

THE NUTRITIVE VALUE OF THE PROTEINS OF LEGUMINOUS SEEDS

S. VENKAT RAO, R. LEELA, M. SWAMINATHAN AND H. A. B. PARIJA.

Central Food Technological Research Institute, Mysore

(Received 15 Sept 1964)

1. Introduction
2. Production
3. Consumption
4. Method of preparation for consumption
5. Amino acid composition of legume proteins
6. Factors affecting the nutritive value of legume proteins:
 - 6.1 Trypsin inhibitor
 - 6.2 Hemagglutinins and growth inhibitors
 - 6.3 Other toxic factors
7. Elimination of toxic factors
8. Effect of heat processing on the nutritive value of legume proteins
9. Amino acid supplementation of legume proteins
10. Supplementary value of legume proteins to cereal proteins
11. Processed protein foods based on legumes
12. Use of processed legumes and protein foods containing legumes in the treatment of protein malnutrition in children
13. Supplementary value of processed legumes and protein foods containing legumes to cereal diets:
 - 13.1. Experiments with animals
 - 13.2. Experiments with preschool children
14. Conclusion

1. Introduction

The edible leguminous seeds are important sources of proteins in the dietaries of millions of people in Asian, African and South American Countries.^{1,2} Legumes have assumed great importance as a supplementary protein food in view of the acute shortage in the production of animal proteins and the wide prevalence of protein malnutrition in the countries mentioned above.^{3,4} During recent years investigations have been carried out by several groups of workers on the utilisation of legumes and oilseed meals in the prevention and treatment of protein malnutrition in children.^{5,6} The problems involved in the production and use of legumes as a source of protein in human diets were discussed in a joint conference of FAO and CTA held in 1958.⁷ A brief account of available information on the nutritive value of the proteins of leguminous seeds is given in this review.

2. Production

The common leguminous seeds consum-

ed in different parts of the world may be broadly grouped under three heads: (i) grams (ii) peas and (iii) beans. The term pulse is commonly used in India for edible legumes.⁸ Data regarding the production of legumes (excluding soyabean and peanut) are given in Table I. It is evident that India is the largest producer of legumes in the world, the average production per head being about 61g/day providing about 12 to 15g of protein.

3. Consumption

Data regarding the consumption of legumes as revealed by diet surveys are given in Table II. It is evident that the quantity of legumes consumed varies widely from country to country.

4. Method of preparation for consumption

Legumes are prepared for consumption in several ways such as whole legumes, dehusked dhal and legume flour. A large number of recipes for the preparation of legumes for consumption either by themselves or mixed with cereal or root flours is available in different countries. The

Table I. Estimated production and availability for human consumption of Legumes*

Country	Total production (thousand metric tons)	g/day/caput
Argentina	82	8.9
Australia	14	4.7
Austria	5	1.4
Belgium-Luxemburg	38	5.2
Canada	66	6.0
Ceylon	4	13.1
Denmark	36	11.2
Egypt	305	24.7
Finland	12	4.6
Germany Federal Republic	15	4.8
Greece	187	30.1
Honduras	201	30.2
India	10,617	60.8
Ireland	2	2.7
Israel	2.6	8.8
Italy	850	16.2
Netherlands	105	7.7
New Zealand	24	5.2
Norway	1	7.1
Pakistan	900	20.5
Philippines	40	5.3
Portugal	55	17.3
Switzerland	—	5.8
Sweden	38	4.1
Turkey	282	26.3
Union of South Africa	58	5.3
United Kingdom	62	11.0
United States of America	914	10.6
Uruguay	3.7	5.0

* Exclusive of peanut and soya bean. (Ref. 4, Patwardhan, V. N. 1962)

different methods of cooking legumes may be grouped as follows.^{7,9-11}

Whole legumes: (i) Soaking and cooking in excess of water and rejecting the water.

(ii) toasting and

(iii) cooking after germination.

Dehusked split legumes: (i) soaking and cooking in water and

(ii) cooking in water to a mash

Legume flour: The legume flours are usually mixed with cereal and tuber flours and made into unleavened bread, pancakes or puddings.

5. Amino acid composition

The essential amino acid composition of a number of leguminous seeds has been

Table II. Consumption of pulses in various countries as ascertained by diet surveys†

Country	Consumption (g/caput/day)	Remarks
Philippines	14	
Western Java	6	
Mexico	42	
Venezuela	80	Rural workers
Venezuela	89	Urban workers
Guatemala	44	Poor Ladinos
Guatemala	57	Other Ladinos
Guatemala	51	Poor Indians
Guatemala	58	Other Indians
Togoland	13-140	
Countries (13)		
South of Sahara	10-150	
India	14-114	Per consumption unit
New Guinea	3	
New Guinea	27	Includes peanuts
Japan	5.5-7.8	Exclusive of 44 to 65 gm. of soya and its products
Haiti	30	

† Ref. 4, Patwardhan, V. N. (1962)

determined by several workers.¹²⁻¹⁴ Some of the available data is summarised in Table III. The legume proteins are, in general, good sources of lysine and threonine in which cereals are deficient. They are, however, poor sources of sulphur amino acids and tryptophan.

6. Toxic factors in certain legumes and their effect on the nutritive value of the proteins

The results of investigations carried out by different workers have shown that many legumes contain toxic factors which affect adversely the nutritive value of their proteins.¹⁵ Most of these factors can be inactivated by optimal heat treatment and/or by leaching out in water. The various toxic factors may be broadly grouped under the following heads: (i) trypsin inhibitors (ii) hemagglutinins and growth inhibitors and (iii) other toxic factors. The available information is summarised in Table IV.

6.1 Trypsin inhibitors: Osborne and Mendel¹⁶ first observed that raw soya bean will not support growth of rats while

Table III. Amino acid composition of legume proteins

Reference No.	Valine	Tryptophan	Threonine	Phenylalanine	Methionine	Cystine	Lysine	Leucine	Isoleucine	Histidine	Arginine
Aconite bean (<i>Phaseolus aconitifolius</i>)	3.2	0.7	—	4.5	0.9	0.5	5.4	6.7	5.0	3.3	—
Bengal gram (chickpea) (<i>Cicer arctinum</i>)	5.4	0.6	4.8	5.0	1.7	0.8	6.4	8.0	6.0	2.3	6.9
Blackeyed pea (<i>Vigna sinensis</i>)	5.4	1.4	3.8	5.2	0.7	—	9.6	10.4	4.8	3.0	7.8
Black bean (<i>Phaseolus vulgaris</i>)	5.8	1.1	5.0	5.7	1.1	1.8	8.0	3.3	5.1	2.8	6.6
Black gram (<i>Phaseolus mungo</i>)	6.4	0.5	4.3	5.4	1.1	0.7	6.0	7.2	5.5	2.7	5.7
Cow pea (<i>Vigna catjang</i>)	6.3	0.6	3.2	5.2	1.0	0.7	6.2	7.5	4.9	3.1	6.9
Field bean (<i>Dolichos lablab</i>)	5.6	0.5	3.3	5.3	0.7	1.0	8.1	8.9	6.0	2.8	9.2
Green gram (<i>Phaseolus radiatus</i>)	6.4	0.4	3.5	5.9	1.0	0.6	7.0	7.7	6.3	2.7	6.3
Horse bean (<i>Vicia faba</i>)	5.6	0.5	3.3	5.2	0.7	0.5	5.5	7.6	5.5	2.9	6.5
Horse gram (<i>Lathyrus sativus</i>)	5.1	0.9	2.6	3.4	1.0	0.4	8.3	6.6	6.6	2.5	7.8
Horse gram (<i>Dolichos biflorus</i>)	4.7	0.4	2.3	4.1	0.4	1.4	9.2	7.9	6.7	3.0	5.4
Kidney bean (<i>Phaseolus vulgaris</i>)	5.4	0.6	3.8	8.5	0.8	—	8.3	7.9	4.9	2.4	5.9
Lentil (<i>Lens esculenta</i>)	5.4	0.3	2.9	5.3	0.8	—	9.2	5.4	5.4	2.1	7.0
Lima bean (<i>Phaseolus limerisii</i>)	5.8	—	4.8	4.1	1.2	0.8	7.6	8.2	5.6	3.0	6.4
Navy bean (<i>Phaseolus vulgaris</i>)	6.4	—	4.8	5.6	1.2	—	7.9	8.0	4.8	2.9	6.9
Pea (<i>Pisum sativum</i>)	7.8	—	4.9	6.1	1.3	—	11.4	10.9	8.4	2.7	17.7
Pinto bean (<i>Phaseolus vulgaris</i>)	6.2	0.7	4.3	5.8	1.0	1.2	6.8	8.2	5.7	2.9	5.4
Red gram or pigeon pea (<i>Cojanius calian</i>)	5.1	0.2	3.4	9.1	0.9	0.8	6.4	7.0	5.7	3.4	5.4

Table IV. The effect of heat on the nutritive value of legumes and the presence or absence of toxic components*

Botanical Name	Common name	Nutritive value	Trypsin inhibitor	Hemagglutinin	Other toxic factors
1	2	3	4	5	6
<i>Cajanus indicus</i> (cajan)	Red gram	+141, 142	+51, 56	-56, 58	—
	Pigeon pea	±100, 143, 144	—	—	—
	Dhal arhar	—	—	—	—
	Jack bean	+55, 145	—	—	—
	Sword bean	?	—	—	—
	Partridge pea	+55	—	—	—
	Bengal gram	+143, 144, 151	—	—	—
	Chickpea	±141, 142, 152	—	—	—
	Garbanzo	-153	—	—	—
					Saponin ⁸⁶
					Saponin ⁸⁸
					Cyanogenetic glucoside ⁷

1	2	3	4	5	6
<i>Cyanopsis psoraleoides</i>	Guar bean	± 55	-26, 55	?	
<i>Delichos lablab</i>	Horse gram, Horse bean Field bean	+ 55, 144, 152, 103	+ 51, 109, 155, -157	+ 51, 158, 68	
<i>Faba vulgaris</i>	Field bean	+ 53	+ 109, -157, 53, 111, 159	+ 63, 71, 160	Goitrogenic factor, saponin, anticoagulant, diuretic prin- ciple, toxic histone ¹¹
<i>Glycine max</i>	Double bean Soya bean	+ 15	+ 15		B-Glutamyl amino-propio- nitrile Human lathyrisim
<i>Lathyrus odoratus</i>	Sweet pea	± 93, 161	+ 26	+ 154	
<i>Lathyrus sativus</i>	Khesari dhal	+ 141, 142	?	?	
<i>Lens esculenta</i>	Lentil	+ 55, 141, 142, ± 144 - 153, 162	+ 51, 109, 163, -26, 55	+ 64	
<i>Lespedeza stipuleacea</i>	Lespedeza	± 55	+ 26	?	
<i>Phaseolus angularis</i>	Adzuki bean	± 164	?	?	
<i>Phaseolus aureus</i>	Mung bean	+ 152	+ 26, 109, 166, 167	+ 154	
<i>Phaseolus (radialis)</i>	Green bean	± 55, 141, 142, 144, 165	+ 26, 54, 171, 52, 172, 173	+ 174, -176	Cyanogenetic glucosides ¹²
<i>Phaseolus lunatus</i>	Lima bean	+ 58, 168, 59, 169, 170	+ 109, 177	? - 78	
<i>Phaseolus mungo</i>	Black gram	± 141, 142, 144, 152	+ 19, 26, 51, 109	+ 154, 63, 65, 75, 179, 76,	Goitrogenic factor ¹³
<i>Phaseolus vulgaris</i>	Navy bean	+ 162, 58, 59, 169, 60, 61 178		180	
<i>Pisum sativum</i>	Kidney bean Pinto bean French bean Black bean White bean Field or garden pea	+ 152 ± 153, 162, 181, 182 - 59, 169, 183, 186 + 49, 55, 187 + 55	-26	± 64, 66	Goitrogenic factor + ¹⁴
<i>Sitizobium deeringianum</i>	Velvet bean		+ 26	?	
<i>Vicia faba</i>	Fava bean Broad bean	± 55	+ 26 -26, 55	+ 154, 160, 188-191	Favism cyanogenetic gluco- side. ^{15, 16}
<i>Vicia sativa</i>	Common vetch	± 55	-26, 55	154, 64, 192	Cyanogenetic glucoside, alkaloid ¹⁷
<i>Vigna sinensis</i>	Southern pea Cow pea	+ 55 ± 169	+ 26, 51, 109	+ 15, 16, 18-11	

1 Ref. 15, Leiner, I. E. (1962)

* + indicates that the nutritive value is improved by heat, or positive evidence for the presence of a trypsin inhibitor or hemagglutinin; - indicates that the nutritive value is adversely affected by heat, or the absence of a trypsin inhibitor or hemagglutinin; ? indicates that information is not available; # indicates that the untritive value is unaffected by heat.

cooked soya bean promoted good growth. Melnick *et al.*¹⁷ reported that the methionine in raw soya bean was liberated more slowly by the proteolytic enzymes of the intestines than the other essential amino acids. This concept was strengthened by the discovery,¹⁸⁻¹⁹ purification²⁰⁻²¹ and characterisation²²⁻²⁷ of a heat labile protein in soya bean which inhibited the proteolytic activity of trypsin. Active antitryptic fractions from unheated soya bean have been shown to retard the growth of rats,²⁸⁻³⁰ mice³¹ and chicks.²⁰⁻³² Because the protein efficiency of partially heated soya bean flours increased in proportion to the destruction of the trypsin inhibitor, Westfall and Hauge³³ concluded that the trypsin inhibitor was the major cause of the poor utilisation of the protein in raw soya bean. Trypsin counteracted the growth depression observed with chicks³⁴⁻³⁶ and rats³⁷ receiving diets containing raw soya bean.

There appeared to be little doubt, therefore, that the poor growth-promoting quality of raw soya bean could be attributed to a large extent to a trypsin inhibitor. Although Melnick's hypothesis,¹⁷ referred to earlier, would appear to explain the mode of action of a trypsin inhibitor, other observations do not support his theory. *In vitro* studies have shown that the trypsin inhibitor does not specifically retard the enzymatic release of methionine, but seems to affect all the amino acids to the same extent.³⁸⁻⁴⁰ Active antitryptic preparations have been shown to retard the growth of rats⁴¹ and mice³¹ even when added to diets containing predigested protein when intestinal proteolysis would normally play a part with respect to the availability of the essential amino acids.

It has been noted that chicks⁴² and rats^{33,44} fed raw soya bean oil meal develop marked hypertrophy of the pancreas. Contrary to what had been generally assumed the amount of trypsin found in the intestines of rats,^{3,45} and chicks⁴⁷ fed raw soya bean was actually greater than that observed in animals fed the heated soya bean. A crystalline preparation of the trypsin inhibitor produced the same effects as raw soya bean oil meal.⁴⁷ It would appear, therefore, that the growth depression

caused by the trypsin inhibitor is little to do with an inhibition of intestinal proteolysis, but may be the result of an endogenous loss of essential amino acids from a hyperactive pancreas which is responding in a compensatory fashion to the effects of the trypsin inhibitor. The loss in methionine would be particularly acute since soya bean protein is deficient in this amino acid. Whether this explanation will withstand the test of further experimentation remains to be seen.

Trypsin inhibitors have also been found in a large number of other legumes and their presence provides the most likely explanation for the observation that heating increases the *in vitro* digestibility of some leguminous proteins.⁴⁸⁻⁵⁰ Jaffe⁵¹ observed that those legumes which had the highest trypsin inhibitor activity were also those in which the digestibility as measured *in vivo* with rats, was most improved by cooking. A direct inhibition of the growth of mice by purified preparations of a trypsin inhibitor from lima beans was demonstrated by Fauber *et al.*⁵² but Sohoni *et al.*⁵³ were unable to show any growth inhibition in rats by an inhibitor purified from the double bean. Even in the case of soya bean, it is not certain that the trypsin inhibitor exerts its deleterious effect on growth by an inhibition of intestinal proteolysis since Klose *et al.*⁵⁴ found that fractions of lima bean protein which were high in antitryptic activity inhibited the growth of rats fed acid hydrolyzed caseins.

6.2 Hemagglutinins and growth inhibitors: An examination of the data in Table IV reveals no obvious relationship between the beneficial effect which heat produces on some legumes and the presence or absence of a trypsin inhibitor, a conclusion which has also been reached by Borchers and Aekerson⁵⁵ and Jaffe.⁵⁶ This discrepancy has served to focus attention on the possible presence of other growth inhibitors in raw legumes which are destroyed by heat. Numerous reports may be found in the older literature describing the toxic effects produced in animals by the ingestion of some legumes. Thus Osborne and Meadel⁵⁷ in 1912 reported that rats would not grow if the source of dietary protein

was derived from the kidney bean, (*Phaseolus vulgaris*) and that prolonged ingestion of raw beans resulted in death of rats. Since that time, numerous workers^{56,58-62} have reported similar observations on a wide variety of beans which are botanically classified as *Phaseolus vulgaris*. In addition to the latter, Jaffe⁵⁶⁻⁶¹ also found a high degree of toxicity in rats fed diets containing *Dolichos lablab*, *Canavalia ensiformis* and *Phaseolus lunatus*. Since this toxicity could not be counteracted by a casein digest, Jaffe¹⁰³ concluded that the toxic principle involved here was not a trypsin inhibitor.

The presence of substances in legumes which have the ability to agglutinate the red blood cells from various species of animals has long been recognised.⁶³⁻⁶⁷ These hemagglutinins are sometimes referred to as phytoagglutinins or lectins⁶⁸ and their distribution among the legumes is shown in Table IV. Although a toxic reaction may be produced in animals by the direct injection of the purified hemagglutinin of the jack bean^{69,70} and the soya bean,⁷¹⁻⁷² Liener⁷³⁻⁷⁴ called attention to the fact that the oral ingestion of the purified hemagglutinin of the soya bean would inhibit the growth of rats. The hemagglutinin of *Phaseolus vulgaris* has been recently purified^{75,76} and Jaffe⁷⁷ and Honavar *et al.*⁷⁸ have shown that purified preparations of this hemagglutinin can markedly inhibit the growth of rats. Jaffe⁷⁷ is of the opinion that the action of the hemagglutinin is to combine with the cells lining the intestinal wall and thus to interfere with the intestinal absorption of nutrients.

At this point, it may be well to point out that there have been several instances reported in which manifestations of toxicity have been observed in subjects who have eaten insufficiently cooked scarlet runner beans⁷⁹ (*Phaseolus coccineus* or *multiflorus*) or inadequately heated kidney bean flour.⁸⁰ Cartwright and Wintrobe⁸¹ described symptoms of nausea, vomiting and diarrhoea in Japanese prisoners of war who were fed raw soya bean. It would appear logical to suspect the hemagglutinins as being responsible at least in part, for the toxic manifestations observed in

human subjects ingesting certain raw legumes.

6.3 Other toxic factors: Other toxic factors present in legumes are goitrogenic factor,⁸²⁻⁸³ cynogenetic glucosides⁸⁴⁻⁸⁶ and saponins.^{87,88} In addition certain classes of legumes contain specific toxic principles, e.g. the toxic principles present in the legumes of *lathyrus* family cause the disease lathyrism in human beings⁸⁹⁻⁹² and *odoratism* in animals⁹³ and the toxic principles present in *vicia faba* cause the disease *savism* characterised by hemolytic anemia in animals and human beings.^{15,94}

7. Elimination of toxic factors

The toxic factors present in legumes can be eliminated by heat processing under controlled conditions and by leaching out the toxic principles by soaking in cold or warm water and discarding the water.^{15,95}

Heat processing: The toxic principles which are inactivated by controlled heat processing are trypsin inhibitors,¹⁵ hemagglutinins and growth inhibitors,¹⁵ goitrogenic agents,⁸⁶ saponins,⁸⁷ and the toxic principles causing *savism*.⁹⁴

Leaching in water: The toxic principles which are leached out in water include cynogenetic glucosides,⁸⁵ and toxic factors present in legumes of the *lathyrus* family.^{91,97}

8. Effect of heat processing on the nutritive value of legume proteins

Most of the antinutritional or toxic effects of legumes which have been described can be partially or wholly eliminated by the proper application of heat.^{15,98} This effect is manifested by a general enhancement of the nutritive value of the proteins of legumes (Table V). It is this relative ease with which these toxic components can be removed, in most cases, by appropriate methods of cooking that has no doubt contributed to the popularity of legumes as a staple food in the diet in many countries. Methods of preparing legumes for human consumption have been described in detail by several workers.^{79-12,99} The nutritive value of the proteins in several legumes have been compared when fed raw and after autoclaving (Table V). Those found to improve on heat processing include the field bean¹⁰⁰ (*Dol-*

Table V. Effect of heat processing on the nutritive value of legume proteins as judged by growth of rats.

Source of protein	Conditions of heat treatment	Protein content of diet (%)	Duration of experiment (days)	Gain in body weight (g/week)	PER	Reference No.
1	2	3	4	5	6	7
Black bean (<i>Phaseolus vulgaris</i>)	Raw	14.1	28	—	—	201
" "	Autoclaved at 16 lbs pressure for 30 mts.	15.5	"	12.5	1.1	"
" "	Autoclaved 16 lbs. pressure for 60 mts.	14.8	"	8.8	0.9	"
Black gram (<i>Phaseolus mungo</i>)	Raw	12	21	6.7	—	141
" "	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	7.0	—	"
Blackeyed pea (<i>Vigna sinensis</i>)	Raw	"	20	9.8	—	55
" "	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	15.4	—	"
Chickpea (sample 1) (<i>Cicer arletinum</i>)	Raw	"	21	11.3	—	141
" "	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	11.6	—	"
" (sample 2)	Raw	"	28	5.6	1.3	143
" "	Autoclaved at 15 lbs. pressure for 15 mts.	"	"	14.7	2.4	"
Cow pea (Sample 1) (<i>Vigna catieng</i>)	Raw	"	21	10.0	—	193
" (Sample 2)	Cooked for 2 hours	"	"	13.6	—	"
" "	Raw	"	"	9.9	—	"
" "	Cooked for 2 hours	"	"	10.0	—	"
Field bean (<i>Dolichos lablab</i>)	Raw	"	35	-1.8	Negative	100
" "	Autoclaved at 15 lbs pressure for 30 mts.	"	"	-1.4	Negative	"
Green gram (Sample 1) (<i>Phaseolus radiatus</i>)	Raw	12	21	5.0	—	141
" "	Autoclaved at 15 lbs pressure for 30 mts.	"	"	7.0	—	"
" (sample 2)	Raw	"	"	9.4	1.4	160
" "	Autoclaved at 15 lbs pressure for 15 mts.	"	"	9.9	1.3	"
Horse bean (<i>Vicia faba</i>)	Raw	"	20	11.0	—	55
" "	Autoclaved at 15 lbs pressure for 30 mts.	"	"	14.7	—	"
Horse gram (<i>Dolichos biflorus</i>)	Raw	12	36	4.4	0.7	100
" "	Autoclaved at 15 lbs. pressure for 15 mts.	"	37	10.7	1.7	"
Jack bean (<i>Canavalia ensiformis</i>)	Raw	"	20	2.1	—	55
" "	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	9.8	—	"
Kesari dhal (<i>Lathyrus sativus</i>)	Raw	21	21	2.0	—	141
" "	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	2.4	—	"
Kidney bean (<i>Phaseolus vulgaris</i>)	Raw	10	56	—	—	59
" "	Autoclaved at 15 lbs. pressure for 45 mts.	"	"	—	0.8	"
Lentil (Sample 1) (<i>Lens esculenta</i>)	Raw	12	21	2.0	—	141
" "	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	5.0	—	"
" (sample 2)	Raw	"	35	7.2	0.9	100
" "	Autoclaved at 15 lbs. Pressure for 15 mts.	"	"	6.5	0.8	"
Lintil bean (sample 1) (<i>Phaseolus lunatus</i>)	Raw	10	28	-2.7	—	169

	1	2	3	4	5	6	7
Lima bean (sample 1) (<i>Phaseolus lunatus</i>)		Autoclaved at 15 lbs. pressure for 30 mts.	"	"	6.2	—	"
Navy bean (<i>Phaseolus vulgaris</i>)		Raw	"	56	—	—	59
"	"	Autoclaved at 15 lbs. pressure for 45 mts.	"	"	—	0.9	"
Pinto bean (<i>Phaseolus vulgaris</i>)		Raw	"	28	-4.5	—	169
"	"	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	2.5	—	"
Pea (sample 1) (<i>Pisum sativum</i>)		Raw	12	21	3.3	—	141
"	"	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	5.5	—	"
"	(sample 2)	Raw	10	56	3.6	1.1	115
"	"	Baked for 90 mts. at 140°C	"	"	2.5	0.8	"
"	"	Autoclaved at 17 lbs. pressure for 90 mts.	"	"	1.9	0.6	"
Pea (Alaska) (<i>Pisum sativum</i>)		Raw	10	42	5.6	1.4	184
"	"	Baked	"	"	5.9	1.1	"
Red gram (sample 1) (<i>Cajanus cajan</i>)		Raw	12	21	-1.6	—	141
"	"	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	7.6	—	"
"	(sample 2)	Raw	"	28	11.8	2.1	143
"	"	Autoclaved at 15 lbs. pressure for 15 mts.	"	"	8.3	1.7	"
Snap bean (<i>Phaseolus vulgaris</i>)		Raw	10	10	—	—	202
"	"	cooked	"	"	0.07	-0.2	"
Velvet bean (<i>Mucuna deeringianum</i>)		Raw	12	20	-1.8	—	55
"	"	Autoclaved at 15 lbs. pressure for 30 mts.	"	"	8.8	—	"

chias lablab), navy bean⁵⁹ (*Phaseolus vulgaris*), Jack bean⁵⁵ (*Canavalia ensiformis*), velvet bean⁵⁵ (*Mucuna deeringianum*), adzuki bean⁵⁵ (*Phaseolus angularis*), horse bean,⁵⁵ horse gram¹⁰⁰ (*Dolichos biflorus*) and *khesari dahi*^{100,101} (*Lathyrus sativus*). Those reported not to so improve include the partridge pea⁵⁵ (*Chamaecrista fasticulata*), guar bean⁵⁵ (*Cyamopsis psoraloides*), Lespedeza (*Lespedeza stipulacea*) and the common vetch⁶⁵ (*Vicia sativa*). There are some reports in the literature¹³ to the effect that the nutritive value of pea proteins is impaired as a result of heat-processing, but there are also a couple of reports to the contrary.¹³ Conflicting data have been reported regarding the effect of heat-processing on the biological value of the proteins present in a number of other legumes such as Bengal gram,^{100,101} pigeon peas and cow peas^{55,59} (*Vigna sinensis*). Parching improves the nutritive value of the proteins in the Bengal gram (chickpea) green gram, black gram, horse gram and dried peas.¹⁰² Data regarding the effect of heat processing on the nutritive value of the legume proteins are given in Tables IV & V.

As noted in Table IV those legume proteins whose nutritive value is enhanced by heat generally contain more than one toxic component, so that it frequently becomes difficult to know which toxic factor is in fact responsible for the poor nutritive value of the unheated legume proteins. In such cases an observed correlation between an improvement in nutritive value and the destruction of a specific toxic component does not necessarily establish a casual relationship. In most instances when heat treatment does produce a positive effect, it seems to make little difference whether the legumes are cooked in water, autoclaved or parched. Notable exceptions, however, are *Phaseolus vulgaris* and *Dolichos lablab* which require preliminary soaking prior to cooking or autoclaving in order to eliminate completely the toxicity of the raw bean.^{103,104} De Vries and vander Lee¹⁰⁵ suggested that a kidney bean paste might be substituted for almond paste in baked foods provided the beans were soaked in water for twelve to twenty-four hours followed by autoclaving for about forty minutes at 105 to 110°C. Recommendations for the use of these

legumes in products intended for human consumption without prior soaking, such as the kidney bean for extending wheat flour^{74,106} and *Dolichos lablab* for making bean cakes¹⁰⁷ should be viewed with caution. That the trypsin inhibitor of soya bean is destroyed by heat is amply supported by experimental evidence,¹⁰⁸ and there appears to be in fact an inverse correlation between the trypsin inhibitor content of partially heated soyabean meals and their nutritive value.³³ The heat lability of trypsin inhibitors from other legumes has not been investigated to any great extent, but the available information would indicate that some of these inhibitors, such as those from the lima bean,⁵² *Phaseolus vulgaris*¹⁰⁹ and *Faba vulgaris*^{110,111} may be more heat stable than the soya bean trypsin inhibitor. The increased digestibility of cooked legumes as measured *in vitro*^{48,49} and *in vivo*⁵¹ is presumably due to the destruction of trypsin inhibitors contained in them. The hemagglutinin of soya bean is destroyed by autoclaving, and the improvement in nutritive value effected by heat parallels the extent to which hemagglutinin has been destroyed.¹¹² Partial heat inactivation of the purified hemagglutinin from *Phaseolus vulgaris* showed a parallel destruction of toxicity and hemagglutinating activity.⁷⁷

9. Amino acid supplementation of Legume Proteins

Legume proteins are good sources of lysine and threonine but are deficient in sulphur amino acids and tryptophan.¹¹³ The nutritive value of several legume proteins is seriously affected by the growth inhibitors present and the proteins of several raw legumes do not promote growth in animals.¹⁵ Optimal heat processing has been shown to inactivate these and bring about an increase in the nutritive value of the proteins.¹⁵ The protein efficiency ratios of optimally heat processed legume proteins range from 1.5 to 2.0 depending on the legume.¹³ When supplemented with methionine, the nutritive value of legume proteins are known to improve to a marked extent.¹¹⁴ For example the nutritive value of the proteins of Alaska

field pea is increased almost to that of casein when supplemented with methionine.¹¹⁵ In the case of split pea, lentil and pigeon pea, addition of tryptophan and threonine along with methionine brought about a marked improvement in the nutritive value of the proteins.¹⁰¹

10. Supplementary Value of Legume Proteins to Cereal Proteins

Since legume proteins are rich in lysine and threonine, they supplement to a marked extent those of cereal proteins which are, in general, limiting in these amino acids. A mixture of cereals and legumes contains proteins of superior nutritive value as compared with that of cereal or legume proteins.^{116,119} The proteins of Bengalgram (chickpea), blackgram, green gram and red gram (Pigeon pea) supplement the proteins of wheat, sorghum and pearl millet to a marked extent.¹¹⁸

11. Processed Protein Foods Based on Legumes

Among the legumes, parched chickpea has been used rather extensively either by itself or in combination with other protein foods as a supplement to the diets of children and for the treatment of protein malnutrition in children.⁴ Other legumes tested so far with children include black bean and cow pea.⁵ A brief account of some processed products based on legumes and suitable for use as a supplement to the diets of children is given below:

Parched chickpea: This is prepared extensively on a cottage industrial basis throughout India. The process is briefly as follows: The chickpea is soaked in water and then parched by keeping it in contact for a few minutes on a hot sand bath in a shallow vessel. The parched pea is dehusked. Since it is a precooked product, it is widely used along with parched cereals as a snack by children and adults in India.¹⁰²

Roasted chickpea dhal: This is prepared by roasting the dehusked dhal in an electric roaster at 120-130°C for 8-10 minutes. The roasted dhal is powdered coarsely and used in the preparation of processed

protein foods. eg. Indian Multipurpose food,¹⁴⁰ Malt food etc.¹²¹

Balanced Malt Food: The balanced malt food consists of a blend of cereal malt (37%) low fat peanut flour (30%), roasted chickpea flour (20%) and skim milk powder (10%) and fortified with essential minerals and vitamins.¹²¹ Two ounces of the product will provide about one third to one half the daily requirements of protein, calcium and certain vitamins of preschool children.

Indian Multipurpose Food: Indian multipurpose food consists of a blend of 3 parts of low fat groundnut flour and one part of roasted chickpea flour fortified with calcium salts and certain vitamins.¹²⁰ It is available in three forms (1) *Formula A*: Seasoned; *Formula B*: Unseasoned and *Formula C*: with added skim-milk powder (80 parts of formula B and 20 parts of skim milk powder). Two ounces of the product will provide about half the daily requirements of protein, calcium and certain vitamins of preschool children

Protein food based on chickpea, coconut meal and low fat groundnut flour: This consists of a 1:1:2 blend of roasted chickpea flour, expeller coconut meal and low-fat groundnut flour, fortified with calcium salts and vitamins.¹²²

Precooked roller dried protein food: This consists of a blend of roasted chickpea flour 30 parts, refined wheat flour 25 parts and groundnut flour 45 parts. The blend is cooked with water fortified with calcium salts and vitamins and dried on a roller drier.¹²³ The product contains about 32% protein. Two ounces of the product will provide about one third the daily requirements of proteins, calcium and certain vitamins of weaned infants.

12. Use of processed legumes and protein foods containing legumes in the treatment of protein malnutrition in children

Several investigators have reported the results of studies on the treatment of kwashiorkor with legumes or blends of legumes with oilseed meals or cereals.^{4,5} The legumes so far tested include chickpea, cow pea, black bean and a mixture of chickpea and blackgram or chickpea and certain oilseed meals. The results are briefly summarised below.

Chickpea and blend of chickpea and skim milk powder: The efficiency of parched chickpea or a combination of chickpea and skim milk powder (4 parts of protein from chickpea and 1 part from skim milk powder) in the treatment of kwashiorkor has been studied by some groups of workers.^{4,124} The results (Table VI) showed that a mixture of chickpea and skim milk powder was more effective than chickpea alone in initiating cure of kwashiorkor.⁴

Chickpea-blackgram dhal blend: A 1:1 mixture of chickpea and blackgram dhal (Table VI) was found to be less effective than chickpea in initiating cure of kwashiorkor.⁴

Chickpea-oilseed meal blend: The efficacy of two blends viz. Mysore Food A (a blend of roasted chickpea flour 25 parts, low fat groundnut flour 74 parts and lucerne powder 1 part) and Mysore Food B (roasted chickpea 25 parts, low fat groundnut flour 49 parts; low fat sesame flour 25 parts and lucerne powder 1 part) in the treatment of kwashiorkor has been tested. The results (Table VI) showed that both the blends were moderately effective in initiating cure of kwashiorkor.⁴

Indian Multipurpose Food: Indian Multipurpose Food based on a 1:3 blend of chickpea flour and low fat groundnut flour has been found to be moderately effective in the treatment of kwashiorkor.^{125,126} Indian Multipurpose Food Formula C (based on a 20:20:60 blend of chickpea, skim milk powder and low fat groundnut flour) has been reported to be highly effective in the treatment of kwashiorkor.¹²⁷

Maize-blackbean: The research group on infantile malnutrition of the children's hospital in Mexico City, have investigated the nutritive value of a mixture of lime-treated maize (as tortillas) and black beans in the 4:5 proportion by weight common in some Mexican diets. The slope of the weight gain of rats fed this mixture was only 0.93 ± 0.037 as compared with 1.84 ± 0.040 when 22% of the protein came from milk.¹²⁸ Similarly, the absorption and retention of nitrogen by malnourished children was variable from child to child much lower than with isocaloric diets containing isonitrogenous quantities of

Table VI. Results of treatment of children suffering from Kwashiorkor with skim milk and vegetable protein mixture*

Diet	Cases (No.)	Disappearance of oedema (No. of days)	Minimum weight reached (No. of days)	one lb. gain in body weight after reaching minimum weight (No. of days)	Diarrhoea controlled (No. of days)	Rise in serum albumin (g/100ml)		
						10th day	30th day	
Mysore food A	21	4-22 12.5	2-18 7.1	1-30 7.4	2-27 7.8	0.14	0.86	Srikantia and Gopalan 1960
Mysore food B	31	2-30 13.1	3-21 8.6	1-17 6.1	2-16 6.3	0.12	0.77	
Mysore food A	25	1-29 5.4	1-20 6.4	7-30 16.8	2-7 4.3	0.5	1.42	Chaudhuri <i>et al.</i> (unpublished 1959)
Mysore food B	25	1-11 4.6	1-15 6.4	4-30 14.2	2-10 4.2	0.6	1.54	
Skim milk powder	28	1-16 6.6	1-20 8.3	6-25 13.8	2-12 6.4	0.8	1.90	Achar <i>et al.</i> (unpublished 1959)
<i>Cicer arietinum</i> and skim milk powder (4:1)	21	9-33 19	2-31 11.7	3-11 6.4	5-15 9.5	0.3	1.22	
<i>Cicer arietinum</i> and <i>Phaseolus mungo</i> (1:1)	7	13-40 28.0	8-37 20.7	—	—	—	1.36	Achar <i>et al.</i> (unpublished 1959)
Skim milk powder	26	9-37 18.8	2-23 9.1	4-10 6.1	8-22 15.3	0.76	1.66	
<i>Cicer arietinum</i>	56	4-45 18	1-18 5.8	0-51 9.5	2-13 5.7	0.4	1.04	Venkatachalam <i>et al.</i> 1956
<i>Cicer arietinum</i> plus rice (1:1.3)	19	4-68 17	3-8 5.2	0-64 14	3-19 8.0	0.20	0.63	
Skim milk powder	49	3-33 12	2-18 5.4	0-24 7.3	2-23 10.5	0.75	1.24	

* Ref. 4 Patwardhan, V. N., 1962.

milk. The addition of tryptophan and lysine to the Maize-bean diet greatly improved nitrogen absorption and retention in all 4 cases.¹²⁹ Scrimshaw *et al.*¹³⁰ also found that a mixture of corn *masa* and black beans was not effective in initiating cure in cases of protein malnutrition in children. The obvious need is to find an inexpensive local protein source which will supply additional protein and also help to correct the tryptophan and lysine deficiency of maize or a mixture of maize and beans as above.

Maize-cowpea-maize germ: In common with other investigators, Hansen and co-workers¹³¹ found that milk initiated cure of kwashiorkor without difficulty. A two-component mixture of 66% maize meal, and 33% cow pea meal, however, did not initiate cure in the 3 cases treated with it while a 3-component mixture made up of equal parts of maize meal, maize germ

and cow pea, did bring about satisfactory recovery in the 1 case in which it was employed. It should be noted that it would require 238g of the dry 3-component mixture and 267 g. of the 2-component mixture to supply the essential amino acids contained in 100g. of skim milk. Since these formulae also required relatively greater dilution after cooking, it was difficult to feed enough of the vegetable formula to supply protein needs. It appears likely that excessive bulk for protein content is the cause for the lack of success in initiating cure in kwashiorkor with mixtures of maize and beans, and this is the same problem encountered with other common legume preparations.¹³² The differences in biological value of the protein are clearly reflected in the nitrogen retentions which averaged 13-14% for milk, 8.8% for the 2-component mixture and only 5.7% for the 3-component

mixture. The percentage of nitrogen absorbed was also low with the vegetable mixtures. It was concluded that either the 2 or the 3-component mixture is satisfactory to promote consolidation of cure and to prevent kwashiorkor, but only the 3-component mixture is of sufficiently good concentration and protein quality to be satisfactory for use in treatment.

Bean-peanut blend: The nitrogen balance data furnished by De Maeyer and Vanderborght on the supplementation of a mixed diet with either skim milk, a combination of beans and peanuts, peanut flour or soyabean fed to children convalescing from kwashiorkor, merit further mention. Their basal diet supplied from 11 to 47% of the nitrogen intake from rice, bread and banana flour, and the remainder from the test supplement. Nitrogen absorption and retention were measured from each supplement at intakes varying from 2.0 to 5.8 g. protein per kg. Skim milk had the highest supplementary value by a considerable margin and the combination of beans and peanuts came next, followed by soyabean flour and peanut flour.

13. Supplementary value of processed legumes and protein foods containing legumes to poor diets based on cereals or a blend of tapioca-cereals

The supplementary value of certain processed legumes and protein foods containing legume to poor diets based on cereals or a blend of tapioca flour and maize has been studied by several workers in albino rats and in children.^{4,5,133} The results are summarised below:

13.1. *Experiments with albino rats:* Desikachar *et al*¹³⁴ showd that chickpea when incorporated at 15% level exerted a significant supplementary value to poor Indian rice diet. Tasker *et al*.¹³⁵ found that chickpea when incorporated at 20% level in a low protein rice-tapioca diet so as to provide about 4% extra protein promoted a significantly greater gain in body weight in albino rats than that observed with the control rice-tapioca diet (Table VII). In a later study, Tasker *et al*.¹³⁶ observed that chickpea when incorporated in a low protein maize-tapioca diet so as to provide about 15% extra protein made up the

protein deficiency in the diet and promoted good growth in albino rats. Processed protein foods based on a blend of chickpea, low fat groundnut flour and coconut meal have been found by the same workers to supplement effectively poor diets based on rice or a mixture of maize and tapioca flours (Table VII).

Table VII. *The supplementary value of chick pea flour and other protein foods to poor tapioca-rice and tapioca-maize diets**
(Results of experiments with rats in 8 week period)

Diets	Protein content of the diet on moisture free basis (%)	Average weekly gain in body weight of rats (g)
<i>Series I</i>		
A. Tapioca-rice	5.3	4.1
B. " +20% chickpea flour	10.3	7.8
C. " +20% groundnut flour	15.6	10.0
D. " +20% coconut meal	10.7	11.6
E. " +20% protein food containing chickpea	12.9	15.0
F. " +20% skim milk powder	12.9	16.0
<i>Series II</i>		
A. Tapioca-maize	5.08	0.6
B. " +27.6% peanut flour	19.40	17.5
C. " +29.1% of 3:1 blend of peanut flour and skim milk powder	19.40	18.5
D. " +53.3% chickpea flour	19.80	17.2
E. " +51.1% of 3:1 blend of chickpea flour and skim milk powder	19.80	17.8
F. " +35.3% Indian multipurpose food (formula C)	19.80	18.3
G. " +38.2% of protein food containing coconut meal	19.60	19.0
H. " +40.6% skim milk powder	19.40	19.1

* Ref. Nos. 135 and 136.

13.2. *Experiments with preschool children:* The growth rate and nutritional status of children fed on a diet based predominantly on cereals and legumes has been reported to be inferior to those of children on a mixed diet containing animal foods.¹³⁷ On the other hand, Baptist and De Mel¹³⁸ reported that the growth rate of children aged 1-6 years on an entirely vegetarian diet containing a varied mixture

of plant proteins from three cereals (rice, ragi and wheat), four legumes (lentil, green gram, black gram and pigeon pea), coconut and several vegetables was of the same order as that observed with a control group receiving the same diet together with half an ounce of skim milk powder daily per child (Table VIII). In a feeding experiment¹³⁹ extending for 9 months, weaned infants (aged 9 to 20 months) fed a supplement of 2 ounces daily of a malt food or Indian Multipurpose Food containing 20% skim milk powder (formula C), showed highly significant increases in height, weight, red cell count and haemoglobin levels as well as improvement in nutritional status, as compared with a con-

trol group receiving a daily supplement of 2 ounces of rice (Table IX). Ganapathy *et al.*¹⁴⁰ conducted two feeding trials in children to assess the value of legumes or protein food based on a mixture of chickpea and low-fat groundnut flour as compared with skim milk powder to the diets of preschool children. In the first experiment three groups of preschool children were given one of the following supplements for a period of one year: (i) a 80:20 blend of parched chickpea and skim milk powder (ii) A protein food containing 75 parts of groundnut flour, 25 parts of roasted Bengalgram flour, 1 part of lucerne powder and 1 part of calcium phosphate and (iii) skim milk powder. The children in each

Table VIII. *The growth rate* of preschool children on a diet containing vegetable proteins and the same supplemented with skim milk powder*

Diet and protein supplement	No. and age of children	Duration of experiment	Protein from protein supplement (g/day)	Protein intake (g/day)	Mean percentage increase in	
					Weight	Height
Basal diet containing vegetable proteins	15 1-6 years	17 weeks	—	(1-3 years) 26.4 (3½-6 years) 29.7	9.87	2.09
Basal diet + 0.5 ounce skim milk powder	15 (1-6 years)	„	5.1	(1-3 years) 31.5 (3½-6 years) 34.8	8.40	2.31

* Ref. 138, Baptist *et al.*, 1955.

Table IX. *The effect of supplementary protein food on the growth rate of undernourished weaned infants and preschool children*

Expt. No.	Diet and protein supplement	No. and age of children	Duration of experiment (months)	Protein from protein supplement (g/day)	Protein intake (g/day)	Increase in		Reference No.
						Weight (kg)	Height (cm)	
I	Rice-diet (control)	8 boys	9	—	21.0	0.74	4.30	
		10 girls			21.3	1.02	3.51	
	(9-20 months)							
	Rice diet + Indian multipurpose food (Formula C)	8 boys	9	20.3	41.3	1.95	6.38	138
		10 girls		19.3	40.6	2.34	6.17	
	Rice diet + malt food	8 boys	9	13.4	34.4	1.91	5.46	
		10 girls		13.6	34.9	2.29	6.18	
II	Basal diet + skim milk powder	36 (6 to 60 months)	12	7.5 to 20.0 g depending on age	—	1.66	8.09	140
	Basal diet + Mysore Food A	36 (6 to 60 months)	12	„	—	2.00	7.36	
	Basal diet + Bengalgram + skim milk powder	37 (6 to 60 months)	12	„	—	2.10	8.36	

group received daily varying quantities of additional protein (depending on their age) from the different supplements as indicated below: (a) 9 to 12 months, 7.5g protein (b) 13 to 24 months, 10g. protein (c) 25 to 36 months, 15.0g protein and (d) 36 to 60 months, 20g. protein. No significant difference was observed in the mean increases in weight and height or in the nutritional status between three groups at the end of the experiment (Table IX). In the second experiment, the rate of growth and nutritional status of two groups of children receiving a vegetarian diet and a mixed diet containing skim milk powder were compared over a period of 9 months. In the first diet cereals contributed about 40% and pulses about 60% of the proteins while in the second diet cereals contributed about 40% and skim milk powder about 60% of the proteins. The mean daily protein intake of the children of different age groups on the two diets was as follows: (a) 12-24 months, 20g (b) 25-36 months 29g (c) 37-48 months, 33g and (d) 49-60 months 38g. The quantity of legumes consumed ranged from 54g for the age group 12-24 months to 100g for the age group 37-48 months and of skim milk powder from 33g for the age group 12-24 months to 71g for the age group 37-48 months. No significant differences were observed in the mean increases in height and weight between the two groups but the increases were significantly greater than those observed in a similar group of control children subsisting on their home diet. (Table X)

14. Conclusion

The data presented in this review have shown that legume proteins possess wide-

ly varying nutritive value. Even though the nutritive value of the proteins of raw legumes is adversely affected by the trypsin and growth inhibitors present in them, cooking inactivates to a large extent the inhibitors and thus brings about an improvement in the nutritive value of proteins.¹⁵ Some of the legumes such as chickpea and peas with a low inhibitor content but containing proteins of fairly high nutritive value deserve to be widely cultivated and used as a cheap source of protein in the diets of people in the tropical and sub-tropical countries^{4,5} Even though legumes are being used widely in the diets of older children and adults, they are not at present being used to any significant extent as a supplement to the diets of preschool children.^{4,5} From the experience gained in feeding experiments using cereals and legumes with preschool children Ganapathy *et al.*¹⁴⁰ concluded as follows: "In the experiment where full feeding was done, wastage of food served was relatively more in the age group 1 to 2 years. It will be necessary to find out whether it would be possible to evolve vegetable protein meals of smaller bulk to meet the needs of this age group and whether a concentrated supplement has to be resorted to". The results reported by several workers have shown that processed protein foods based on legumes and oilseed meals eg Indian Multipurpose Food and containing about twice as much proteins as common legumes could be used as effective supplements to the diets of preschool children.^{125-126,140} Such foods contain also about two to four times as much calcium, and riboflavin as common pulses. They are also good sources of vitamin A in which pulses are deficient. In view of this, processed protein foods

Table X. The growth rate* of preschool children on diets containing vegetable proteins and a mixture of vegetable proteins and milk proteins (experimental period—9 months)

Diet and protein supplement	No. and age of children	Protein from protein supplement (g/day)	Protein intake (g/day)	Increase in	
				Weight (kg)	Height (cm)
Basal diet + legumes	44 (12 to 60 months)	10 to 20 (g) depending on age	20 to 30 (g) depending on age	1.90	5.4
Basal diet + skim milk powder	54 (12 to 60 months)	"	"	1.79	6.4

* Ref. 140, Ganapathy *et al.*, 1961.

based on oilseed meals and legumes would be ideally suited for supplementing the diets of weaned infants. Consumption of even one ounce of the food will provide about 10g. of additional protein to the child's diet. Further these foods can be readily manufactured on a large scale at low cost and used to overcome the high incidence of malnutrition among preschool children in the developing countries.

REFERENCES

1. Winton A. L. and Winton, K. B. (1949), *The Structure and composition of Foods*, Vol. 2, New York, John Wiley and Sons.
2. *FAO Year Book of Production*, FAO Rome, 1959.
3. Trowell, H. C., Davies J. N. P. and Dean, R. F. A. (1954), *Kwashiorkor*, Edward Arnold Publishers, London.
4. Patwardhan, V. N. (1962), *Amer. J. Nutr.*, **11**, 12.
5. Scrimshaw, N. S. and Bressani, R. (1961), *Fed. Proc.* **20 Suppl.** 7, 80.
6. Parpia, H. A. B., Narayana Rao, M. and Swaminathan, M. (1963), *J. Nutr. Dietet.* **1**, 114.
7. Report of the FAO/CCTA Technical Meetings on Legumes in Agriculture and Human Nutrition in Africa, FAO, Rome, 1959.
8. Aykroyd, W. R., Patwardhan, V. N. and Ranganathan, S. (1956), *The nutritive value of Indian Foodstuffs and the planning of Satisfactory Diets*, 5th Edn., Manager of Publications, Delhi.
9. Neela, R. (1950), *South Indian Recipes*, S. Vishwanathan, Madras.
10. All India Women's Food Council, *Annapurna recipes of supplementary foods*, New Delhi, 1952.
11. Chowdhury, S. (1954), *Indian Cookery*, Deutsch publishers, London.
12. Block, R. J. and Weiss, K. W. (1956), *Amino acid Handbook*, Charles C. Thomas, Springfield, Illinois.
13. Kuppaswamy, S., Srinivasan, M. and Subrahmanyam, V. (1958), *Proteins in Foods* Spl. Rep. Ser. No. 33, *Indian Council of Med. Res.*, New Delhi.
14. Patwardhan, V. N. and Ramachandran, M. (1960), *Sci. & Cult.* **25**, 401.
15. Liener, J. E. (1962), *Amer. J. Clin. Nutr.*, **11**, 281.
16. Osborne, T. B. and Mendel, L. B. (1917), *J. Biol. Chem.* **32**, 369.
17. Melnick, D., Oser, B. L. and Weiss, S. (1946) *Science*, **103**, 326.
18. Ham, W. E. and Sandstedt, R. M. (1944), *J. Biol. Chem.*, **154**, 505.
19. Bowman, D. E. (1944), *Proc. Soc. Exptl. Biol. & Med.* **57**, 139.
20. Kunitz, M. (1945), *Science*, **101**, 668.
21. Kunitz, M. (1946), *J. Gen. Physiol.* **29**, 149.
22. Kunitz, M. (1947), *J. Gen. Physiol.* **30**, 291.
23. Kunitz, M. (1947), *J. Gen. Physiol.* **30**, 311.
24. Kunitz, M. (1948), *J. Gen. Physiol.* **32**, 241.
25. Borchers, R. and Ackerson, C. W. (1947), *Arch. Biochem.*, **13**, 291.
26. Bowman, D. E. (1946), *Proc. Soc. Exptl. Biol. Med.*, **63**, 547.
27. Bowman, D. E. (1948), *Arch. Biochem.* **16**, 109.
28. Borchers, R., Ackerson, C. W., Mussehl, F. E. and Moehl A. (1948), *Arch. Biochem* **19**, 317.
29. Klose, A. A., Hill, B. and Fevold, H. L. (1946) *Proc. Soc. Exptl. Biol. Med.* **62**, 10.
30. Liener, J. E., Deuel, H. J. and Fevold, H. L. (1949), *J. Nutr.*, **39**, 325.
31. Westfall, R. J., Bosshardt, D. K. and Barnes, R. H. (1948) *Proc. Soc. Exptl. Biol. Med.*, **68**, 498.
32. Ham, W. E., Sandstedt, R. M. and Mussehl, F. E. (1945), *J. Biol. Chem.*, **161**, 635
33. Westfall, R. J., and Hauge, S. M. (1948), *J. Nutr.*, **35**, 352.
34. Almquist, H. J., and Merritt, J. B. (1952), *Arch. Biochem.*, **35**, 359.
35. Almquist, H. J. and Merritt, J. B. (1953) *Proc. Soc. Exptl. Biol. Med.* **79**, 277.
36. Almquist, H. J. and Merritt, J. B. (1953), *Proc. Soc. Exptl. Biol. Med.* **83**, 269.
37. Borchers, R. and Ackerson, C. W. (1951), *Proc. Soc. Exptl. Biol. Med.* **78**, 81.
38. Riesen, W. H., Clandinin, D. R., Elvehjem, C. A. and Craven, W. W. (1947), *J. Biol. Chem.* **167**, 143.
39. Hou, H. C., Reisen, W. H. and Elvehjem, C. A. (1949), *Proc. Soc. Exptl. Biol. Med.*, **70**, 416.
40. Liener, J. E., and Fevold, H. L. (1949), *Arch. Biochem.*, **21**, 395.
41. Desikachar, H. S. R. and De., S. S. (1947), *Science*, **106**, 421.
42. Chernick, S. S., Lepkovsky, S. and Chaikoff, I. L. (1948), *Am. J. Physiol.* **155**, 33.
43. Alumot, E. and Nitsan, Z. (1961), *J. Nutr.* **73**, 71.
44. Booth, A. N., Robbins, D. J., Ribelin, W. E. and De Eds F. C. (1960), *Proc. Soc. Exptl. Biol. Med.*, **104**, 681.
45. Lyman, R. L., and Lepkovsky, S. (1957), *J. Nutr.*, **62**, 269.
46. Lyman, R. L., (1957), *J. Nutr.*, **62**, 285.
47. Lepkovsky, S., Bingham, E. and Pencharz, R. (1959), *Poultry Sc.*, **38**, 1289.
48. Waterman, H. C. and Johns, C. O. (1921), *J. Biol. Chem.*, **46**, 9.
49. Waterman, H. C. and Jones, D. B. (1921), *J. Biol. Chem.* **47**, 285.
50. Jones, D. B. and Waterman, H. C., (1922), *J. Biol. Chem.*, **52**, 357.
51. Jaffe, W. G. (1950), *Proc. Soc. Exptl. Biol. Med.*, **75**, 219.
52. Tauber, H., Kershaw, B. B. and Wright, R. D. (1949), *J. Biol. Chem.*, **179**, 1155.
53. Sohonie K., Apte, U. and Ambe, K. S. (1958), *J. Sci. & Indust. Res.* **17C**, 42.
54. Klose, A. A., Greaves, J. D. and Fevold H. L. (1948), *Science*, **108**, 88

55. Borchers, R. and Ackerson, C. W. (1950) *J. Nutr.* **41**, 339.
56. Jaffe, W. G. (1950), *Acta Cient. Venezolana*, **1**, 62.
57. Osborne, T. B. and Mendel, L. B. (1912), *Ztschr. Physiol. Chem.*, **80**, 307.
58. Johns, C. O. and Finks, A. J. (1920), *Science*, **52**, 414.
59. Everson, G. and Heckert, A. (1944), *J. Am. Dietet. Assoc.* **20**, 81.
60. Johns, C. O. and Finks, A. J. (1920), *J. Biol. Chem.*, **41**, 379.
61. Luning, O. and Bartels, W. (1926), *Ztschr. Lebensm. Unterschu Forsch*, **51**, 220.
62. McCollum E. V., Simmonds, N. and Pitz, W. (1917), *J. Biol. Chem.* **29**, 521.
63. Wienhaus, O. (1908), *Biochem. Ztschr*, **18**, 228.
64. Landsteiner, K. and Rautscheck, H. (1907), *Centralbl. Baktr.*, **45**, 660.
65. Goddard, V. R. and Mendel, L. B. (1929), *J. Biol. Chem.* **82**, 447.
66. Mendel, L. B. (1909), *J. Biol. Chem.*, **6**, 19.
67. Schneider, E. C. (1911), *J. Biol. Chem.*, **11**, 47.
68. Boyd, W. C. and Shapleigh, E. (1954), *Science*, **119**, 419.
69. Ham, T. H. and Castle, W. B. (1940), *Trans. Am. Physicians* **55**, 127.
70. Dameshak, W. and Miller, E. B. (1943), *Arch. Int. Med.*, **72**, 1.
71. Liener, I. E. (1951), *J. Biol. Chem.* **193**, 183.
72. Sjollem, B. (1925), *Deutsche tierarztl. Wchnschr.*, **33**, 470.
73. Liener, I. E. and Pallansch, M. J. (1952), *J. Biol. Chem.* **197**, 29.
74. Liener, I. E. (1953), *J. Nutr.* **49**, 527.
75. Rigas, D. A. and Osgood, E.E. (1955), *J. Biol. Chem.* **212**, 607.
76. Jaffe, W. G. and Gaede, K. (1959), *Nature*, **183**, 1329.
77. Jaffe, W. G. (1961) *Arztl. Forech*, **12**, 1012.
78. Honavar, P. M., Shih, C. V. and Leiner, I.E. (1962), *J. Nutr.* **77**, 109.
79. Faschingbaur, H. and Kofler, L. (1929), *Wien. Klin Wchnschr*, **42**, 1069.
80. Griebel, C. (1950), *Ztschr. Lebensm. Untersuch. U. Forsch* **90**, 191.
81. Cartwright, G. E. and Wintrobe, M. M. (1946) *J. Lab. & Clin. Med.* **31**, 886.
82. McCarrison, R. (1933), *Indian J. Med. Res.* **21**, 179.
83. Greer, M. A. and Ashwood, E. B. (1948) *Endocrinology*, **43**, 105.
84. Dunstan, W. R. and Henry, T. A. (1949), *Proc. Roy. Soc. Med.*, **72**, 85.
85. Rahman, S. A., De, S. S. and Sabrahmanyam, V. (1947), *Current Sci.* **76**, 357.
86. Viehoever, A. (1940), *Thailand Sc. Bull.* **2**, 1.
87. Potter, G. C. and Kummorow, F. A. (1954), *Science*, **120**, 224.
88. Charavanapvan, C. (1943), *Trop. Agriculturist Ceylon*, **99**, 157.
89. Dastur, D. K. and Iyer, C.G.S. (1922) *Nutrition Rev.* **17**, 33.
90. Subrahmanyam V., Narayana Rao, M. and Swaminathan, M. (1957), *Food Science*, **6**, 156.
91. Roy, D. N., Nagarajan, V. and Gopalan, C. (1963), *Current Sci.* **32**, 116.
92. Gardner, A. F. (1959), *Am. J. Clin. Nutr.*, **7**, 213.
93. Geiger, G. J., Steenbock, H. and Parsons, H. T. (1933), *J. Nutr.* **6**, 427.
94. Luisada, A. (1941), *Medicine*, **20**, 229.
95. Miller, D. S. (1961), *Proc. Nutr. Soc.* **20**, XXI.
96. Wilgus, H. S., Jr., Gassner, F. X., Patton, A. R. and Gustavson, R. G. (1941) *J. Nutrition*, **22**, 43.
97. Acton, H. W. and Chopra, R. N. (1922), *Indian Med. Gaz.* **57**, 412.
98. Liener, I. E. (1958), in *Processed Plant Protein Foodstuffs*, Ed. By Altschul, Chapt. 5, Academic Press incorporated Publishers., London.
99. Dean, R. F. A. (1958), in *Processed Plant Protein Foodstuff*, Ed. Altschul, Chap. 9, Academic Press Publishers, London.
100. Hirwe, R. N. and Magar, N. G. (1953), *Indian J. Med. Res.* **41**, 191.
101. Esh, G. C. and Som, J. M. (1953), *Indian J. Physiol. All. Sci.*, **7**, 158.
102. Acharya, B. N., Niyogi, S.P. and Patwardhan, V. N. (1942). *Indian J. Med Res.*, **30**, 73.
103. Jaffe, W. G. (1949), *Experientia*, **5**, 81.
104. Jaffe, W. G. (1949), *Prac. Soc. Exptl. Biol. & Med.* **71**, 398.
105. De Vries, H. and Van der. Lee, J. (1949), *Bakkerj-Wetenschap*, **2**, 109.
106. Anonymous, (1948) *Indust. Eng. News*, **26**, 2156.
107. Morcos, S. R. and Doctor, A. M. (1959), *Brit. J. Nutr.* **13**, 163.
108. Borchers, R. Ackerson, C. W. and Sandstedt, R. M. (1947), *Arch. Biochem.*, **12**, 367.
109. Sohonic, K. and Bhandarkar, A. P. (1955), *J. Sci. Industr. Res.*, **14C**, 100.
110. Sohonic, K. and Bhandarkar, A. P. (1954), *J. Sci. Industr. Res.*, **13B**, 500.
111. Kothary, K. and Sohonic K. (1960), *J. Sci. Industr. Res.* **19C**, 16.
112. Liener, I. E. and Hill, E. G. (1953), *J. Nutr.* **49**, 609.
113. Block, R. J. and Mitchell, H. H. (1946), *Nutr. Abs. Rev.*, **16**, 249.
114. Venkat Rao, S., Joseph, A. A., Swaminathan, M. and Parpia, H. A. B. (1964), *J. Nutr. Dietet.* **1**, 192.
115. Woods, E., Beeson, W. M. and Bolin, D. W. (1943), *J. Nutr.* **26**, 327.
116. Baptist, N. G. (1956), *Ceylon, J. Sci.* **9**, 15.
117. Venkata Rao, S., Swaminathan, M. and Parpia, H. A. B. (1964), *J. Nutr. Dietet.*, **1**, 128.
118. Phansalkar, S. V., Ramchandran, M. and Patwardhan, V. N. (1957), *Indian J. Med. Res.*, **45**, 611.
119. Bressani, R., Valiente, A. T. and Tejada, D. E. (1962), *J. Food Science*, **27**, 394.
120. Subrahmanyam, V., Rama Rao, G., Kuppuswamy, S., Narayana Rao, M. and Swaminathan, M. (1957), *Food Sci.*, **6**, 76.
121. Chandrasekhara, M. R., Swaminathan, M. and Subrahmanyam, V. (1955), *Bull. Cent. Food. Tech. Res. Inst.*, *Mysore*, **5**, 25.

122. Krishnamurthy, K., Tasker, P. K., Indira K., Rajagopalan, R., Swaminathan, M. and Subrahmanyam, V. (1958), *Ann. Biochem. Exptl. Med.*, **18**, 175.
123. Narayana Rao, M., Joseph, K., Swaminathan, M. and Subrahmanyam, V. (1961), *Proc. Symposium on Proteins*, CFTRI, Mysore, p. 286.
124. Venkatachalam, P. S., Srikanthia, S. G., Mehta, G. and Gopalan, G. (1956), *Indian J. Med. Res.*, **44**, 539.
125. Bharucha, P. E. and Edibam, B. C. (1960), *Indian J. Child. Hlth.*, **9**, 261.
126. Purshowthama Rao, G. (1960), *Indian J. Child. Hlth.*, **9**, 207.
127. Subrahmanyam, V., Doraiswamy, T. R., Joseph, K., Narayana Rao, M., and Swaminathan, M. (1957), *Indian J. Pediat.*, **24**, 112.
128. Gravioto, R. O., Messieu, P. G. and Guzman, J. G. (1955), *Bol. Ofic. Sav. Panam.*, **38**, 148.
129. Gravioto, J. (1958), *Biol. Med. Hosp. Inf. Mexico*, **15**, 825.
130. Scrimshaw, N. S., Behar, M., Willson, D., Viteri, F., Arroyave, G. and Bressani, R. (1961), *Amer. J. Clin. Nutr.*, **9**, 196.
131. Hansen, J. D. L., Schendel, H. E., Wilkins, A. and Brock, J. F. (1960), *Pediatrics*, **25**, 258.
132. De Maeyer, E. M. and Venderborgh, H. (1958), *J. Nutr.*, **65**, 335.
133. Narayana Rao, M. and Swaminathan, M. (1960), *Ann. Rev. Food Tech.*, **1**, 73.
134. Desikachar, H. S. R., Sankaran, A. N. and Subrahmanyam, V. (1956), *Indian J. Med. Res.*, **44**, 741.
135. Tasker, P. K., Narayana Rao, M., Indiramma, K., Swaminathan, M. and Subrahmanyam, V. (1961), *Ann. Biochem. Exp. Med.*, **21**, 17.
136. Tasker, P. K., Narayana Rao, M., Paul Jayaraj, A., Indiramma, K., Swaminathan, M., Sreenivasan, A. and Subrahmanyam, V. (1962), *Indian J. Med. Res.*, **50**, 468.
137. Mack, P. B., Shevock, V. D. and Tomasetti, M. R. (1947), *J. Amer. Diet. Assoc.*, **23**, pp. 488., 588, 677.
138. Baptist N. G. and De Mel, B. V. (1955), *Brit. J. Nutr.*, **9**, 157.
139. Subrahmanyam, V., Doraiswamy, T. R., Bhagavan, R. K., Narayana Rao, M., Sankaran, A. N. and Swaminathan, M. (1959), *Indian J. Pediat.*, **26**, 406.
140. Ganapathy, R., Swaminathan, M. C., Tasker, A. D. and Someswara Rao, K. (1961), *Indian J. Med. Res.*, **49**, 306.
141. Esh, G. C. and Som, J. M. (1952), *Indian J. Physiol. and Allied Sci.*, **6**, 61.
142. Esh, G. C. (1952), *J. Proc. Inst. Chemists, (India)*, **30**, 21.
143. Hirwe, R. N. and Magar, N. G. (1951), *Current Sci.*, **20**, 40.
144. Esh, G. C. and Som, J. M. (1955), *Proc. Nat. Inst. Sci. (India)*, **21B**, 68.
145. Orru, A. and Demel, V. C. (1941), *Quadrini Nutriz.*, **7**, 273.
146. Ubata, F. B. (1955), *Rev. Brasil Biol.*, **5**, 1.
147. Sumner, J. B. and Howell, S. F. (1935), *J. Immunol.*, **29**, 133.
148. Sumner, J. B. and Howell, S. F. and Seissig, A. (1935), *Science*, **82**, 65.
149. Sumner, J. B. and Howell, S. F. (1936), *J. Bacteriol.*, **32**, 227.
150. Desouza, M. A. (1948), *Rev. Brasil. Farm.*, **30**, 189.
151. Adolph, W. H., Shammas, E. I. and Holaby, S. H. (1955), *Food Res.*, **20**, 31.
152. Indian Council of Medical Research (1946), *Report on soya bean. Spl. Rep. No. 13*. New Delhi.
153. Blaizot, J. (1947), *Bull. Soc. Scient. hyg. aliment.*, **35**, 23.
154. Krupic, M. (1953), *Biol. Zentralbl.*, **72**, 424.
155. Gaitonde, M. K. and Sohoni, K. (1951), *Current Sci.*, **20**, 27.
156. Jaffe, W. G. Panchart, A., Pacz Purma, J. I., Torrealba, R. and Franceschi, N. D. (1955), *Arch. Venezolanas Nutrie.*, **6**, 195.
157. Sohoni, K. and Ambe, K. S. (1955), *Nature*, **175**, 508.
158. Bird, G. W. G. (1951) *Current Sci.*, **20**, 298.
159. Sohoni, K., Huprikar, S. V. and Joshi, M. R. (1959), *J. Sc. & Industr. Res.*, **18c**, 95.
160. Boyd, W. C. and Reguera, R. M. (1949), *J. Immunol.*, **63**, 333.
161. Lewis, H. B., Fajans, R. S., Esterer, M. B., Shen, C. and Oliphant, M. (1948) *J. Nutr.*, **36**, 537.
162. Jacquot, R., Matet, J. and Fridenson, O. (1947), *Ann. Nutrition of aliment.*, **1**, 157.
163. Gontea, I. and Gorder, M. (1958), *Commun. Acad. Rey. Populare Romine.*, **8**, 723.
164. Johns, C. O. and Finks, A. J. (1921) *Am. J. Physiol.*, **56**, 208.
165. Milby, T. T. (1945) *Proc. Oklahoma Acad. Sc.*, **25**, 50.
166. Honovar, P. M. and Sohoni, K. (1959), *Ann. Biochem Exptl. Med.*, **19**, 57.
167. Honovar, P. M. and Sohoni, K. (1959) *J. Sci. & Industr. Res.*, **18c**, 202.
168. Finks, A. J. and Johns, C. O. (1921), *Am. J. Physiol.*, **56**, 202.
169. Richardson, L. R. (1948), *J. Nutr.*, **36**, 451.
170. Metta, V. C., Norton, H. W., and Johnson, B. C. (1957), *J. Nutr.*, **63**, 143.
171. Klose, A. A., Hills, B., Greaves, J. D. and Fevold, H. L. (1949), *Arch. Biochem.*, **22**, 213.
172. Fraenkel-Contrat, H., Bean, R. C., Ducay, E. D. and Olcott, H. S. (1952), *Arch. Biochem.*, **37**, 393.
173. Apte, V. and Sohoni, K. (1957), *J. Sci. & Industr. Res.*, **16C**, 225.
174. Bird, G. W. C. (1954) *Nature*, **174**, 1015.
175. Boyd, W. G., Shapleigh, E. and McMaster, M. (1955), *Arch. Biochem.*, **55**, 226.
176. Schertz, K. F., Jurzelsky, W., Jr. and Boyd, W. C. (1960), *Proc. Nat. Acad. Sci.*, **46**, 529.
177. Chattopadhyay, H. and Banerjee, S. (1953), *Indian J. Med. Res.*, **41**, 185.
178. Brozetti, P. (1948), *Ann. Fac. Agrar. Univ. Studi. Perugia.*, **5**, 170.
179. Saint Paul, M., Daoule-Le-Bourdelle, F. and Fine, J. M. (1956), *Compt. rend. soc. biol.*, **150**, 1742.
180. Coulet, M., Bezon, M. J. and Cognet, B. (1959), *Bull. Soc. Chim. Biol.*, **41**, 1385.
181. Johns C. O. and Finks, A. J. (1921), *J. Biol. Chem.*, **46**, 15.

182. Finks, A. J., Jones, D. B. and Johns, C. O. (1922), *J. Biol. Chem.* **52**, 403.
183. Woods, E., Beeson, W. M. and Bolin, D. W. (1943), *J. Nutr.*, **26**, 327.
184. Murray, H. C. (1948), *J. Nutr.*, **35**, 257.
185. Armbuster, G. and Murray H. C. (1951), *J. Nutr.*, **44**, 205.
186. Schneider, B. H. and Miller, D. F. (1954), *J. Nutr.*, **52**, 581.
187. Sure, B. and Read, J. W. (1921), *J. Agr. Res.*, **22**, 5.
188. Creiger, W. B. and Gifford, H. (1952), *Blood*, **7**, 721.
189. Deshpande, P. D. and Radhakrishna Rao, M. V. (1954), *Indian J. Med. Res.* **42**, 77.
190. Gugudda, E., Biggli, C. and Massenti, S. (1953), *Minerva Med.*, **441**, 140.
191. Ottensooser, P. (1955), *Acad. Bras. Cienc.*, **27**, 519.
192. Sherman, W. C. (1941), *51st Annual Report, Alabama Agricultural Experimental Station.*
193. Sherwood, F. W., Weldon, V. Peterson, W. J. *J. Nutr.*, **52**, 199.
194. Schilling, E. D. and Strong, F. M. (1854), *J. Am. Chem. Soc.* **76**, 2848.
195. Vijayaraghavan, P. K. and Srinivasan, P. R. (1953), *J. Nutr.*, **51**, 261.
196. Williams, H. H. (1955), *Cornell Univ. Agr. Expt. Sta. Mem.*, 337.
197. Ramachandran, M. and Phansalkar, S. V. (1956), *Indian J. Med. Res.*, **44**, 501.
198. Mahon, J. H. and Common, R. H. (1950), *Sci. Agric.*, **30**, 43.
199. Horn, M. J., Blum, A. E., Gersdorff, C. E. F. and Warren, H. W. (1955), *Cereal Chem.* **32**, 64.
200. Hirsch, J. S., Niles, A. D. and Ketmerer, A. R. (1952), *Food Res.*, **17**, 442.
201. Bressani, R., Elias, L. G. and Valiente, A. E. (1963), *Brit. J. Nutr.*, **17**, 68.
202. Russel, W. C. Taylor, M. W., Mehrohof, T. G., and Hirsch, R. R. (1946), *J. Nutr.*, **32**, 313.