Effect of Harvest Date and Maturity upon Free Amino Acid Levels in Three Varieties of Peanuts[1,2]

C.T. YOUNG, Department of Food Science, University of Georgia, Georgia Station, Experiment, Georgia 30212, and **R.S. MATLOCK, M.E. MASON**[3], and **G.R. WALLER**, Departments of Agronomy and Biochemistry, Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74074

ABSTRACT

An improved method for the extraction of free amino acids and a peptide of unknown composition with a methanol:chloroform:water mixture (60:25:15; v:v:v) was described. The largest variations were related to maturity. If the amino acids were ranked in descending order, glutamic acid, followed by asparagine (including asparagine, glutamine, threonine, and serine), topped the list in the mature and low intermediate groups, except in Valencia at 141 days. The peptide and phenylalanine were usually in the top six, while aspartic acid occurred there fairly often. Arginine was the highest in immature peanuts. Proline was found in higher concentrations in immature peanuts than previously reported. The presence of two nonprotein amino acids (γ-methyleneglutamine and γ-methyleneglutamic acid) in mature kernels was confirmed.

INTRODUCTION

Recent research (1-3) has indicated that the unique nutty flavor of roasted peanuts results largely from the reactions of glucose and fructose (from hydrolysis of sucrose) with free amino acids. The majority of these amino acids were believed to be released from a large peptide during the roasting operation (2). Only very limited information (1, 2) is available on the factors affecting the quantitation of these flavor precursors in peanuts.

One of the problems in such studies has been to find a simple and reproducible method for extracting maximum quantities of both peptide and free amino acids. A perchloric acid extraction was used by Newell (1) and Mason, et al, (2) in some of the earlier research on peanut flavor. A milder and simpler extraction with methanol, chloroform, and water mixture (MCW) had been reported, however, as excellent for obtaining free amino acids from plant material (4). Although not complete, methanol alone was found by the senior author to be suitable for the extraction of the flavor precursors of peanuts. Some further investigation of methods and selection of an efficient procedure was, therefore, considered prerequisite to the main objective in the present study. This objective was to measure some of the environmental and genotypic effects upon the peptide and free amino acid contents of raw peanuts to provide basic information relating to the conditions necessary to produce and maintain the peanut flavor precursors which are thought to be related to good roasted flavor.

EXPERIMENTAL PROCEDURES

Various solvents and procedures, as listed in Table I, were examined for extraction of free amino acids and peptide, and Table II lists the μmoles of each amino acid recovered from an equivalent amount of a standard peanut sample of Spanhoma variety (P-112, 1967, Ft. Cobb, Okla.). Based upon these results, the method described below as adopted for the main portion of the study.

A 10 gm ± 1 mg peanut sample of known moisture content was ground thoroughly (ca. 20-30 sec) using a Sorval Omni-Mixer equipped with a 250 ml stainless steel container, at a power-stat setting of 80 V. A 100 ml portion of hexane was added with mixing, and the slurry was filtered with suction using a coarse sintered glass disk. The

[1]Presented at the AOCS Spring Meeting, New Orleans, May 1973.

[2]Journal article 2635 of the Oklahoma Agricultural Experiment Station.

[3]Present address: International Flavors and Fragrances, Union Beach, New Jersey.

TABLE I

Extraction Methods for Free Amino Acids and Peptide

Method	Description of procedure
1.	Perchloric acid method of Newell (1). Samples (10 g) were homogenized cold at high speed in 100 ml 3N $HC1O_4$ for 9 min, filtered, pH adjusted to 8.0 with 2N KOH, and refiltered to remove precipitate.
2.	Peanuts (10 g) extracted with 100 ml 95% ethanol, filtered, evaporated to dryness, taken up in 10 ml pH 2.2 citrate buffer, and filtered for analysis.
3.	Peanuts (10 g) extracted with 100 ml 95% ethanol, filtered, evaporated to dryness, extracted with ether, and taken up in 5 ml 2.2 citrate buffer.
4.	Same as 2, except methanol is used in the extraction.
5.	Same as 2, except using 70% aqueous methanol.
6.	Peanuts (10 g) extracted with 100 ml hexane, extracted 3 min wtih 100 ml methanol chloroform, and water mixture (MCW) in Sorval Omni-Mixer, filtered, repeated, evaporated, and lyophilized to dryness, taken up in 20 ml 2.2 buffer, filtered, and analyzed.
7.	Same as 6, except after the MCW extraction, two extractions for 3 min with 100 ml 80% aqueous ethanol were added.
8.	Same as 6, except used 250 ml MCW (one extraction).
9.	Peanuts (10 g) were ether extracted and then extracted once with 250 ml MCW.
10.	Same as 9, except no ether extraction.

TABLE II

Recovery of Amino Acid and Peptide from Spanhoma Peanuts by Various Extraction Procedures

Amino acid	Procedure									
	1	2	3	4	5	6	7	8	9	10
					μm/g					
Aspartic acid	.317	.058	.059	.206	.326	.346	.570		.400	.365
Threonine	.084	.019	.014	.017	.109	.198	.248		trace	nil
Serine	.228	.044	.053	.073	.169	.198	.278		.158	.165
Asparagine	.474	.106	.104	.147	.448	.542	.490		.238	.255
Proline	.324	.104	.028	.084	.400	.494	.358		.302	.083
Glutamic acid	1.521	.331	.248	.073	1.735	1.336	2.582	1.980	2.335	1.770
Glycine	.101	.015	.009	.063	.090	.146	.288		.115	.135
Alanine	.243	.069	.041	.099	.310	.264	.598		.300	.287
Valine	.114	.029	.008	.023	.155	.162	.264		.110	.087
Cysteine	nil	slight trace	nil	nil	.010	nil	nil		trace	nil
Methionine		slight trace	nil	trace		.022	.052		.025	trace
Isoleucine	.047	.009	.016	.009	.064	.090	.136	.057	.055	.055
Leucine	.045	.008	.015	.006	.048	.090	.172		.048	.040
Phenylalanine	.436	.031	.016	.039	.344	.494	.620	.394	.435	.367
Tyrosine	.062	.006	.004	.007	.037	.044	.076		.043	.032
Unknown-1	.024	.049	.779	.028	.011	.146	.176			
Unknown-1a					.025	.024	.054			
Unknown-2	.028	.076	.066	.235	.296	.072	.074			
Unknown-3	.078	.015	trace	trace	.031	.032	.038			
Unknown-2a						.118	.124			
Peptide	.238	.086	.056	.151	.111	.424	.432	.307	.365	.350

residue was washed twice with hexane dispensed from a wash bottle. The hexane was evaporated from this extract in a rotary evaporator and a portion of the oil was reserved for fatty acid analysis. The residue from the disk was returned to the same container, and, using the same power-stat settings, was extracted further by blending for 3 min with 150 ml MCW (60:25:15; v:v:v), filtered on the same disk, and washed once with a small amount of MCW. Extraction then was repeated by blending 1 min with 100 ml MCW, filtering, and washing twice with MCW, after which the residue was discarded.

The combined filtrate was evaporated to near dryness with a rotary evaporator at 45 C and lyophilized to dryness. The dried extract was taken up in 20 ml pH 2.2 citrate buffer, which then was centrifuged, decanted, and stored at -20 C until analyzed. Amino acid analyses were made using the ion-exchange column chromatographic technique of Spackman, et al., (5) in a Beckman model 120-C amino acid analyzer.

The standard Beckman physiological procedure for acidic amino acids and neutral amino acids was modified to accelerate the analyses, which were performed at 56 C with no change in temperature. A 0.9 x 56 cm column containing the PA-28 resin was used. Buffer A was at pH 3.250 and required accurate measurement, and the timer setting to start B buffer (pH 4.26) was adjusted accordingly. These steps were necessary to separate the peptide from the other amino acids. The buffer change occurred at ca. 120-125 min, with the total run requiring ca. 260 min.

The analysis of the basic amino acids on the short PA-35 column (0.9 x 13 cm) was found to be adequate. A pH 5.28 buffer was used, the run requiring only ca. 1 hr, instead of the recommended physiological run of 6-¼ hr. The time thus saved on each sample resulted in only a slight loss of information on the free amino acid content of peanuts. Standards were analyzed to obtain the appropriate color constants for these operating conditions on this analyzer. An average color constant was used for the peptide and unknowns as recommended by the analyzer operation instructions.

The samples used in this study were Argentine, Spanhoma, and Valencia peanuts obtained from a harvesting date test at Perkins, Okla., similar to those reported for a recent investigation of fatty acid variation by Young, et al. (6). As inspected and separated for stages of maturity at each date of harvest, mature peanuts were classified as those with a dark colored interior pericarp surface or some white on interior pericarp and a thin pink or very thin faded pink colored testa (skin). Low intermediate peanuts were those with considerable white on interior pericarp, testa not completely collapsed, and a slight wrinkling of skin. The immature peanuts had a white pericarp, thick fleshy white-pink testa, and undersized, shriveled kernels. This method was designed to establish three discrete levels of maturity. An earlier study (7), having shown that mature and high intermediate peanuts were similar in maturity based upon the arginine and fatty acid contents, these two groups were combined as mature peanuts to provide fewer samples for analysis.

The harvesting test was set up as a randomized block experiment with several replications, but, due to varying proportions of available pods within the three maturity classifications at succeeding dates of harvest. it was necessary to combine peanuts from all reps to obtain sufficient amounts of samples for the investigations being conducted. Thus, only single determinations were made in the maturity and variety portion of the amino acid studies.

TABLE III

Columns and Chromatographic Conditions Used for Analysis of Free Amino Acids

Component	Acidic and neutral amino acids	Basic amino acids
Column	0.9 x 56 cm	0.9 x 13 cm
Resin	PA-28	PA-35
Buffer flow	50 ml/hr	68 ml/hr
Ninhydrin flow	25 ml/hr	25 ml/hr
Sample applied	0.3 ml	0.3 ml
Column temperature	56 C	56 C
pH buffer A	3.250	5.28
pH buffer B	4.26	---
Buffer change	See text	---

RESULTS AND DISCUSSION

Methods of Extraction

Details of the procedures which were compared for

TABLE IV

Effect of Maturity and Harvest Date upon Free Amino Acid Composition of Peanuts

Cultivar: Argentine-(Okla. P-No. 0002, Entry No. 01)

Amino acid	Maturity	Harvest date and no. of days 9/10 113	9/24 127	10/8 141	10/22 155	11/5 169
		μm/gm				
Aspartic acid	Mature	.69	.90	1.16	1.19	1.97
	Low Int.[a]	2.18	1.63	.83	1.16	1.63
	Immature	3.34	3.97	1.32	.11	.17
Asparagine[b]	Mature	3.34	2.62	2.74	2.23	2.81
	Low Int.	6.51	5.54	6.34	3.68	4.41
	Immature	19.72	16.55	19.59	27.07	21.42
Proline	Mature	1.11	2.15	1.31	.89	.90
	Low Int.	4.32	2.57	1.28	.95	1.23
	Immature	20.83	5.79	4.29	14.56	15.15
Glutamic acid	Mature	4.95	6.45	7.33	6.81	7.02
	Low Int.	7.82	8.14	9.53	6.48	8.44
	Immature	11.52	10.73	13.47	14.56	16.86
Glycine	Mature	.38	.32	.45	.17	.18
	Low Int.	.67	.56	1.13	.29	.32
	Immature	1.24	.91	2.56	1.02	1.71
Alanine	Mature	1.14	1.02	1.43	.54	.68
	Low Int.	2.55	2.22	6.23	.78	.97
	Immature	5.07	3.59	10.92	4.07	7.14
Valine	Mature	.55	.42	1.43	.28	.39
	Low Int.	.81	.67	1.44	.36	.44
	Immature	1.94	.78	2.33	1.69	1.46
Methionine	Mature	.03	.03	.05	.01	.06
	Low Int.	.06	.08	.07	.07	.06
	Immature	.10	.13	.17	.14	.12
Isoleucine	Mature	.17	.18	.36	.23	.17
	Low Int.	.26	.22	.66	.17	.16
	Immature	.70	.24	1.05	.68	.43
Leucine	Mature	.15	.13	.24	.12	.13
	Low Int.	.23	.21	.50	.17	.14
	Immature	.51	.25	.72	.53	.39
Tyrosine	Mature	.08	.08	.17	.11	.10
	Low Int.	.10	.09	.48	.11	.10
	Immature	.24	.14	.70	.35	.25
Phenylalanine	Mature	.51	1.07	1.43	1.43	1.32
	Low Int.	.35	.40	2.44	1.37	1.55
	Immature	1.30	.41	3.53	3.42	1.37
Peptide	Mature	.63	1.63	2.40	1.79	1.47
	Low Int.	.30	.68	1.03	1.37	1.03
	Immature	.34	.66	.94	1.64	.34
Ammonia	Mature	.43	.33	.40	.28	.43
	Low Int.	.95	.77	1.23	.49	1.20
	Immature	2.91	2.08	2.42	2.45	3.11
Lysine	Mature	.08	.09	.10	.08	.09
	Low Int.	.33	.30	.49	.16	.18
	Immature	1.84	1.48	2.14	2.50	3.20
Histidine	Mature	.17	.19	.28	.19	.20
	Low Int.	.53	.43	1.05	.35	.36
	Immature	1.99	1.14	2.12	3.54	3.34
Arginine	Mature	.60	.69	.54	.43	.52
	Low Int.	3.62	2.90	3.00	1.10	1.27
	Immature	25.52	17.93	21.44	33.28	38.31
Tryptophan	Mature	.05	.06	.14	.09	.07
	Low Int.	.07	.07	.37	.30	.15
	Immature	.35	.08	.54	.61	.30

[a]Low Int. = low intermediate group.

[b]A combination of asparagine, glutamine, threonine, and serine.

relative efficiency are given in Table I, with corresponding data for recovery of free amino acid and peptide in Table II. Methanol and 95% ethanol (methods 2, 3, and 4), as extracting solvents, did not remove enough of the free amino acids, although repeated extractions might have improved the results. In method 5, 70% aqueous methanol was used, but the method was rejected because of poor peptide extraction. The method using MCW (method 6) extracted about the same amount of free amino acids as the perchloric acid extraction, with nearly twice as much peptide. Because of considerable interest in the peptide as a major precursor of roasted peanut flavor (1-3), the MCW method, thus, 'assumes importance. A more complete extaction was obtained by method 7, which apparently afforded recovery of all free amino acids (8), but several time consuming steps employing additional extractions were necessary. Also, there was no significant increase in the extraction of the peptide. Several simplications were tired in methods 8, 9, and 10, but poorer extraction, particularly of the peptide, made them less desirable. Thus, method 6 was selected for these studies as giving better results than the accepted perchloric acid extraction, though obviously not the ultimate method.

A comparison of results in Table II with those published by Newell (1) will show that the MCW method consistently extracted twice as many μmoles of each amino acid, except for methionine. The obtaining of higher values by method 1, than those reported by Newell, was possibly due to variety and storage conditions of the raw peanuts before analysis. Calculations of the 440/570 ratios were not made

TABLE V

Effect of Harvest Date upon Free Amino Acid Composition of Mature Spanhoma and Valencia Peanuts

	Spanhoma-Okla. P-No. 0112 Harvest date and no. of days					Valencia-Okla. P-No. 0161 Harvest date and no. of days				
Amino acid	9/10 113	9/24 127	10/8 141	10/22 155	11/5 169	9/10 113	9/24 127	10/8 141	10/22 155	11/5 169
			μm/g					μm/g		
Aspartic acid	.71	.80	.93	1.35	2.32	.89	1.27	.52	.88	1.09
Asparagine[a]	2.71	2.14	2.97	2.26	2.64	3.65	3.69	4.67	3.30	3.50
Proline	.88	1.92	1.17	.77	.82	.90	2.41	2.68	.94	1.66
Glutamic acid	6.25	6.55	6.84	6.35	7.69	6.32	7.14	6.46	6.53	4.53
Glycine	.37	.29	.79	.22	.18	.43	.41	.86	.39	.63
Alanine	.91	.81	2.23	.76	.61	1.27	1.69	5.25	1.23	2.60
Valine	.47	.41	.86	.33	.34	.74	.67	1.62	.54	.43
Methionine	.05	.07	.07	.07	.06	.10	.10	.17	.06	.01
Isoluecine	.18	.19	.43	.16	.18	.36	.27	.89	.28	.23
Leucine	.14	.13	.38	.12	.13	.25	.29	.82	.24	.18
Tyrosine	.09	.08	.28	.07	.12	.16	.14	.59	.13	.15
Phenylalanine	.94	1.02	1.14	.89	2.03	1.60	.69	1.41	1.25	1.34
Peptide	1.66	1.96	1.72	1.68	2.06	1.75	1.38	1.23	1.07	1.33
Ammonia	.57	.29	.73	.40	.31	.48	.44	.93	.47	.90
Lysine	.05	.05	.14	.06	.09	.10	.12	.30	.13	.12
Histidine	.13	.14	.03	.19	.22	.24	.22	.41	.20	.19
Arginine	.22	.25	.78	.34	.57	.50	.60	1.36	.65	.69
Tryptophan	nil	.05	.10	.06	.10	.12	.09	.18	.20	.08

[a]A combination of asparagine, glutamine, threonine, and serine.

in this study because of numerous small 440 peaks and lack of scale expansion on the Beckman 120 C analyzer.

Effect of Harvest Date, Maturity, and Variety

Figure 1 and Table III shows a typical ion-exchange column chromatogram of the extract of mature peanuts. This cultivar of Argentine peanut samples was harvested at 141 days after planting. The peptide peak between valine and methionine, at just beyond 3 hr, is located in the zone of buffer change from pH 3.250-pH 4.25. The peptide indicated is the one which was called peptide II in the previous report (2). It was postulated that the peptides partially hydrolyze during the roasting reaction to produce free amino acids for reactions with the sugars. Present research indicates the the peak previously designated as peptide I may have been at least partially an artifact of the extraction procedures, but attempts to isolate and fully characterize these components have not been completely successful (7).

Tables IV and V give the results obtained on the free amino acid composition of the 1968 peanuts grown at Perkins, Okla. as influenced by harvest date and maturity. These were a part of the same peanut samples which were analyzed for effect of maturity on fatty acid composition (6).

Table IV shows the values in the three maturity classes over the five harvest dates for the Argentine (P-2) variety. Usually, except for aspartic acid and the peptide, the amounts of amino acid decreased with increasing maturity for a given harvest date. Aspartic acid content early in the season (113 days) decreased from 3.34 μm/g for immature to 0.69 μm/g for mature, but the reverse trend was observed late in the season (169 days) with an increase from 0.17 μm/g for immature to 1.97 μm/g for mature. The peptide content increased with maturity at each date except the 155 day harvest, at which the level was about

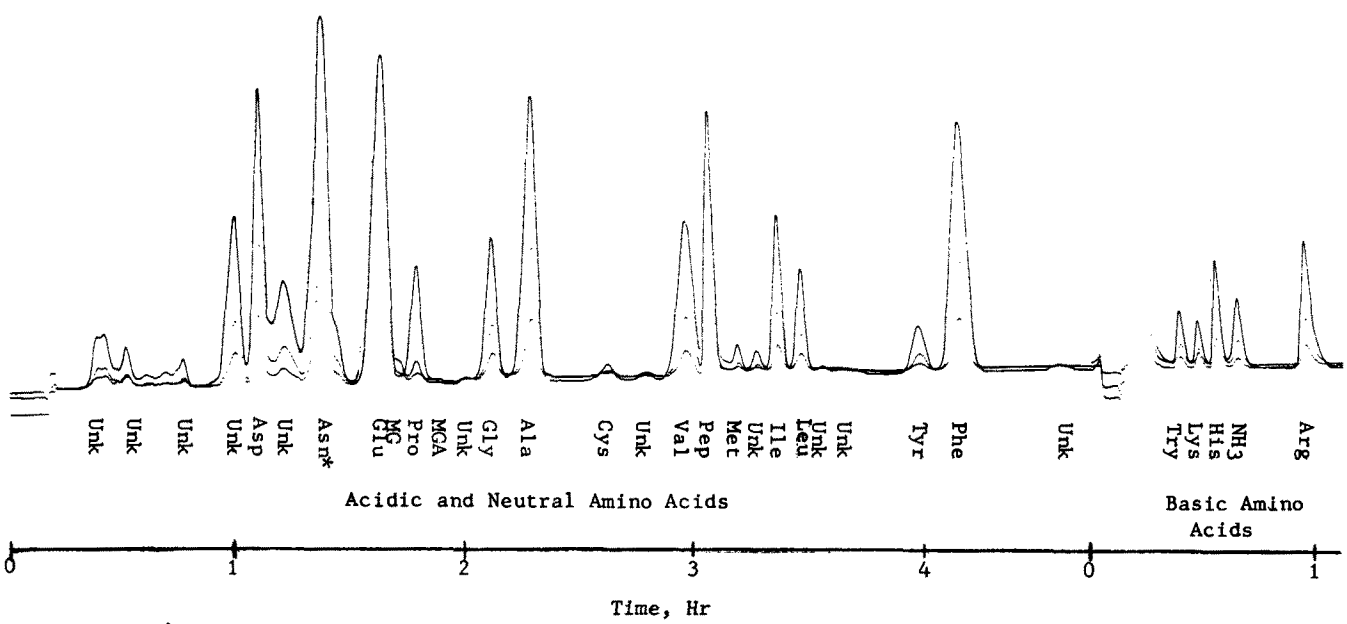

FIG. 1. Chromatogram of free amino acids from mature peanuts, variety Argentine P-2. The peak used for calculations has been traced over for photographing purposes. Conditions were as follows: harvested, Oct. 8, 1968, 141 days; concentration of sample, 10 g peanuts/20 ml pH 2.2 citrate buffer.

the same in all three maturity groups.

With the accelerated analysis procedure, the asparagine and glutamine peaks occurred between threonine and serine, giving one large peak which is referred to as asparagine (including asparagine, glutamine, threonine, and serine) peak, since it is predominately asparagine. If the amino acid values of Tables IV and V were ranked in descending order, glutamic acid followed by asparagine (including asparagine, glutamine, threonine, and serine) would top the list in the mature and low intermediate groups, except in Valencia (P-161) at 141 days. The peptide and phenylalanine were usually in the top six, while aspartic acid occurred there fairly often. In the immature peanuts, there was more arginine (18-38 μm/g) than any other amino acid. This continues to support the theory that the high level of arginine is an indication of immaturity (1, 2, 7, 9, 10).

Proline in the immature peanuts was found in higher concentrations than previously reported, although there was considerably more variation in proline content than in arginine. It has been reported (11) that high proline may be indicative of disease or other adverse conditions. Further studies are needed on the proline variation of mature and immature peanuts and its relation to immaturity.

An examination of the alanine values in Tables IV and V shows that the content at the 141 day harvest was 2-3 times higher than at the other harvest periods. These peanut samples were cured at a higher temperature (110 F instead of 90 F) due to failure of the temperature control and were scored lower on the organoleptic test. There is a possibility that the high alanine content may have resulted from the increased drying temperature due to anaerobic respiration. Alanine would be an expeced product of glycolytic respiration under conditions which inhibit Krebs cycle oxidation of the end products of glycolysis. Five other amino acids (glycine, valine, isoleucine, leucine, and tyrosine) apparently were affected similarly. Because of the potential importance of this observation in explaining basic problems of off-flavors in peanuts dried above 95 F, further studies are needed.

In addition to the normal amino acids, the chromatogram shown in Figure 1 and Table III has several unidentified peaks designated as unknown, and two peaks near proline identified as γ-methyleneglutamine and γ-methyleneglutamic acid. Done and Fowden (12) reported γ-methyleneglutamine and γ-methyleneglutamic acid in germinating peanut seedlings in 1952, but, prior to 1970 (7), the presence of these two nonprotein amino acids in mature kernels had not been confirmed. The other unknowns remain unidentified, although the possibility that one was γ-aminobutyric acid, which is common in plant material, was not examined.

ACKNOWLEDGMENTS

The financial assistance of Anderson Clayton and Company, Richardson, Tex., Oklahoma Peanut Commission, Madill, Okla., Best Foods, CPC International, Union, N.J., and the technical assistance of D. Rebouche are acknowledged.

REFERENCES

1. Newell, J.A., Ph.D. dissertation, Oklahoma State University, Stillwater, Okla., 1967.
2. Mason, M.E., J.A. Newell, B.R. Johnson, P.E. Koehler, and G.R. Waller, J. Agr. Food Chem. 17:728 (1969).
3. Koehler, P.E., and G.V. Odell, Ibid. 18:895 (1970).
4. Bieleski, R.L., and N.A. Turner, Anal. Biochem. 17:278 (1966).
5. Spackman, D.H., W.H. Stein, and S. Moore, Anal. Chem. 30:1190 (1958).
6. Young, C.T., M.E. Mason, R.S. Matlock, and G.R. Waller, JAOCS 49:314 (1972).
7. Young, C.T., Ph.D. dissertation, Oklahoma State University, Stillwater, Okla., 1970.
8. Nowacki, E., Z. Pflanzenzueht. 61:232 (1969).
9. Young, C.T., S.R. Cecil, and D.H. Smith, J. Amer. Peanut Res. Educ. Assoc. 4:52 (1972).
10. Young, C.T., and M.E. Mason, J. Fd. Sci. 37:722 (1972).
11. Palfi, G., Acta Agron. Acad. Sci. Hung. 17:381 (1968).
12. Done, J. and L. Fowden, Biochem. J. 51:451 (1952).

[Received January 31, 1973]