During recent years there has been increasing emphasis on augmenting the protein intake of the people in countries suffering from protein malnutrition. Among the edible oilseed meals as a source of protein, groundnut is of considerable importance in our country where the annual production is of the order of 5.2 million tons. The residual cake contains a high percentage of protein (45 to 50 per cent).

As the amino acid composition is the most practical initial way of predicting the nutritive value, nine important groundnut varieties of Punjab were studied for their amino acid composition.

Ion-exchange chromatographic technique, using Amberlite IR-120 resin, was employed for determining all the amino acids except methionine and tryptophan, as reproducibility of this method was found to be high.

The recoveries of aspartic acid, threonine, serine, glutamic acid, glycine, valine, isoleucine, leucine, phenylalanine, lysine and histidine were within ±3 per cent and of proline, alanine, tyrosine and arginine within ±4 per cent. (Table 9). Bender et al. (1969) reported wide variations up to 8 per cent in the recoveries of proline, alanine, leucine, isoleucine and arginine by ion-exchange chromatographic technique using Dowex X-8 resin columns. However, Esther (1962) used Amberlite IR-120 resin columns and reported a reproducibility of ±2 per cent for majority of the amino acids except proline, tyrosine and arginine which showed variations of ±4 per cent in the recoveries by this technique.
In the author's exploratory experiments an overall average recovery based on five determinations of 15 amino acids and ammonia contained in a model mixture was 100.4 per cent ± 4.5 (Table 9). The overall recovery of amino acids incorporated with the test sample before hydrolysis was of the order of 99.3 per cent ± 3.8 (Table 10).

Based on 102 determinations of the 16 amino acids and ammonia from a mixture of amino acids, VanEtten, Miller, Wolff and Jones (1961) reported a recovery of 100.2 per cent ± 5.68.

According to Evans, Bendemer and Bauer (1960) the ion-exchange chromatographic method for determining cysteine and cystine as cysteic acid was the best. Cysteic acid formed by oxidation with performic acid is stable on hydrolysis with hydrochloric acid in the presence of carbohydrates. It was, therefore, adopted in this investigation. The average recovery of cysteine and cystine determined as cysteic acid, in a model mixture, was low i.e. 89.4 per cent (Table 9). Similar recovery of 90 per cent has been reported by Schram, Moore and Bigwood (1954) as against a recovery of 85 per cent by Vervack (1960). The average recovery of amino acids when incorporated with the test sample before oxidation and hydrolysis was 89 per cent (Table 10). The values of cysteic acid were, therefore, corrected by using the factor 100/89.

When used as internal standards with the test samples, methionine and tryptophan exhibited losses of 18.6 and 15.3 per cent respectively (Table 12).
The magnitude of destruction of these amino acids during hydrolysis was less than that reported by Chihna (quoted by Wolfe and Fowden, 1957). Losses similar to what have been observed were reported by McCarthy and Paille (1959) for methionine and by Kuiken, Lyman and Hale (1947), and van Etten et al. (1961) for tryptophan.

The values of methionine and tryptophan were, therefore, corrected on the basis of respective recoveries.

The distribution of nitrogen after hydrolysis, in humin, ammonia and amino acids is given below:

<table>
<thead>
<tr>
<th>Variety</th>
<th>As per cent of total nitrogen</th>
<th>Total Nitrogen Recovered Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Humin Nitrogen</td>
<td>Amino acids Nitrogen</td>
</tr>
<tr>
<td>331/2</td>
<td>1.9</td>
<td>85.2</td>
</tr>
<tr>
<td>511/28</td>
<td>2.2</td>
<td>84.9</td>
</tr>
<tr>
<td>145/12-P</td>
<td>1.4</td>
<td>85.0</td>
</tr>
<tr>
<td>142/15</td>
<td>2.2</td>
<td>84.6</td>
</tr>
<tr>
<td>69/9</td>
<td>1.7</td>
<td>85.4</td>
</tr>
<tr>
<td>P.G.No.I</td>
<td>1.8</td>
<td>86.0</td>
</tr>
<tr>
<td>5/10</td>
<td>1.6</td>
<td>86.3</td>
</tr>
<tr>
<td>A 20</td>
<td>1.4</td>
<td>85.2</td>
</tr>
<tr>
<td>A 23</td>
<td>1.3</td>
<td>84.7</td>
</tr>
</tbody>
</table>

The nitrogen found as humin, ammonia and amino acids varied from 97.5 (A 23) to 99.0 (P.G.No.I) per cent of the nitrogen of the sample. Esther (1962) also reported recovery of nitrogen as amino acids and ammonia to be 95 to 100 per cent of the nitrogen in wheat varieties. Van Etten et al. (1961), on the other hand, observed wide variations (74.7 to 103.9) per cent in nitrogen content of
hydrolysates of 27 selected seed meals in the form of humin nitrogen, amino acid nitrogen and as ammonical nitrogen.

The variations in percentage of nitrogen in the varieties were not of a very high order but the differences between the varieties were statistically significant (Table 12). Variety 5/10, P.G.No.1 and A 20 were found to contain significantly lower nitrogen than the varieties 321/2, 145/12-P, 142/16 and 69/9. Spreading varieties, on the whole, were found to contain higher nitrogen than erect varieties. As the varieties were grown under similar conditions the differences in the nitrogen content can be primarily attributed to the genetic differences in the varieties. This is further supported by the fact that in the varieties derived from crosses in which one parent was common, no such differences were observed.

Busson et al. (1960) reported that nitrogen content in groundnut varied considerably but was influenced more by the ecological conditions than by variety. In other words these workers obtained varietal differences in the nitrogen content but as these were not outstanding these might have been masked by considerable variation due to ecological conditions. On the other hand, appreciable differences in the protein content of ten varieties of soybeans were observed by Carter and Hopper (1942). Similar variations in protein content of 20 strains of soybeans were reported by Kuiken and Lyman (1942). Differences in the protein content in corn, wheat, oats etc. due to genetic differences have been reported by a number of workers. Wolfe and Powden, 1957; Lawrence et al. (1958), and Frey (1958). The results of amino acid composition of the groundnut varieties have been presented in
Table 13 to Table 31. The representative chromatograms as obtained by ion-exchange chromatographic procedure for the varieties are shown in Fig. 2 to Fig. 10.

Of the 19 amino acids determined in different varieties, the differences in the mean values of serine, glutamic acid, proline, alanine, leucine, tyrosine, phenylalanine, lysine, methionine and cysteine and cystine contents were statistically significant at 5 per cent level of probability. The differences in the mean values of aspartic acid, threonine, glycine, valine, isoleucine, histidine, arginine and tryptophan contents of the varieties were statistically not significant. Busson et al. (1960) found no significant differences in the amino acid content of twenty varieties of groundnut (Arachis hypogaea) by ion-exchange chromatographic method. Jain also studied the amino acid composition of two varieties of groundnuts viz., a Spanish type from Georgia (U.S.A.) and the other from South Africa, using the method of Moore et al. (1958). The African variety was found to contain higher lysine content (3.72 gms.) than the U.S. variety (3.18 gms. per 16 gram nitrogen). As the information regarding conditions under which these two varieties were grown is lacking, this difference in lysine content may be due to the combined effect of genetic and environmental factors. Kotasahane and Narayana (1937), however, observed no appreciable differences in the six amino acids viz., tyrosine, lysine, histidine, arginine, tryptophan and cystine in the case of Spanish variety grown in India, a local variety and the Virginia groundnuts grown in India. Kuiken and Lyman (1940) determined essential amino acids of
TYROSINE
PHENYL ALANINE

ACIDIC & NEUTRAL AMINO ACIDS
LYSINE
HISTIDINE
ARGININE

AMMONIA
SERINE
THREONINE
ONINE SULFOOXIDE
ASPARTIC ACID
PROLINE
GLUTAMIC ACID
GLYCINE
ALANINE
Cystine

METHIONINE SULFOOXIDE
METHIONINE
PHENYL ALANINE
TYROSINE

AMINO ACIDS

CYSTEIC ACID
20 strains of soybean meals by microbiological methods and found some variation in the methionine contents of the strains. However, as the differences in methionine contents were not very large, these authors observed that selection from the varieties for development of a superior protein strain was not possible. Alderks (1949), on the other hand, obtained no significant differences between 20 soybean varieties for any of the essential amino acids, by microbiological methods. 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 varieties studied would permit development of a superior protein variety, as the differences observed were not very outstanding.

Survey of 70 strains of groundnut for lysine and methionine

The results of lysine and methionine contents of 70 strains (Table 34) revealed that differences in the lysine and methionine contents were not of a very high order and hence for breeding a variety of groundnut with high methionine or lysine, these strains have a very limited utility. The reasons for not observing great variation in these strains may be attributed to their genetic similarity, since almost all these strains owe their evolution to the varieties received from Madras (India).

Interrelations between Nitrogen and Amino Acids in Groundnut

Any interpretations of the correlations is limited by the small ranges of variations observed for most of
the amino acids studied. The results suggest that the contents of serine, glutamic acid, proline, lysine and methionine tend to be negatively but weakly correlated with the nitrogen content while alanine, leucine, phenylalanine and cysteine + cystine tend to be positively but weakly correlated with the nitrogen content. However, none of these correlations proved significant at 5 per cent level of probability. Bressani et al. (1962) also did not obtain significant relationship between nitrogen - leucine, nitrogen - lysine and nitrogen - methionine in 10 varieties of corn. Esther (1962), however, found inverse relationship between protein content and lysine in wheat. Lawrence, Katherine, Huey and Lee (1958) also obtained similar results in 286 samples representing broad spectrum of wheat varieties having protein content below 13.5 per cent. Above this level, however, the correlation was not significant.

The author has not come across literature reporting correlation of various amino acids to total nitrogen in groundnut. However, from the observations made during the present investigation the absence of this relationship may be due to very high content of nitrogen in groundnut.

Interrelations between Amino Acids in Groundnut

The correlations between amino acids were heterogeneous both in magnitude and direction and only the positive correlations reached the level of significance. These were between tyrosine - phenylalanine and methionine - cysteine + cystine. Although negative correlations were observed in case of lysine - methionine, and lysine - cysteine + cystine these were of small magnitude and were not significant. The
results suggest that methionine and cysteine + cystine were closely related to one another in the groundnut. The same was true of phenylalanine and tyrosine. The regression coefficients of these amino acids were worked out and are as follows:

\[ Y = 220.8 + 0.409X \]

Where \( Y \) = Phenylalanine in mg.

and \( X \) = Tyrosine in mg.

\[ Y = 26.4 + 0.209X \]

Where \( X \) = Cysteine + cystine in mg.

and \( Y \) = Methionine in mg.

These equations can be applied in predicting the level of one amino acid if the other is known, especially for methionine, the determination of which is not very accurate as compared with cysteine + cystine as cysteic acid. Such results have not been reported in the literature so far.

Frey (1951) showed that valine, isoleucine and leucine were more closely related to one another than to tryptophan in the corn grain. Bressani et al. (1962), on the other hand, found positive significant correlations between isoleucine - valine, leucine - lysine, valine - lysine, isoleucine - tryptophan and valine - tryptophan in corn grain.

The positive correlation obtained during the present investigation, between phenylalanine and tyrosine is understandable because it is known that both these amino acids are synthesized from a common precursor prehenic acid in microorganisms (quoted by West and Todd, 1961) and it is likely that same synthetic pathway may be operative in
LEUCINE
ISOLEUCINE
METHIONINE
VALINE

Tyrosine
Phenylalanine

Acidic & Neutral Amino Acids
Lysine
Histidine
Arginine

Cystine + Cysteine as Cystic Acid
plants. Similarly the relationship between methionine – cysteine + cystine is quite likely because the latter two amino acids are formed from methionine through cystathionine (quoted by Cantarow and Schepartz, 1962).

The correlation between nitrogen – lysine and nitrogen – methionine in 70 strains of groundnut were also negative and not significant at 5 per cent level of probability.

The correlation coefficient between nitrogen and oil contents was found to be inverse and highly significant. Viljoen (1937) (quoted by Cravens and Endre, 1968) also obtained inverse coefficient of correlation between nitrogen and oil in soybean. Nijhawan (1955) has also reported similar findings in groundnut.

The dependence of protein quality on the essential amino acid contents of the foods is now well established. However, quantitative analysis of the amino acids may not be adequate to specify the quality of a protein as the enzymic hydrolysis in the stomach and intestine does not yield products of the same availability as does acid hydrolysis in the laboratory. Lea and Hannan (1950) showed that amino acid may be present chemically but not be biologically available. Further the rate of release of a most limiting amino acid by enzymes in the digestive system affects considerably the utilization of the protein. Soybean protein is an example where the presence of active trypsin inhibitor decreases the available level of the essential amino acids to a point as to precipitate a deficiency of most limiting
amino acid namely methionine (Ham, Sandstedt and Mussel, 1945, quoted by Almquist, 1951) and thus affecting its nutritive value. In view of the presence of such an inhibitor in the groundnut (Borchers, Ackerson and Kimmell, 1947) and Lord and Wakely (1950) and very low status of methionine it was, therefore, considered essential that the final proof of protein quality must be found by biological methods.

Defatted groundnut meals of nine varieties were, therefore, tested by feeding to weanling rats at a dietary level of 10 per cent protein. The biological values of the varieties were determined in accordance with the nitrogen balance method of Mitchell (1923-24). The average values ranged from 50.9 to 52.8 showing that the varieties had no significant effect on the biological values of groundnut. The values are slightly lower than the value of 57.9 reported by Pian (1930) and later by Mitchell et al. (1935). Everson, Steenbok, Cederquist and Parsons (1944) also evaluated nutritive value of five varieties of soybeans and observed no appreciable differences between the varieties. Alderks (1949) studied the quality of protein of 20 strains of soybean by 'in vitro' enzyme digestion method and found no significant differences between the varieties.

As the differences observed in the methionine contents of these varieties were statistically significant, though not very outstanding biological values of these varieties were also calculated using chemical score method of Block and Mitchell (1946). The values ranged between 56.4 to 57.4 showing no significant effect of small
variations in the methionine contents even on calculated biological values. These results agree closely with the biological values obtained by actual feeding trial. Almquist (1947), Gran (1948), and Schweigert (1948) also reported that the calculated biological value from amino acid composition agree closely with the biological results in most of the proteins.

The digestibility coefficient of these varieties, as evaluated by Mitchell's method (1923-24) ranged between 81.9 to 83.0 showing that the varieties had no significant effect on the digestibility coefficient. These figures are slightly lower than the value of 89 reported by Morrison (1935). Plane (1930) and Mitchell and Beadle (1937), however, obtained a value of 97.4. The differences in digestibility coefficient may be attributed to variations in the experimental conditions.

Since methionine is limiting amino acid in groundnut and significant differences were obtained by the author in the methionine content of the varieties three varieties viz., 511/28, 5/10, A 20 representing respectively the lowest, intermediate and the highest amounts of methionine and cysteine + cystine were further studied for the differences in the quality of protein as measured by protein efficiency ratio (PER) at a 10 per cent level of protein in the diets. The mean PER values ranged from 1.56 to 1.58 and indicated no correspondence with the magnitude of differences in the essential amino acid contents.

Maynard et al. (1923) obtained a PER of 1.45 at 9 per cent level of protein while Rao et al. (1953)
reported a PER of 1.45 at 10 per cent level. PER as high as 1.82 and 1.72 have also been reported by Jones and Widness (1945) and Block and Mitchell (1946) respectively. Sure (1955), however, reported a PER of 1.40 at 10 per cent level of protein.

The results discussed above have shown that although significant intervarietal differences were observed in most of the essential amino acids, yet these differences were not very wide. It was, therefore, not possible to detect these differences in the biological value (BV) and protein efficiency ratio (PER) of these varieties.

The differences in the nitrogen contents, when the groundnut is grown under similar ecological conditions, were not so marked though in some cases the values were significantly higher. However, the higher level of nitrogen content has not affected their nutritive value as is the case with corn where varieties with higher nitrogen content has been shown to be inferior in quality as compared to varieties with lower nitrogen content.

The present investigation has shown that the total protein content and amino acid composition especially most of the essential amino acids were influenced significantly by genetic factors when groundnut is grown under similar environmental and agronomic conditions. The observed intervarietal differences were small because most of the varieties studied were derived from a closer genetic stock. However, if varieties with widest genetic differences are studied it may be possible to observe
considerable intervarietal variations in the quality of their protein.

It has been observed that it is possible to predict the biological value of groundnut from its methionine content by chemical score method with reasonable accuracy and without resorting to actual biological tests. This is due to the fact that availability of all the essential amino acids (Kuiken and Lyman, 1948) and especially of methionine is not affected by the trypsin inhibitor known to be present in the groundnut, unlike soybean where trypsin inhibitor retards the liberation of methionine in the early stages of digestion.

Compared with whole egg protein, groundnut is particularly deficient in methionine, lysine and isoleucine (Rosen, 1958) and the low biological value of groundnut is due to lower content of methionine. Mitchell (1923-24), Block and Mitchell (1945) and Cama and Morton (1950). Marked improvement has been affected in its nutritive value by supplementation with Dl-methionine (Cama and Morton, 1950). Threonine (Sure, 1955) and Lysine (Cama et al., 1955) improve further the methionine fortified protein.

Research work carried out has shown that racemic methionine is fully as effective for growth purposes as the natural L-methionine when added to rat diets (Jackson and Block, 1933) and Rose (1938) quoted by Almquist (1951). In man, D-methionine, is also utilized as readily as the natural form (Albanese, 1944).

With large scale production of synthetic
methionine and its efficient utilization by animal body, use of DL-methionine as a supplement to animal feeds is rapidly increasing. Synthetic methionine can play equally important role in human nutrition in country like India in view of the findings of Sure (1955) who obtained an increase of 44 per cent in the protein efficiency of groundnut when supplemented with DL-methionine at 1 per cent level. Further improvement of 17.5 per cent was obtained (Sure, 1955) by supplementing with the next limiting amino acid namely threonine when its nutritive value came at par with casein. However, to achieve maximum efficiency, optimum level of methionine supplementation and the economics involved will have to be studied.