Some Environmental Factors Affecting Free Amino Acid Composition in Six Varieties of Peanuts[1,2]

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ABSTRACT

This study involved six Spanish type entries (five commercial varieties and a plant introduction) grown in the National Variety Tests in Oklahoma and Georgia under both irrigated and nonirrigated conditions. Significant effects (Georgia vs Oklahoma) were observed on aspartic acid, proline, glycine, valine, isoleucine, peptide, ammonia, and histidine. Significant differences for irrigated vs nonirrigated in the two states for aspartic acid, threonine, proline, glutamic acid, isoleucine, leucine, tyrosine, phenylalanine, peptide, ammonia, and histidine were observed. Significant differences among the six entries were observed for glutamic acid, leucine, tyrosine, phenylalanine, ammonia, histidine, arginine, tryptophan, and total amino acids. None of the treatments produced significant changes in measured amounts of serine, alanine, methionine, and lysine. Significant differences for Georgia vs Oklahoma and irrigated vs nonirrigated for Kjeldahl nitrogen of the whole peanut were noted.

INTRODUCTION

There is considerable evidence that the unique nutty flavor of roasted peanuts results largely from the reactions of glucose and fructose (formed from the breakdown of sucrose) with free amino acids (1-3). The majority of the free amino acids are believed to be released from a large peptide during the roasting operation (2).

In this study, an effort was made to measure statistically some of the effects of genotype and environment upon the free amino acid concentration of raw Spanish type peanuts, to provide a better understanding of the conditions necessary to produce a peanut with good roasted flavor.

EXPERIMENTAL PROCEDURES

This study involved six Spanish type entries (five commercial varieties and a plant introduction) grown in the National Variety Tests in Oklahoma and Georgia in 1968. Mature, sound, machine shelled peanuts were used for analyses. In Oklahoma, the nonirrigated (NIR) peanut samples were grown at the research station near Perkins on a Taller loam soil and received 21.99 in. of rainfall (5/1-11/30). The irrigated (IRR) peanut samples were produced on the research station near Fort Cobb on a sandy loam soil and received 28.10 in. of rainfall (5/1-11/30), plus 10 in. of irrigation water in 5 equal applications. The samples from Georgia were grown at Tifton on a Tifton loam soil. The NIR peanuts received 16.12 in. of rainfall (5/2-8/19), and the IRR peanuts received 15.41 in. of rainfall (5/2-8/19), plus 4.25 in. of irrigation water applied in 4 applications. The shelled peanut samples were received in late fall and stored at 34 F and 60% relative humidity until analyzed. Nitrogen was determined by macro-Kjeldahl analysis on peanut samples ground in a Laboratory Wiley Mill using a 10 mesh screen.

A 10 g ± 1 mg ground peanut sample was extracted with hexane to obtain oil for fatty acid analysis (which are reported in the sequent publication) and with a methanol: chloroform:water mixture (60:25:15; v:v:v), as described in a previous publication (4). The residue then was discarded. The combined filtrate was evaporated to near dryness with a rotary evaporator at 45 C, diluted to 25 ml with water, and centrifuged; and a portion of the clear liquid (between the fatty layer and residue) was diluted with an equal volume of pH 2.2 citrate buffer and stored at -20 C until analyzed.

The modified, accelerated physiological procedures, as previously described (4), were used, except an unknown amount of Aminex A-5 resin (Bio-Rad Laboratories, Richmond, Calif.) had been added to the PA-28 column (Beckman Instruments, Palo Alto, Calif.). Separation, although acceptable, was not as complete as shown previously.

RESULTS AND DISCUSSION

Table I shows the free amino acid contents of peanuts from the 1968 National Variety Test. These data were analyzed statistically (5) and the variance results recorded in Table II along with the coefficients of variation (CV). CV(a) is a measure of between plot variation, and CV(b) is within plot variation. These shelled peanuts from the 1968 National Variety Test were stored at 34 F and 60% relative humidity until July 1969 at which time they were extracted for the free amino acids. This storage system was similar to that used by some commercial peanut storage companies.

The most notable characteristics were the complete absence of asparagine and glutamine, absence of most of the peptide (ca. a 75% reduction), and an increase in the ammonia content (ca. a sixfold increase), when results were compared to those previously reported (1,2,4). Earlier work by Young and Holley (6) showed increasing amounts of ammonia in the peanut volatiles of roasted peanuts after the peanuts were shelled and stored at 42 F, but they did not speculate on the source of the ammonia. Based upon these results and those of Young and Holley, it appeared that most of this ammonia probably came from the breakdown of asparagine and glutamine. Mason and Matlock (7) examined the amino acid content of aleurone grains stored at 70 F and found that with 0-6 months of storage, the asparagine and glutamine contents did not change significantly. However, since the peanuts used in the present study were still viable, the asparagine and glutamine probably were metabolized. Prentice, et al., (8) and Burger, et al., (9) demonstrated peptide hydrolyses in wheat and barley, respectively. Enzymes with similar activity are thought to be responsible for the disappearance of asparagine, glutamine, and the peptide in peanuts. Further research is needed to explain these changes completely.

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[2]Journal paper 2636 of the Oklahoma Agricultural Experiment Station.

[3]ARS, USDA.

TABLE I

Environmental Effects (Variety, Planting Location, and Soil Moisture Condition) upon Free Amino Acid Composition of Peanuts[a]

State	Treatment	Aspartic acid	Threonine	Proline	Glutamic acid	Glycine	Valine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Peptide	NH_3	Histidine	Arginine	Tryptophan	Total[b]
										μM/gm							
										Argentine							
OK	NIR	1.07	.32	.94	5.35	.39	.61	.32	.37	.45	1.24	.48	1.55	.31	.47	.40	16.10
GA	NIR	1.58	.35	.75	6.47	.42	.60	.33	.38	.49	1.74	.18	1.27	.31	.47	.40	17.48
OK	IRR	1.36	.35	.68	5.06	.40	.53	.34	.39	.43	1.87	.29	2.54	.34	.56	.42	18.00
GA	IRR	0.51	.32	.41	5.65	.51	.81	.35	.36	.41	3.33	.19	1.14	.34	.39	.36	16.87
										Tifspan							
OK	NIR	0.78	.33	.88	5.21	.40	.56	.32	.38	.48	1.72	.37	1.83	.32	.50	.45	16.38
GA	NIR	1.18	.29	.64	6.65	.50	.70	.30	.34	.38	1.04	.18	0.86	.30	.47	.36	15.98
OK	IRR	2.58	.36	.85	5.80	.37	.55	.34	.39	.43	2.24	.26	2.16	.35	.62	.42	19.55
GA	IRR	1.05	.34	.46	5.60	.51	.67	.39	.38	.48	2.79	.17	1.30	.34	.48	.42	16.95
										Spantex							
OK	NIR	1.57	.32	.95	6.10	.39	.50	.33	.38	.49	2.70	.30	1.62	.32	.54	.42	18.48
GA	NIR	0.77	.33	.53	6.85	.52	.70	.35	.37	.45	1.54	.19	1.09	.38	.65	.43	17.25
OK	IRR	1.94	.33	.61	5.45	.39	.54	.35	.40	.45	2.15	.19	1.70	.33	.54	.43	17.50
GA	IRR	1.12	.36	.50	5.37	.53	.61	.42	.42	.57	4.85	.17	1.58	.48	.87	.42	20.15
										Starr							
OK	NIR	1.60	.34	1.11	6.01	.40	.66	.33	.38	.47	2.25	.24	1.67	.32	.56	.42	18.21
GA	NIR	0.86	.31	.49	6.44	.52	.50	.34	.38	.55	2.25	.19	1.31	.42	.78	.47	17.68
OK	IRR	2.49	.37	.59	5.94	.40	.58	.35	.39	.44	2.18	.20	2.48	.33	.59	.42	19.54
GA	IRR	0.86	.34	.45	6.25	.51	.69	.41	.40	.50	4.21	.19	1.52	.41	.65	.44	19.71
										Spancross							
OK	NIR	1.51	.33	1.14	9.80	.43	.54	.35	.38	.65	4.51	.25	2.65	.33	.59	.53	25.85
GA	NIR	0.60	.35	.60	7.81	.57	.54	.36	.41	.61	2.93	.21	2.49	.33	.52	.64	21.66
OK	IRR	2.50	.37	.71	8.62	.44	.61	.38	.43	.53	4.17	.24	5.60	.38	.85	.54	29.32
GA	IRR	0.80	.34	.44	6.35	.52	.60	.41	.41	.58	4.54	.20	1.80	.37	.50	.47	20.33
										PI 268684							
OK	NIR	1.70	.33	1.06	9.65	.43	.49	.34	.39	.65	4.73	.26	3.66	.33	.51	.52	26.99
GA	NIR	1.06	.32	.51	8.77	.57	.49	.33	.38	.61	3.61	.22	2.16	.33	.45	.58	22.61
OK	IRR	3.22	.36	.70	7.57	.41	.57	.35	.40	.45	3.15	.21	4.21	.36	.73	.44	25.11
GA	IRR	1.03	.35	.48	6.62	.54	.58	.40	.40	.53	4.32	.18	2.56	.38	.59	.45	21.71

[a]NIR = nonirrigated; IRR = irrigated; OK = Oklahoma, and GA = Georgia.

[b]Includes serine, alanine, methionine, and lysine.

TABLE II

Summary of Analysis of Variance on Pooled Data of Free Amino Acid Composition and Protein Content of Peanuts[a]

Amino acid	Ga vs Ok (s)	IRR vs NIR (L)	Variety (E)	SxL	SxE	LxE[b]	SxLxE[b]	CV(a) %	CV(b) %
Nirtogen	*	**	NS	*	NS			2.4	2.5
Aspartic acid	**	*	NS	**	NS			29.5	37.2
Threonine	NS	**	NS	NS	NS			4.2	6.6
Serine	NS	NS	NS	NS	NS			35.4	22.5
Proline	**	**	NS	NS	NS			19.8	21.3
Glutamic acid	NS	*	**	NS	NS			16.1	18.2
Glycine	**	NS	NS	NS	NS			3.9	9.6
Alanine	NS	NS	NS	NS	NS			62.3	47.4
Valine	*	NS	NS	NS	NS			13.8	16.6
Methionine	NS	NS	NS	NS	NS			5.3	4.3
Isoleucine	**	**	NS	*	NS			5.5	8.8
Leucine	NS	**	*	NS	NS			2.2	5.1
Tyrosine	NS	*	**	*	NS			8.5	11.8
Phenylalanine	NS	*	**	**	NS			24.9	31.1
Peptide	**	*	NS	NS	NS			23.0	24.4
NH_3	**	*	**	NS	NS			27.3	31.5
Lysine	NS	NS	NS	NS	NS			12.9	14.6
Histidine	**	**	**	NS	**			5.0	7.5
Arginine	NS	NS	*	NS	**			20.2	19.2
Tryptophan	NS	NS	**	NS	NS			9.5	17.2
Total	NS	NS	**	NS	NS			11.8	14.7

[a]NS = Not significant, * = 5% level, ** = 1% level, Ga = Georgia, Ok = Oklahoma, CV(a) = measure of between plot variation, CV(b) = within plot variation.

[b]Entire column not significant.

Since peanuts are indeterminate in their growth habit, the peanuts in this study, although defined as mature by commercial terms, would have some degree of immaturity, because the fruits were not examined and separated as done in the previous study on maturity (4). This probably contributes to the fact that ca. one-half of the coefficients of variation are above 10% (Table II). Also Aminex A-5 resin had been added to the column containing the PA-28 resin to maintain its length which resulted in poorer, but acceptable, resolution of amino acids from the extracts of the peanuts when compared to the chromatograms for a related study (4,10). The variances (Table II) for the following were significantly different between the samples grown in Georgia vs Oklahoma: aspartic acid, proline, glycine, valine, isoleucine, peptide, ammonia, and histidine. The variances (Table II) for the free amino acids were significantly different for the IRR vs NIR tests in the two states for aspartic acid, threonine, proline, glutamic acid, isoleucine, leucine, tyrosine, phenylalanine, peptide, ammonia, and histidine. The variances for the other free amino acids did not differ significantly. It should be noted, however, that the wider differences between IRR and NIR observed in Oklahoma may have been related, in part, to the fact that the IRR plots were ca. 150 miles from the NIR plots. Further study is needed to delineate these differences. In Georgia, these plots were located on the same farm. The variances for the following were significantly different among the six entries in the four tests: glutamic acid, leucine, tyrosine, phenylalanine, ammonia, histidine, arginine, tryptophan, and total amino acids. The variances between entries for the other free amino acids were not significantly different. The dry matter content, ammonia and histidine were the only items that had significant variances for state, irrigation, and entry (variety). Only the varieties effect was observed for the total of all free amino acids measured. Significant differences for Georgia vs Oklahoma and IRR vs NIR for Kjeldahl nitrogen of the whole peanut were noted.

No significant differences were found among the three variables examined nor their interactions for serine, alanine, methionine, and lysine. Thus, the data on these four amino acids have been deleted from Table I.

Much of the within plot variation (CV(b) in Table II) is most likely due to lack of precision of the extraction method. Since several amino acids show large CVs (such as alanine), it is quite possible that this lack of precision has obscured significant differences that otherwise might have been detected. In future studies, improved precision and accuracy might be accomplished by measuring degree of immaturity, by further refinement of the extraction method, i.e. more uniform cell disruption, and by improved amino acid analyzer analytical procedures.

Assuming glutamic acid to be the predominant amino acid flavor precursor, the effect of variety was most significant followed by irrigation, which was also significant. Growth of peanuts in Georgia or Oklahoma had no significant effect upon the glutamic acid content of the varieties of peanuts tested. If the peptide postulated by Mason, et al., (2,11) is the predominant amino acid precursor, then growth location would be most important (highly significant) contributor to the roasted flavor. Arginine, which is high in immature peanuts (2,12) has been proposed to be related to maturity (2,11,13) which appears to be associated with poor or off-flavors of roasted peanuts. In this study, the variety effect was significant, whereas the location and irrigation effects were not significant. A similar examination could be made for each of the amino acids, and, ultimately, a more complete evaluation might be based upon a selected combination of several of the amino acids. The further study of arginine in a model system, as used by Newell, et al., (1) and Koehler and Odell (3), would provide a better understanding of the possible role of arginine in off-flavor immature peanuts.

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