

Nutritive value of groundnut (*Arachis hypogaea*)

1. Amino acid composition of different varieties of groundnut grown in the Punjab

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1. Nine important varieties of groundnut (*Arachis hypogaea* Linn.) were analysed for amino acid composition by an ion-exchange chromatographic procedure. Cystine, as cysteic acid, and methionine and tryptophan were determined by colorimetric methods.
2. Small, but statistically significant, differences in the contents of nitrogen, serine, glutamic acid, proline, alanine, leucine, phenylalanine, tyrosine, lysine, methionine and cystine were found in the varieties.
3. A positive correlation ($P < 0.05$) between methionine and cystine was observed.
4. There was, however, no indication that selection from the varieties studied would permit development of a variety of superior protein quality.

The work carried out over the past 50 years on the groundnut proteins reveals little information about the influence of genetic factors on their amino acid composition (Rosen, 1958). Bolhuis (1963) suggested that the N content of various food crops, including groundnut, is influenced to a considerable extent by genetic constitution. Busson, Carbiener, Georgin, Lanza & Dubois (1960), however, observed that the N content of groundnut was influenced more by ecological than by genetic factors; the amino acid composition was not altered by either of these factors. The investigation now described was therefore undertaken to study more fully the genetic differences in the composition of groundnut protein as revealed by the contents of essential and non-essential amino acids when different varieties were grown under identical conditions of culture, soil and climate.

EXPERIMENTAL

Nine varieties of groundnut, namely 321/2, 511/28, 145/12-P, 142/16, 69/9, Punjab Groundnut (PG) no. 1, 5/10, A 20 and A 23, were selected from several varieties on the basis of their high oil content and yield per acre. Representative samples of these varieties were grown under identical conditions at the Experimental Farm of the College of Agriculture, Ludhiana in 1960 and 1961. The origin of these varieties is presented in Table 1.

The varieties were grown without irrigation on sandy-loam soil. The total rainfall during the period of crop growth was 39.6 cm in 1960 and 32.8 cm in 1961. Ammonium

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sulphate at the rate of 11.4 kg nitrogen/acre was applied. Thirty-six samples representing nine varieties were collected from four out of six replicates in each year for analysis.

Preparation of samples. Kernels were removed from the pods and shrivelled immature seeds were rejected. Weighed samples of the kernels were ground in a pestle and mortar and defatted with light petroleum (b.p. 40°–60°) in a Soxhlet apparatus. The defatted samples were ground finely to pass through a 40-mesh sieve and preserved in air-tight containers for use as required.

Table 1. *Origin of the groundnut varieties*

Variety	Origin*	Habit
321/2	D ₃ × A.H. 477	Spreading
511/28	D ₃ × A.H. 477	
145/12-P	A ₁₋₁ × D ₃	
142/16	A ₁₋₁ × D ₃	
69/9	A ₁₋₁ × D ₃	
Punjab Ground-nut no. 1	Single plant selection from local collection	
5/10	PG no. 1 × A.H. 259	Erect
A 20	Natal common × Aut	
A 23	Philippine Pink × Natal common	

* D₃, Virginia Runner; A.H. 477, *Arachis hypogaea* from Madras (India); A.H. 259, *Arachis hypogaea* from Madras (India); A₁₋₁, mutant from variety Akola, Madhya Pradesh (India); Aut, South African variety.

Acid hydrolysis. Samples weighing 250 mg of the defatted kernels of each variety were hydrolysed with 200 ml hydrochloric acid of constant b.p. (redistilled in all-glass Pyrex apparatus) for 24 h under reflux. The excess acid was removed by evaporation to near dryness followed by further evaporation of three small volumes of added distilled water. The humin was removed by filtration and the volume of the filtrate made to 100 ml. The nitrogen content of humin was determined by the Kjeldahl method. The hydrolysates were stored at 0° if not immediately used.

Alkali hydrolysis. For the determination of tryptophan, 250 mg of the defatted sample were hydrolysed with 50 ml 5 N-sodium hydroxide for 5 h under reflux. The contents were transferred to a 100 ml volumetric flask and made to volume with water. The hydrolysates were then filtered and, unless immediately used, were stored in a refrigerator.

Methods of analysis. All the amino acids except cystine, methionine and tryptophan were determined by the procedure of Moore, Spackman & Stein (1958) with 150 cm and 15 cm columns of Amberlite IR-120 with resin particles passing sieves of between 250 and 300 mesh. Fractions of 2 ml were collected manually. The modified ninhydrin colour reaction of Moore & Stein (1954) was employed, 1 ml reagent with 2 ml fractions being used.

In the first series of experiments conditions for determination of various amino acids were standardized. A model mixture of fifteen amino acids containing 0.5 μ-mole/ml aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine,

valine, isoleucine, leucine, phenylalanine, tyrosine, lysine, histidine and arginine and ammonia was prepared. In the second series of recovery experiments these amino acids and ammonia were added to the test material before hydrolysis.

Cystine was determined as cysteic acid by the ion-exchange chromatographic method of Vervack (1960).

Methionine and tryptophan were determined by the colorimetric procedure of Horn, Jones & Blum (1946) and Steers & Sevag (1949) respectively.

N was estimated by the micro-Kjeldahl method of the Association of Official Agricultural Chemists (1960).

Expression of results. The values for amino acid contents are based on mg amino acid/g N of defatted meals, as recommended by FAO (1957).

Table 2. Total nitrogen (%) and amino acid composition (mg/g N) of the kernels of nine varieties of groundnut after extraction with light petroleum†

Code number of variety	...	321/2	511/28	145/12-P	142/16	69/9	PG.no.1	5/10	A 20	A 23	SED‡
Total N (%)	...	10.76	10.75	10.77	10.78	10.81	10.70	10.69	10.70	10.73	0.03**
Aspartic acid		711	710	710	710	709	711	710	710	711	2.3
Threonine		155	158	157	155	156	159	160	159	154	2.4
Serine		311	310	318	314	315	320	318	305	305	4.0**
Glutamic acid		1127	1122	1117	1113	1112	1116	1121	1115	1118	3.6**
Proline		253	255	244	242	244	249	247	240	232	4.6**
Glycine		356	361	357	362	357	361	362	359	362	2.3
Alanine		254	259	257	259	262	266	265	254	250	3.3**
Valine		238	236	233	238	240	238	236	239	238	2.1
Methionine		55	55	57	56	56	56	57	58	58	0.6**
Isoleucine		207	205	205	205	208	207	205	205	206	2.7
Leucine		410	406	417	415	417	414	411	408	408	2.8**
Tyrosine		241	241	258	257	252	254	253	245	247	4.9**
Phenylalanine		308	313	328	330	328	323	317	331	329	4.8**
Lysine		242	242	242	242	245	247	248	242	239	2.8*
Histidine		178	173	169	175	177	176	177	172	172	3.9
Arginine		658	650	668	667	665	670	664	676	671	11.5
Tryptophan		60	60	60	60	60	60	59	61	61	0.8
Cystine as cysteic acid		100	100	101	102	102	100	101	104	102	1.3**
Ammonia		140	141	142	143	144	136	133	137	139	1.6**
Total (g/g N)		6.004	5.997	6.040	6.045	6.049	6.063	6.044	6.020	6.002	

* $P < 0.05$.

** $P < 0.01$.

† On the dry-matter basis. Each value is the mean of eight replicates for the years 1960 and 1961.

‡ Standard error deviation of values as obtained from †.

RESULTS

The mean N content and amino acid contents of nine varieties of groundnut in the years 1960 and 1961 are given in Table 2. The analysis of variance indicated significant differences due to variety for total N, serine, glutamic acid, proline, alanine, leucine, tyrosine, phenylalanine, lysine, methionine, cystine and ammonia. However, differences due to replication, year and variety \times year interaction were not significant for all the constituents studied. The mean values over the eight replicates

for each variety were used in calculating the correlation coefficients given in Table 3. The results showed that some amino acids increased as the N content increased and vice versa, but none of the coefficients of correlation proved significant. However, the positive correlation coefficient between values for methionine and cystine proved significant at the 5% level.

Table 3. *Correlation coefficient between contents of nitrogen and amino acids in nine varieties of groundnut*

Amino acid	Nitrogen	Tyrosine	Lysine	Methionine
Serine	+0.03	—	—	—
Glutamic acid	-0.26	—	—	—
Proline	-0.06	—	—	—
Alanine	-0.19	—	—	—
Leucine	+0.62	—	—	—
Tyrosine	+0.16	—	—	—
Phenylalanine	+0.22	+0.53	—	—
Lysine	-0.43	—	—	—
Methionine	-0.25	—	-0.49	—
Cystine	+0.03	—	-0.32	+0.72*

* $P < 0.05$.

DISCUSSION

The recoveries of aspartic acid, threonine, serine, glutamic acid, glycine, valine, isoleucine, leucine, phenylalanine, lysine and histidine were within $\pm 3\%$ and of proline, tyrosine and arginine within $\pm 4\%$. Bender, Palgrave & Doell (1959) reported variations of $\pm 8\%$ in the recoveries of proline, alanine, leucine, isoleucine and arginine by ion-exchange chromatographic technique using Dowex X-8 resin columns. However, Sihlbom (1962) used Amberlite IR-120 resin columns and reported a reproducibility of $\pm 2\%$ for the majority of the amino acids, except for proline, tyrosine and arginine for which recovery by this technique varied by $\pm 4\%$.

The mean recovery of cystine determined as cysteic acid, in a model mixture, was 89.4%; a recovery of 90% was reported by Schram, Moore & Bigwood (1954). The mean recovery of amino acids when incorporated with the test sample before oxidation and hydrolysis was 89%. The values for cysteic acid were therefore corrected by the factor 100/89. When used as internal standards with the test samples, the losses of methionine and tryptophan were 18.6 and 16.3% respectively. The destruction of these amino acids was less than that reported by Chibnall (quoted by Wolfe & Fowden, 1957). The values for methionine and tryptophan were therefore corrected on the basis of their respective recoveries.

The N found as humin, ammonia and amino acids varied from 97.5 (A 23) to 99.0% (PG no. 1) of the N of the samples. Sihlbom (1962) also reported the recovery of N as amino acids and ammonia to be 95–100% of the N in wheat varieties. VanEtten, Miller, Wolff & Jones (1961), on the other hand, observed wide variation (74.7–103.9%) in the N content of hydrolysates of twenty-seven selected seed meals in the form of humin N, amino-N and ammonia-N.

The variations in the N content of the varieties were not wide, but the differences

between the varieties were statistically significant. Varieties 5/11, PG no. 1 and A 20 were found to contain significantly less N than varieties 321/2, 145/12-P, 142/16 and 69/9. The spreading varieties tended to have a higher N content than the erect varieties. As the varieties were grown under similar conditions the differences in the N content may be attributed to the genetic differences in the varieties. This is supported by the fact that no such differences were observed in the varieties derived from crosses in which one parent was common.

Busson *et al.* (1960) reported that the N content of groundnut varied considerably, but was influenced more by ecological conditions than by variety. In other words, these workers obtained varietal differences but, as these were not outstanding, they may have been masked by considerable variations due to ecological conditions.

Of the nineteen amino acids studied, the differences in the mean contents of serine, glutamic acid, proline, alanine, leucine, tyrosine, phenylalanine, methionine and cystine were significant at the 1% level and those of lysine at the 5% level. The differences in the mean contents of aspartic acid, threonine, glycine, valine, isoleucine, histidine, arginine and tryptophan in the varieties were statistically not significant.

Table 4. *Comparison of mean values (g/16 g N) for amino acid contents of groundnut reported by different workers*

Amino acid	This work	Busson <i>et al.</i> (1960)	Mauron (1961)
Aspartic acid	11.36	11.69	11.05
Threonine	2.51	2.70	2.90
Serine	5.01	4.95	4.95
Glutamic acid	17.95	18.85	18.65
Proline	3.92	4.69	—
Glycine	5.75	5.87	5.45
Alanine	4.13	4.04	3.70
Valine	3.80	4.38	4.35
Methionine	0.90	1.42	1.10
Isoleucine	3.29	3.60	3.45
Leucine	6.59	6.56	6.25
Tyrosine	3.98	3.98	4.25
Phenylalanine	5.17	5.03	5.35
Lysine	3.89	3.46	3.45
Histidine	2.78	2.20	2.44
Arginine	10.65	11.59	10.70
Tryptophan	0.96	1.03	1.15
Cystine	1.62*	1.45	—

* As cysteic acid.

Busson *et al.* (1960), using the ion-exchange chromatographic method, found no significant differences in the amino acid content of twenty varieties of groundnut (*Arachis hypogaea*). Mauron (1961), on the other hand, found the content of lysine in the South African variety to be 3.72 g/16 g N, as against 3.18 g/16 g N in the Spanish variety from Georgia, USA. As the conditions under which these varieties were grown have not been discussed, this difference in lysine content may have been due to the combined effect of genetic and environmental factors.

Experimental evidence indicates that genetic and climatic factors influence the

quality of vegetable crops. It has been shown that the N content of cereals is influenced by variety and ecology, though environment has, on occasion, been shown to alter the concentration of certain amino acids. Such changes were observed for wheat (Miller, Aurand & Flach, 1950), oats (Frey, 1951) and maize (Flynn, Zuber, Leweke, Grainger & Hogan, 1954). The chemical composition of soya beans is influenced more by variety than by environment (Cartter & Hopper, 1942).

The mean amino acid contents of nine varieties of groundnut compare favourably with those reported by Busson *et al.* (1960) and Mauron (1961) (Table 4).

Ellinger & Boyne (1963) determined cystine as cysteic acid and reported a value of 1.40 g/16 g N in groundnut. Similar values of 1.50 and 1.60 g/16 g N have also been reported by Desikachar & De (1947) and Block & Mitchell (1946-7) respectively in groundnut. Ahuja (1964) working in this laboratory determined cystine as cysteic acid in Punjab Groundnut no. 1 variety and obtained a value of 1.64 g/16 g N.

Interrelation between contents of N and amino acids

Any interpretation of the correlations we have observed is limited by the small ranges of variation observed for most of the amino acids studied. There were no correlations significant at the 5% level of probability. Bressani, Elias, Scrimshaw & Guzman (1962) also did not find a significant relationship between the contents of N and lysine or N and methionine in ten varieties of maize. Sihlbom (1962), however, found an inverse relationship between protein content and lysine content in wheat. Lawrence, Day, Huey & Lee (1958) also found an inverse relationship in wheat varieties having a protein content below 13.5%.

Interrelations between amino acids in groundnut

The correlations between amino acids were heterogenous both in magnitude and direction and only one correlation was significant. This was between methionine and cystine. Negative correlations were observed between lysine and methionine and between lysine and cystine and positive correlation between tyrosine and phenylalanine but were not statistically significant. These results suggest that methionine and cystine were closely related to one another in the groundnut.

The results obtained during this study have shown significant differences for most of the essential amino acids. There is, however, no indication that selection from the varieties studied would permit development of a variety of greater protein content, as differences observed were not wide.

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