Essential Amino-acids of some Common Tropical Legumes and Cereals

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In spite of numerous amino-acid analyses of foodstuffs recorded in recent years, there is little reliable information about the amounts of essential amino-acids in the legumes commonly consumed in tropical countries. Early studies (Jones, 1938) were directed to the preparation of the α- and β-globulins present in some legumes and to the determination of their lysine, tryptophan and cystine contents. More recently, figures for some of the essential acids in a variety of *Phaseolus vulgaris* eaten in Mexico have been published (Massieu, Guzman, Cravioto & Calvo, 1949), and several reports on the amino-acids in soya beans (Riesen, Clandinin, Elvehjem & Cravens, 1947; Kuiken & Lyman, 1949) have appeared. Again, whereas rice, wheat and maize have been the subject of numerous amino-acid analyses, the literature contains few or no data for the indigenous millets and sorghums of tropical countries. The present shortage of food, particularly of rice, in the rice-eating areas of the world has focused attention on such cereals and it therefore becomes of interest to know their contents of essential amino-acids.

The present study was directed to ascertaining the supplementary value of legumes in diets composed largely of mixtures of cereals and vegetables; as a first step the determination of the eight amino-acids shown to be indispensable for man (Rose, 1949) was carried out on three legumes, lentil or Mysore dhall (*Lens esculenta*), green gram or mung (*Phaseolus aureus* Roxburgh) and black gram or ulundu (*Phaseolus mungo* Linn.), and on two cereals, finger millet or kurakkan (ragi, *Eleusine coracana*) and sorghum (*Andropogon sorghum* var. Kelo). Preliminary values for some of the essential acids in whole lentil and green gram (Baptist, 1952*a*) and finger millet (Baptist, 1950, 1951, 1952*b*) have been reported earlier; the present paper reports more detailed analytical findings.

EXPERIMENTAL AND RESULTS

Preparation of vegetable for analysis. Representative samples of each of the food materials were freed carefully from grit and other foreign matter and the testa (legumes), hulls (sorghum) and outer chaffy material (millet) were removed by rubbing and lightly pounding in a mortar, with subsequent winnowing. The cleaned samples were then ground to a fine powder and exhaustively extracted with light petroleum in a Soxhlet apparatus. The dried defatted powder was passed through a 90-mesh Monel sieve. The resulting homogeneous powder was used for analysis. The nitrogen content of each preparation was determined by the micro-Kjeldahl method as described by Chibnall, Rees & Williams (1943). Table 1 gives the nitrogen contents of the anhydrous preparations. The moisture contents of the foodstuffs were determined simultaneously on separate portions of the raw materials.

Table 1. *Nitrogen contents of legumes and cereals*

Vegetable	Nitrogen in anhydrous defatted preparation (%)
Lentil	4·74
Green gram	4·59
Black gram	4·78
Finger millet	1·26
Sorghum	1·93

Preparation of the hydrolysate. Direct acid hydrolysates were prepared as described previously (Baptist, 1954) by boiling with 20% HCl for 20 h. Alkaline hydrolysates for the estimation of tryptophan were prepared with baryta by the method of Greene & Black (1944).

Microbiological analysis. The amino-acid determinations were carried out by microbiological assay on suitable neutralized portions with the organisms and media shown in Table 2. The assay technique was essentially as described previously (Baptist, 1954).

As cereal-vegetable diets tend to be lacking in the amino-acids lysine and tryptophan, and as the legume proteins usually are deficient in methionine, determinations of these three amino-acids were carried out more extensively than in the previous study (Baptist, 1954). Several media and two different organisms were used for each of these amino-acids. The following modifications were made in the media. In the estimation of methionine with *Leuconostoc mesenteroides* P 60, amino-acids were used in place of the oxidized peptone solution used for the assays with *Streptococcus faecalis*. In the medium of Kuiken, Lyman & Hale (1947) for tryptophan assay with *Strep. faecalis*, the oxidized peptone solution was replaced by a casein hydrolysate to which cystine, tyrosine and methionine had been added. This assay has been found preferable to that with *Lactobacillus arabinosus* 17-5 (Wooley & Sebrell, 1945) used previously. The assay with *Strep. faecalis* eliminates the need for ether extraction of the neutralized hydrolysate; further, the agreement at different assay levels as well as between replicate determinations on different hydrolysates proved to be better than in the *Lb. arabinosus* 17-5 assay. Though the methionine contents obtained by the use of different organisms and media showed good agreement, lysine assays with *Strep. faecalis* gave, as previously observed (Baptist, 1954), values lower by some 7–12% than those obtained with *Leuc. mesenteroides* P 60. Table 3 shows the values obtained for lysine and methionine with the different organisms and media quoted in Table 2. The experience gained with microbiological assay in this study has led me to prefer for the corresponding amino-acids the organisms and media indicated by an asterisk in Table 2. These yielded in our hands better results than the others, as judged by low blanks, close agreement at different assay levels in triplicate determinations and quantitative recoveries of added amino-acid within the limits of accuracy ±5%

Table 2. *Organisms and media used in estimations of amino-acids in vegetables*

(The organisms and media preferred and used to obtain the values in Table 4 are indicated by an asterisk)

Amino-acid	Organism	Medium (reference)	Standard range† (μg)
Lysine	*S.f.*	Stokes, Gunness, Dwyer & Caswell (1945)	0–100
	S.f.	Greenhut, Sirny & Elvehjem (1948)	0–120
	*L.m.**	Henderson & Snell (1948)*	0–80
	*L.m.**	Schweigert, Guthneck, Kraybill & Greenwood (1949)*	0–100
	L.m.	Greenhut *et al.* (1948)	0–80
Methionine	*L.m.**	Schweigert *et al.* (1949)*	0–80
	L.m.	Lyman, Moseley, Butler, Wood & Hale (1946); Lyman, Butler, Moseley, Wood & Hale (1946)	0–60
	S.f.	Henderson & Snell (1948)	0–100
	S.f.	Lyman, Butler *et al.* (1946); Lyman, Moseley *et al.* (1946)	0–80
	*S.f.**	Greenhut *et al.* (1948)*	0–80
Tryptophan	*S.f.**	Kuiken *et al.* (1947)*	0–16
	L.a.	Wooley & Sebrell (1945)	0–16
Threonine	*S.f.**	Greenhut *et al.* (1948)*	0–120
	S.f.	Henderson & Snell (1948)	0–120
Isoleucine	*L.m.**	Schweigert *et al.* (1949)*	0–80
	S.f.	Henderson & Snell (1948)	0–100
	L.a.	Henderson & Snell (1948)	0–100
Leucine	*S.f.**	Henderson & Snell (1948)*	0–120
	L.a.	Schweigert *et al.* (1949)	0–80
Valine	*S.f.**	Henderson & Snell (1948)*	0–120
Phenylalanine	*L.m.**	Schweigert *et al.* (1949)*	0–100
	L.m.	Henderson & Snell (1948)	0–100
	S.f.	Henderson & Snell (1948)	0–100

S.f. = *Strep. faecalis*, *L.m.* = *Leuc. mesenteroides* P60, *L.a.* = *Lb. arabinosus* 17-5. The micro-organisms were obtained from the National Collection of Industrial Bacteria, D.S.I.R., Teddington, England.

† Test extracts were compared with standard solutions of recrystallized amino-acids which in turn were checked against authentic samples in the author's possession. The figures indicate the sensitivity of the test.

Table 3. *Values for lysine and methionine obtained with* Leuconostoc mesenteroides *P60 and* Streptococcus faecalis

(Amino-acid N as percentage of total N)

Vegetable	Lysine *L.m.*	Lysine *S.f.*	Methionine *L.m.*	Methionine *S.f.*
Lentil	7·7	6·7	0·4	0·4
Green gram	8·2	7·5	0·7	0·8
Black gram	8·0	7·4	0·9	0·9
Finger millet	3·1	2·8	1·9	1·8
Sorghum	1·8	1·6	0·8	0·8

L.m. = *Leuconostoc mesenteroides* P60, *S.f.* = *Streptococcus faecalis*.

claimed for this method when applied to the crude protein of foodstuffs. Table 4 shows the values obtained for eight essential amino-acids in each of the materials studied, with these organisms and media. The amino-acid contents are shown along with figures for soya bean (Riesen *et al.* 1947), whole rice (Baptist, 1953) and ovalbumin (Block & Bolling, 1951) for comparison.

Table 4. *Values for essential amino-acids in lentil, green gram, black gram, finger millet and sorghum obtained by the methods indicated in Table 2*

(Amino-acid N as percentage of total N)

Amino-acid	Soya bean	Lentil	Green gram	Black gram	Finger millet	Sorghum	Rice	Oval-bumin*
Lysine	7·9	7·7	8·2	8·0	3·1	1·8	4·1	7·8
Methionine	0·8	0·4	0·7	0·9	1·9	0·8	1·0	3·2
Tryptophan	1·1	0·7	0·9	0·9	1·1	0·8	1·0	1·3
Threonine	2·8	2·6	2·6	2·6	2·9	2·4	2·6	3·1
Isoleucine	3·4	3·0	3·2	3·3	3·1	2·9	3·0	5·0
Leucine	5·1	4·6	5·5	5·9	5·9	8·7	5·4	6·3
Valine	4·0	3·7	4·5	4·5	6·3	5·2	5·0	4·8
Phenylalanine	2·7	2·4	3·2	3·1	2·7	2·6	2·8	4·0

* Values for ovalbumin (Block & Bolling, 1951) are quoted for comparison.

DISCUSSION

The nutritive value of the legume proteins is related to two factors, the content of essential amino-acids and the presence of tryptic inhibitors that interfere with the digestive processes (Borchers, Ackerson & Kimmett, 1947). The figures in Table 4 reveal a closely similar amino-acid pattern in the legumes and indicate that they are all uniformly rich in lysine and low in methionine and tryptophan. The early studies of Osborne (1924) and of Jones & Murphy (1924) revealed that diets containing legumes as sources of proteins produced as good growth in rats when supplemented with cystine as when casein was substituted for the legume proteins. It is evident now that the chief deficiency in the legume proteins is a deficiency of total sulphur amino-acids rather than of cystine alone, the added cystine in the early experiments serving to supplement the small amounts of methionine present (Russell, Taylor, Mehrhof & Hirsch, 1946; Richardson, 1948). Of the three materials studied, lentil has the lowest content of both methionine and tryptophan, which would suggest that the proteins of lentil have a low biological value, though Russell *et al.* (1946) were unable to find a direct correlation between nutritive value and methionine content of several varieties of peas and beans studied by them. It is necessary to note, however, that the legumes are low in cystine as well as in tryptophan and that there are appreciably different contents of some of the other amino-acids, such as valine and phenylalanine, which might cause differences in biological values. Of the legumes studied, black gram usually commands the highest price in the market and is popularly regarded as the most nutritious of locally available legumes. This view may find some support from the fact that its methionine content is a little higher than that of green gram and twice that of lentil.

In the cereals the chief differences are found in the leucine, valine, lysine and methionine contents. The leucine and valine contents are not, but the differences in lysine and methionine contents are, of great significance to rice-eating populations. Thus the replacement of rice protein by finger-millet protein would lower the lysine intake but considerably increase the methionine intake. It is of interest that finger millet is traditionally recommended by practitioners of indigenous systems of medicine as a rice substitute for diabetic sufferers. The coarse texture of the flour prepared from this millet usually brings about a smaller carbohydrate intake. Sorghum is low in lysine and is also low in methionine and tryptophan, but its higher protein content in some measure offsets this deficiency. However, a diet based on cereals in which sorghum is either the only or the predominating cereal would tend to be seriously deficient in those essential acids usually lacking in the diets of rice-eating populations.

SUMMARY

1. Eight essential amino-acids have been determined in three legumes (lentil, green gram and black gram) and in two cereals (finger millet and sorghum) commonly consumed in tropical countries.

2. The results indicate a similar essential amino-acid pattern for the legumes. Black gram had the highest content of methionine, and lentil had a somewhat lower content of the essential acids than the others.

3. Among the cereals, finger-millet protein had the highest methionine content reported hitherto for any cereal. Sorghum protein had a lower content of lysine than rice protein, and its methionine and tryptophan contents also appeared slightly lower.

4. The nutritive value of these foodstuffs is briefly discussed in relation to their contents of essential amino-acids.

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