (34%), isoleucine (ILE) (42%), PHE (34)% and ammonia (NH_3) (57%). Eight other compounds, serine (SER), GLU, valine (VAL), TYR, HIS, LYS, ARG and total had an average difference of 5.6%. Since our conditions were more typical of commerical handling practices

Table 1. Means of free amino acids (µmoles/g) among seed size and storage time.[1].

Storage Time (Months)	UNK	ASP	THR	SER	GLU	PRO	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	NH_3	ARG	TOTAL
SEED SIZE - 5.95 mm (Rides 5.95 mm - Passes 6.35 mm)																			
0	0.78	2.52	3.65	1.82	11.24	1.95	5.54	0.18	1.16	0.13	0.47	0.42	0.70	0.61	1.79	0.64	1.40	9.51	44.51
3	0.93	3.00	3.74	2.25	12.29	2.13	5.43	0.17	1.26	0.18	0.52	0.49	0.74	0.76	2.16	0.77	2.00	12.60	51.43
5	0.83	2.36	3.13	1.99	9.88	1.66	3.66	0.18	1.06	0.08	0.48	0.40	0.68	0.67	1.86	0.67	1.33	8.47	39.35
6	0.68	2.05	3.05	2.01	10.04	1.63	3.79	0.18	0.93	0.03	0.38	0.32	0.63	0.61	1.48	0.65	3.30	9.32	41.07
7	1.26	2.96	4.59	3.17	17.88	2.29	5.72	0.25	1.61	0.20	0.66	0.52	0.96	0.94	2.52	1.20	1.46	7.39	55.56
8	1.11	2.72	3.82	2.31	12.94	2.06	4.40	0.24	1.24	0.12	0.55	0.46	0.78	0.81	2.46	0.89	1.33	11.84	50.06
9	1.38	2.87	4.43	2.66	15.73	1.93	5.26	0.26	1.52	0.15	0.58	0.53	0.86	0.99	2.43	0.94	1.31	9.43	53.27
Mean	1.00	2.64	3.77	2.32	12.86	1.95	4.83	0.21	1.26	0.12	0.52	0.45	0.76	0.77	2.10	0.82	1.73	9.79	47.89
SEED SIZE - 7.14 mm (Rides 7.14 mm - Passes 7.54 mm)																			
0	0.72	1.22	1.72	1.21	7.76	1.06	2.94	0.14	0.87	0.05	0.35	0.29	0.47	0.89	1.07	0.27	1.18	3.37	25.58
3	0.69	1.15	1.49	1.08	6.86	0.92	2.46	0.13	0.75	0.04	0.32	0.27	0.43	1.02	0.97	0.21	1.36	3.38	23.54
5	0.80	1.25	1.54	1.09	7.54	1.07	2.16	0.14	0.73	0.04	0.33	0.24	0.42	1.04	0.90	0.25	1.00	2.99	23.52
6	0.86	1.51	1.78	1.78	8.60	1.15	3.74	0.15	0.95	0.02	0.40	0.34	0.56	1.04	1.10	0.37	3.43	3.90	31.68
7	0.97	1.57	1.12	0.97	8.31	0.93	2.12	0.14	0.76	0.02	0.35	0.24	0.43	1.29	0.82	0.23	1.07	2.22	23.56
8	1.07	1.62	1.96	1.62	9.74	1.52	3.40	0.17	1.02	0.02	0.41	0.32	0.51	0.99	1.14	0.35	2.11	4.43	32.42
9	0.97	1.53	1.68	1.22	9.08	1.11	3.27	0.17	0.90	0.03	0.36	0.29	0.48	1.16	1.05	0.30	1.16	4.01	28.75
Mean	0.87	1.41	1.61	1.28	8.27	1.11	2.87	0.15	0.85	0.03	0.36	0.28	0.47	1.06	1.01	0.28	1.62	3.47	27.00
SEED SIZE - 7.94 mm (Rides 7.94 mm - Passed 8.34 mm)																			
0	0.66	1.06	1.40	0.76	6.96	0.62	1.66	0.11	0.63	0.02	0.26	0.20	0.34	1.11	0.65	0.13	1.02	1.52	19.10
3	0.62	0.88	1.47	0.87	7.45	0.77	2.44	0.13	0.77	0.04	0.31	0.28	0.43	1.58	0.97	0.17	1.21	1.81	22.17
5	0.59	0.72	1.05	0.92	7.80	0.77	2.30	0.15	0.81	0.02	0.33	0.28	0.47	1.21	0.95	0.20	1.28	1.94	21.80
6	0.75	0.96	0.93	0.69	7.28	0.67	2.23	0.15	0.63	0.02	0.27	0.21	0.39	1.11	0.73	0.15	2.19	1.81	21.19
7	1.03	1.36	1.98	1.59	10.70	0.97	3.37	0.18	1.06	0.03	0.41	0.33	0.57	1.21	1.47	0.26	2.48	1.96	30.96
8	0.91	1.08	1.68	1.04	9.63	0.82	3.11	0.16	0.85	0.02	0.33	0.27	0.48	1.51	0.86	0.24	1.66	2.26	26.91
9	0.88	1.17	1.53	1.10	8.74	0.78	2.42	0.17	0.87	0.03	0.36	0.30	0.49	1.41	0.96	0.24	1.28	2.38	25.11
Mean	0.78	1.03	1.44	1.00	8.36	0.77	2.50	0.15	0.80	0.02	0.26	0.26	0.45	1.30	0.94	0.20	1.59	1.96	23.89
SEED SIZE - 8.74 mm (Rides 8.74 mm - Passes 9.14 mm)																			
0	0.65	0.66	0.90	0.58	6.41	0.50	2.14	0.11	0.46	0.01	0.19	0.14	0.28	1.82	0.49	0.07	1.06	0.77	17.25
3	0.57	0.76	0.86	0.46	6.01	0.47	2.11	0.12	0.47	0.02	0.22	0.16	0.29	2.67	0.51	0.07	1.08	0.62	17.47
5	0.46	0.61	0.58	0.39	5.24	0.54	1.67	0.13	0.31	0.01	0.14	0.13	0.22	0.92	0.42	0.08	1.54	0.83	14.72
6	0.48	0.74	0.54	0.36	3.96	0.36	1.31	0.12	0.31	0.01	0.14	0.13	0.22	0.92	0.42	0.09	1.54	0.83	12.48
7	0.70	0.77	0.67	0.40	6.32	0.47	2.36	0.13	0.47	0.02	0.22	0.16	0.32	1.82	0.54	0.08	0.85	0.70	16.97
8	0.85	0.88	0.92	0.74	7.23	0.91	2.16	0.16	0.61	0.02	0.25	0.21	0.36	1.68	0.58	0.11	1.02	0.96	19.65
9	0.74	0.88	0.81	0.62	6.69	0.51	2.48	0.13	0.54	0.01	0.23	0.18	0.33	1.91	0.64	0.10	1.38	0.90	19.08
Mean	0.63	0.76	0.76	0.61	5.98	0.54	2.03	0.13	0.47	0.01	0.20	0.16	0.29	1.81	0.62	0.08	1.14	0.78	16.80
SPLIT SEED																			
0	0.73	1.11	1.08	0.59	6.07	0.90	1.19	0.10	0.47	0.02	0.23	0.17	0.30	2.03	0.63	0.10	1.16	1.34	18.21
3	0.81	1.11	1.26	0.53	6.28	0.78	1.94	0.10	0.44	0.01	0.21	0.15	0.30	1.69	0.56	0.12	1.16	1.44	18.89
5	0.52	0.83	0.80	0.43	5.46	0.53	1.85	0.13	0.39	0.01	0.19	0.13	0.30	1.39	0.50	0.09	0.80	1.22	15.59
6	0.63	0.69	0.62	0.44	5.92	0.45	1.63	0.13	0.46	0.01	0.20	0.15	0.25	1.79	0.52	0.09	2.16	0.80	16.94
7	0.93	1.21	0.95	0.52	7.05	0.60	2.72	0.14	0.52	0.01	0.29	0.18	0.34	1.91	0.61	0.12	1.67	1.51	21.30
8	1.00	1.24	0.86	0.47	7.19	0.81	2.55	0.16	0.55	0.01	0.25	0.19	0.35	2.55	0.65	0.10	1.73	1.09	21.74
9	.74	1.29	0.96	0.47	6.27	0.73	2.24	0.13	0.47	0.02	0.24	0.16	0.31	2.15	0.59	0.10	0.92	1.08	18.88
Mean	0.77	1.07	0.93	0.49	6.32	0.69	2.02	0.12	0.47	0.01	0.23	0.16	0.31	1.93	0.58	1.10	1.37	1.21	18.79
MEANS - TIME																			
0	0.71	1.31	1.75	0.99	7.69	1.01	2.69	0.13	0.72	0.04	0.30	0.24	0.42	1.29	0.92	0.24	1.16	3.30	24.93
3	0.72	1.38	1.76	1.04	7.78	1.01	2.88	0.13	0.74	0.06	0.32	0.27	0.44	1.54	1.03	0.27	1.36	3.97	26.70
5	0.64	1.15	1.42	0.96	7.18	0.91	2.33	0.14	0.69	0.03	0.30	0.23	0.43	1.23	0.94	0.25	1.08	3.06	23.00
6	0.68	1.19	1.38	1.06	7.16	0.85	2.54	0.15	0.66	0.02	0.28	0.23	0.41	1.09	0.85	0.27	2.52	3.33	24.67
7	0.98	1.58	1.86	1.33	10.06	1.05	3.26	0.17	0.88	0.06	0.38	0.28	0.52	1.43	1.19	0.38	1.50	2.75	29.67
8	0.99	1.51	1.85	1.23	9.34	1.22	3.12	0.18	0.85	0.04	0.36	0.29	0.49	1.51	1.14	0.34	1.57	4.11	30.16
9	0.94	1.54	1.88	1.21	9.30	1.01	3.13	0.17	0.86	0.05	0.35	0.29	0.49	1.52	1.13	0.34	1.21	3.56	29.02

[1] Means of three replications.

Table 2. Mean squares and components of variance from the analysis of variance of free amino acids in peanuts.

Source	d.f.	UNK	ASP	THR	SER	GLU	PRO	ALA	CYS	VAL	MET
Size	4	0.38**	1.54**	30.71**	11.78**	157.75**	6.72**	28.38**	0.02**	2.22**	0.05**
Linear	1	1.35**	45.84**	118.19**	46.22**	582.00**	26.80**	109.61**	0.08**	7.75**	0.18**
Quad.	1	0.12**	0.22	1.77**	0.00	2.06	0.01	1.62	0.00	0.06	0.01**
Dev.	2	0.02	0.06	1.44	1.25	23.46	0.03	1.15	0.01	0.53	0.00
Time	6	0.36**	0.43**	0.67**	0.30**	20.85**	0.20**	1.79**	0.01**	0.13**	0.00**
Linear	1	1.00**	0.64**	0.17	0.93**	46.89**	0.09	2.67*	0.03**	0.27**	0.00
Quad.	1	0.40**	0.62**	1.59**	0.11	19.76**	0.20*	2.03*	0.00	0.14*	0.00
Dev.	4	0.18	0.33	0.56	0.19	14.61	0.23	1.51	0.00	0.09	0.01
Size x Time	24	0.03**	0.09**	0.29**	0.24**	4.75**	0.07**	1.00**	0.00**	0.05**	0.00**
Lin. x Lin.	1	0.16*	0.00	0.79	0.78**	16.73*	0.00	2.47*	0.00*	0.04	0.00
Dev.	23	0.03	0.09	0.27	0.22	4.23	0.08	0193	0.00	0.05	0.00
Residual	70	0.021	0.063	0.159	0.046	1.388	0.020	·0.237	0.001	0.013	0.001

Source	d.f.	ILE	LEU	TYR	PHE	HIS	LYS	NH_3	ARG	TOTAL
Size	4	0.33**	0.30**	0.75**	5.06**	8.47**	1.91**	1.17**	286.79**	3241.67**
Linear	1	1.21**	1.07**	2.79**	14.78**	32.21**	7.38**	2.91**	1125.47**	12633.63**
Quad.	1	0.01	0.00	0.00	1.18**	0.30*	0.16**	1.07	16.97**	83.34
Dev.	2	0.04	0.06	0.11	2.13	0.69	0.04	0.35	2.36	129.86
Time	6	0.22**	0.01**	0.03**	0.45**	0.25**	0.04**	0.35**	2.36**	703.23**
Linear	1	0.05**	0.02*	0.09**	0.22	0.51*	0.14**	1.14	0.10	264.10**
Quad.	1	0.01	0.01	0.01	0.32	0.12	0.01	2.86**	0.64	105.29*
Dev.	4	0.02	0.01	0.02	0.54	0.21	0.04	4.41	5.04	83.46
Size x Time	24	0.01**	0.01**	0.01**	0.24**	0.12**	0.03**	0.54**	2.15**	32.25**
Lin. x Lin.	1	0.00	0.00	0.01	0.22	0.33*	0.14**	0.29	1.25	23.55
Dev.	23	0.01	0.01	0.01	0.24	0.11	0.02	0.55	2.19	32.63
Residual	70	0.002	0.002	0.004	0.004	0.040	0.005	0.068	0.853	11.759

* Significant at the 0.05 level.
** Significant at the 0.01 level.

where delay between shelling and cold storage usually occurs, our results probably represent the more typical changes that occur in commercial peanuts.

Seed Size

Increase in seed size resulted in a significant decrease in the concentration of the individual free amino acid fractions except for PHE which significantly increases with seed size (Tables 1 and 2). Using seed number per kg (Table 3) as a variable in regression analysis positive regression was observed for all fractions except PHE which showed a negative regression. Only the unknown (UNK), THR, MET, PHE, HIS, LYS and ARG had significant quadratic effects (Table 2). The effects of seed size on the atypical roasted flavor substrates THR, LYS, and ARG are highly significant and demonstrate a quadratic effect probably resulting from their high concentrations in the 5.95 mm seed size. These high concentrations could contribute to a potential poor quality in this seed size.

The highly significant linear effect in all free amino acid fractions suggests that it may be possible to detect roasted flavor quality differences between each seed size. The ability to associate flavor quality with chemical composition would be a major advancement in quality control for peanuts.

Storage Time

Although significant linear and/or quadratic changes in free amino acid concentration occurred in 15 of the 18 individual amino acid fractions over storage time, they are smaller than the seed size effects. With the UNK, ASP, GLU, alanine (ALA), VAL, significant linear and quadratic changes with time were observed (Table 2). It may be important to peanut quality during storage, even within the same seed size, that the linear-quadratic effects predominate for amino acids associated with typical roasted peanut flavor (8) rather than for the atypical components. These results are the first to document a change in amino acids with storage time.

Table 3. Relationship between seed size and seed count per Kg in commercially obtained 1978 crop peanuts.

Seed Size	Seed Count Kg^{-1}
5.95	2547[a/]
7.14	1663
7.94	1288
8.73	1028
Splits	1268

a/ Ave. of five samples.

Seed Size by Storage Time

Measurement of the seed size-storage time interaction indicated that the rate of change with storage time is not related to seed size. This is confirmed by the general lack of significance in the linear by linear trends (Table 2). Thus the changes that are occurring during storage appear to be independent of maturity factors even though maturity has a highly significant effect on the concentration of the free amino acids present (4).

The trends observed in changes of free amino acid concentration with seed size and storage time will be the basis of additional studies to relate these trends to changes in the roasted flavor quality of peanuts.

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