STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART II. The Proteins of the Pigeon Pea (*Cajanus Indicus*).

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Pigeon pea is a legume raised throughout India and other tropical countries for its seeds, which are used for human consumption, and for its foliage which serves as fodder or as green-manure (Krauss, *Hawaii Agric. Exptl. Stn.*, 1921, Bull. 46; Miller, *J. Agric. Sci.*, 1928, **18**, 569).

Only two varieties of the plant, (1) *Cajanus Indicus bicolor* (arhar) and (2) *Cajanus Indicus flavus* (tur, tuar) are cultivated extensively in India. The former is an annual crop reaching 6–8 ft. and produces light grey seeds which are faintly speckled: the latter is a seasonal, shorter plant and produces small, red seeds. Though their identity in species is still doubted (Watt, *Commercial Products of India*, 1908) yet the varieties are prized equally for their peas, which form the chief protein diet of the people. The peas are prepared by husking after either gentle frying or incipient germination of the seed and used in various ways.

A legume depends for nutritional value largely on the quality of its protein, and since the investigations of Osborne and others have shown that there is a definite relation between the composition of a protein and its nutritional efficiency, an attempt was made to estimate the latter for the *bicolor* and *flavus* varieties by chemical examination of their proteins.

EXPERIMENTAL.

CAJANUS INDICUS BICOLOR (*arhar*).

The seeds, obtained from the Pusa Agricultural Research Institute, were sun-dried, decorticated by being passed through a disintegrator and the cotyledons powdered to a fine meal passing through the 90-mesh sieve. Analysis of the meal gave the following percentages:—Moisture, 8·73; Ash, 3·57; Ether extractives, 2·05; Crude protein (N × 6·25), 22·52; Crude fibre, 3·10; Carbohydrates (by difference), 60·03.

Extraction of albumin and globulins.—To determine the optimum conditions for the extraction of the salt-soluble proteins, trials were

conducted with solutions of sodium chloride and varying (1) concentration of salt, (2) time, (3) temperature of extraction, and (4) reaction of extractant.

1. The meal (10 gms.) passing the 90-mesh sieve was mixed with 250 c. c. of salt solutions of different concentrations, and shaken for one hour at the room temperature, 25°. After filtration, nitrogen in the extracts was determined by the Kjeldahl method, Table I showing the percentages involved.

TABLE I.

Conc. of salt	Nitrogen extracted	Conc. of salt	Nitrogen extracted
0 (water)	46·0	6	60·9
1	51·0	7	59·9
2	58·5	8	59·3
3	62·2	9	58·7
3·5	63·1	10	56·8
4	61·4	15	54·9
5	61·2	20	52·9

Salt solution of 3·5 per cent. was evidently the best suited, the other concentrations extracting less nitrogen.

2. Using a 3·5 per cent. solution of sodium chloride, the periods of extraction were varied, and the corresponding amounts of extracted nitrogen determined (Table II).

TABLE II.

Period of extraction in hours ...	¼	½	¾	1	2	3	7	14
Percentage of nitrogen extracted ...	62·1	63·1	63·0	63·0	62·8	62·8	62·2	54·5

The maximum amount of protein was extracted in half an hour: the diminution observed on increasing the time was evidently due to partial denaturation of globulins generally observed on continued shaking with salt solution at room temperature.

3. It was observed that 70 per cent. of the total nitrogen was extracted by 3·5 per cent. salt solution on shaking at the room temperature for only 15 minutes and then transferring to the refrigerator (–2°) for 24 hours. Similar extractions carried out six times, successively using fresh portions of saline, yielded 74 per cent.

4. The reaction of an extract prepared as above was acid to litmus, the P_H measured electrometrically with hydrogen electrode being 5·91. Though rendering of the salt suspension neutral to litmus prior to extraction yielded 78 per cent. of protein, addition of alkali was avoided owing to possibilities of (*a*) denaturation and (*b*) racemisation (Dakin, *J. Biol. Chem.*, 1913, **13**, 357) of proteins, isoelectric points of which were far removed from the P_H of the alkali to be used.

Nitrogen extracted by different solvents.—The residue after successive extraction with 3·5 per cent. salt solution at – 2° was treated with boiling 70 per cent. ethyl alcohol and filtered. The residue was then extracted thrice, in the cold, with 0·2 per cent. sodium hydroxide. Determination of nitrogen in different extracts showed percentages passing into the different solvents:—Salt solution, 74·00; alcohol, 0·95; alkali, 21·00.

Distribution of nitrogen in the saline extract.—The extract from 20 gms. of the meal was dialysed in a large parchment cone against distilled water at 5–6° for 10 days when no more salt was found in the dialysate. The precipitated globulins were separated. The supernatant liquid gave no precipitate on saturation with carbon dioxide showing that it contained no globulins. When heated on the water-bath, it began to show turbidity at 43° and yielded a coagulum at 55°. Further heating did not increase coagulation, indicating that probably only one albumin was present. Adding alcohol to the filtrate after removing coagulum gave a minute quantity of white precipitate when the concentration of alcohol reached about 30 per cent.

The precipitates obtained at different stages were separated and their nitrogen contents determined. Percentage distribution of nitrogen in the saline extract was then found to be (1) precipitable by dialysis (globulins), 44·6; (2) coagulable from salt-free dialysate (albumins), 3·75; (3) precipitable with alcohol (proteose), 2·62; (4) other forms, 23·23.

Heat-coagulation.—To determine the number of heat-coagulable fractions in the saline extract, 100 c.c. was treated with 2 drops of 2 per cent. acetic acid, placed in a long thin-walled test tube fitted with thermometer and stirrer, and heated by immersion in a large beaker of water the temperature of which was raised at the rate of 0·5°

per minute. Observations were made with a dark background and diagonal illumination. Turbidity began at 45° and at 52° a coagulum (1) was formed, settling in 2 minutes; the suspension was kept at the same temperature for ½ hour more and filtered. When the filtrate was heated as before opalescence was again noticed at 65°, 78° and 88°, the corresponding coagula (2), (3), and (4) being separated at 70–74°, 80–85° and 94–95° respectively; (2) was small in bulk while (3) and (4) were large.

The experiment was repeated seven times and showed that reproducible coagulations occurred only at 46–55°, 78–85°, and 88 – 95°. Remembering the occurrence of numerous foreign accompaniments to the coagulable proteins, it cannot be stated that the ranges of temperature cited all represented definite proteins in the extract.

Evidence has already been obtained to show that the fraction separating at 46–55° was an albumin. To determine whether the others were globulins, the precipitate obtained on dialysis was washed repeatedly with distilled water, shaken with 3·5 per cent. salt solution, the mixture centrifuged to free it from suspended matter and the temperatures of coagulation noted as already described. It was observed that (1) the major part of the precipitate redissolved in salt solution indicating that it had not altered much in character and (2) opalescence appeared at 78° and 88°, with the corresponding fractions separating at 85° and 95–96°, respectively. It may therefore be inferred that the coagula separating at the higher temperatures were globulins, and that there were two at least.

Preparation of globulin.—Considerable difficulty was experienced in preparing a clear extract of the meal, particularly because of the large amount of sticky matter contained in it. After several attempts the following method was found suitable. The meal (500 gms.) was treated with 2·5 litres of 3·5 per cent. saline and a few c.c. of toluene; after being stirred vigorously for 2 hours by an electrically driven glass stirrer the mixture was allowed to stand in the refrigerator for 24 hours. It was then pulped with scrap filter-paper, and when suitable consistency had been attained was squeezed out through coarse cloth by a hand-press. The extract contained much suspended matter and was passed twice through Sharple's supercentrifuge; on filtering under pressure through thick mats of paper pulp, 2250 c.c. of a clear extract containing 65 per cent. of the total nitrogen was obtained.

Preparation by dialysis.—The extract from 500 gms. of meal was dialysed in parchment cones against distilled water for 15 days.

Globulin, precipitated in large spheroids, was freed from albumin and last traces of chlorides by washing repeatedly with distilled water followed by dilute alcohol. It was dehydrated by standing overnight with absolute alcohol, washing with absolute ether and finally drying in vacuum over sulphuric acid. A crisp white powder was obtained, representing 4·64 per cent. of the meal extracted.

Precipitation by ammonium sulphate.—A similar extract of the meal was saturated with ammonium sulphate by gradually adding the powdered salt with constant stirring, the protein being precipitated slowly in granular flocks and separated by filtration. On shaking with distilled water the precipitate was completely repeptised by the adsorbed salt. This extract was filtered through paper mat and dialysed in parchment cones until free from salt. The precipitated globulin was washed free from albumin, dehydrated and dried in the manner described already, forming a white powder resembling that obtained by dialysis and representing 4·8 per cent. of the meal.

Analysis of the globulin preparations.—The two preparations were dried at 110° and analysed for their constituent elements (Table III). Carbon and hydrogen were determined by combustion: sulphur by Hoffman and Gortner's modification (*J. Amer. Chem. Soc.*, **45**, 1033) of the original method of Benedict and Denis: and nitrogen by the Kjeldahl process.

TABLE III.

Percentages on ash-free basis.

	C	H	N	S	O	P
By dialysis (ash, 0·52 per cent.)	52·90	6·91	15·94	0·46	23·79	Nil
By precipitation (ash, 0·42 per cent.)	52·78	6·84	16·01	0·45	23·92	Nil

Differences being within the range of experimental error, it may be inferred that the two preparations were identical in their elementary composition.

Fractional precipitation.—With a view to dividing the globulins into several fractions by differential precipitation with ammonium sulphate, trials were carried out with the saline extract from 500 gms. of the meal by adding to it powdered ammonium sulphate so as to raise the concentration of salt gradually from 10 to 100 per cent.

saturation. Precipitation began at 30–40 per cent. saturation, the protein separating in 15 minutes. On filtering and adding more salt, increasing amounts of protein separated at different stages up to 90 per cent. saturation. The different fractions thus obtained were separated, washed with ammonium sulphate of the same concentration, redissolved and precipitated by dialysis. They were then dehydrated, dried and examined for the following factors, (1) yield of moisture-free protein, (2) percentage of sulphur and (3) response to glyoxylic acid test for tryptophan (Hopkins and Cole). The same fractions were obtained from another portion of extract, redissolved in 3·5 per cent. saline, and the corresponding temperatures of coagulation determined. The different observations are recorded in Table IV.

TABLE IV.

Percentage saturation	Yield in grams	Percentage of sulphur	Tryptophan reaction	Solubility in saline	Coagulation temperature
0–40	3·1	0·99	Strong	Sparing	95° on prolonged heating
40–50	2·5	0·58	Faint	Partial	86° and again 95°
50–60	2·1	0.45	,,	Complete	85—88°
60–70	5·9	0·39	Very faint	,,	,,
70–80	5·8	0·38	,,	,,	,,
80–90	5·4	0·39	,,	,,	,,

It may be inferred from the above that the fractions contained at least two globulins, one precipitated between 0 and 40 per cent. saturation, coagulating at 95°, rich in sulphur and tryptophan and the other precipitated between 60 and 90 per cent. saturations, coagulating at 85–88°, poor in sulphur and tryptophan. Since the previous coagulation experiments also gave evidence of the presence of only two distinct globulins, it is probable that the fractions separating between 40 and 60 per cent. saturation were varying mixtures of the two, as indicated by their intermediate sulphur contents.

Refractionation.—To obtain pure specimens, refractionation of the portions separating between the two definite ranges was attempted, constancy of sulphur-content being taken as evidence of purity. It was observed that the fraction separating between 0 and 40 per cent. saturation was already denatured and could not be redissolved in saline. The one separating between 60 and 90 per cent. saturation was however readily peptised and on refractionation was found to contain 0·37 per cent. of sulphur. This being nearly identical with

those obtained for the preparations by single fractionation (Table IV) it may be inferred that the latter was pure.

Preparation of globulins I *and* II.—Globulin I separated in flocks on adding to a clear salt extract of the meal solid ammonium sulphate to 40 per cent. saturation. The precipitate was collected on a paper filter and washed repeatedly with 40 per cent. ammonium sulphate. It was then repeptised by the adsorbed salt on shaking with distilled water and dialysed in parchment cones against distilled water until free from sulphate; the precipitated protein was washed successively with distilled water and dilute alcohol, and dehydrated by washing with absolute alcohol followed by ether. The yield was 0·65–0·80 per cent. of the meal.

In the filtrate from globulin I the saturation of ammonium sulphate was increased to 60 per cent. After removing the precipitate of mixed globulins the saturation of ammonium sulphate was raised to 90 per cent. when globulin II separated. The precipitate was repeptised, filtered, dialysed free from salts, and dehydrated as already described for globulin I. The yield was 3·2–3·5 per cent. of the meal.

Composition of globulins I *and* II.—Three independent preparations (A, B and C) of each globulin were made and their individual composition determined (Table V).

TABLE V.

Percentages on ash-free basis.

	Globulin I				Globulin II			
	A	B	C	Average	A	B	C	Average
C ...	53·17	53·84	53·65	53·55	52·41	52·84	51·98	52·41
H ...	6·92	7·16	6·99	7·02	6·88	6·98	6·84	6·90
S ...	0·99	1·01	1·04	1·01	0·37	0·39	0·39	0·38
N ...	15·21	15·01	15·34	15·19	16·22	16·10	16·25	16·19
O ...	23·71	22·98	22·96	23·23	24·12	23·69	25·54	24·12

The percentage of ash in the preparations was as follows:—Globulin I: A, 0·64; B, 0·72; C, 0·82. Globulin II: A, 0·13; B, 0·14; C, 0·24.

Cajanin and Concajanin.—The foregoing observations indicate that globulins I and II have definite composition and are distinct from each other in their percentage of sulphur and nitrogen. In accordance with convention, globulin II which was present in the larger quantity and was thus the principal protein of the seed was named *cajanin* after the genus, and globulin I which accompanied it, *concajanin*.

Cajanin when dried is snow-white, dissolves freely in alkalis, but less readily in acids; it dissolves sparingly in neutral solvents although before treatment with alcohol and ether the globulin was freely soluble. It gave all the general reactions for proteins but only feeble response to Hopkins and Cole's and Molisch's tests. The isoelectric point as determined by the method of Csonka, Murphy and Jones (*J. Amer. Chem. Soc.*, 1926, **48**, 763) using the acetate buffer is P_H 4·95.

Concajanin, is a grey powder resembling cajanin in properties except that it (1) was sparingly soluble in neutral solvents even before dehydration with alcohol and ether and (2) responds to the tests for tryptophan and carbohydrate. The isoelectric point approximates to that of cajanin being P_H 5·22.

Protein extracted by alkali.—To identify the protein left after exhaustive salt extraction, the residue from 500 gms. of meal was extracted with 0·2 per cent. sodium hydroxide and a clear filtrate obtained on passing the suspension through paper pulp. The protein in the extract was precipitated on rendering slightly acid to litmus. It was filtered, redissolved in alkali, again precipitated with acid, filtered, washed and dried. A pale-red powder representing 0·9 per cent. of the meal and containing 0·52 per cent. of ash was obtained. Its ash-free composition was C, 52·94; H, 6·98; S, 0·41; N, 15·86 and O, 23·81 per cent.

The isoelectric point of this protein was P_H 5·0. It was precipitated from alkaline solution only on adding ammonium sulphate up to at least 40–50 per cent. saturation. The properties correspond more with those of the globulins already described than with those of typical glutelins, which are characterised by (1) precipitation from alkaline solutions by ammonium sulphate even at 1 to 18 per cent. saturation and (2) isoelectric points approximating to P_H 6·5 (Csonka and Jones, *J. Biol. Chem.*, 1927, **73**, 321). In view of the foregoing considerations it may be inferred that the protein extracted by alkali was merely the seed-globulin not fully extracted by salt solution, but readily peptised by dilute alkali. Calculated on the basis of its sulphur-content it appears to have contained cajanin to the extent of 95·2 per cent.

A revised estimate of the approximate percentage distribution of nitrogen in the pea would therefore be :—albumin, 3·8 ; cajanin, 57·2 ; concajanin, 8·4 ; proteose, 2·6 ; other forms, 28·0.

Albumin.—The meal (500 gms.) was extracted with distilled water and the clear extract (2 litres) dialysed for 10 days at 5–6° against distilled water, when the dialysate gave only faint opalescence with silver nitrate. After separation from globulins, the extract containing albumin was saturated with carbon dioxide to precipitate any residual globulin and the acidified filtrate heated at 55° for half-an-hour. The small coagula thus obtained from several extracts were collected, washed with water and alcohol, dehydrated and dried, yielding 4 gms. of a pale grey substance representing 0·4 per cent. of the meal extracted and containing 0·45 per cent. of ash.

The isoelectric point of the albumin was P_H 4·2, and the composition (ash-free) :—C, 54·82 ; H, 6·84 ; N, 14·80 ; S, 1·18 ; O, 22·36 per cent.

Nitrogen distribution.—About 3 gms. each of cajanin, concajanin and the albumin were used for determining the distribution of nitrogen by the Van Slyke method as improved by Plimmer and Rosedale (*Biochem. J.*, 1925, **19**, 1004) with the following modifications : (1) after precipitation with phosphotungstic acid the diamino-fraction was separated by centrifuging and (2) arginine nitrogen was determined by Kœhler's method (*J. Biol. Chem.*, 1920, **42**, 267). Sulphur in the diamino-fraction was determined by the method of Plimmer and Lowndes (*Biochem. J.*, 1927, **21**, 247).

Cystine, tryptophan and tyrosine.—The cystine figures obtained by the Van Slyke method cannot be taken as correct. Being readily racemised during the hydrolysis, it should have been incompletely precipitated, the phosphotungstate of the racemised acid being more soluble than that of the optically active one. Independent determinations were therefore carried out in the acid hydrolysate of the proteins by the methods of (1) Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 421) making use of sodium hydroxide instead of sodium carbonate as modified by Hunter and Eagles (*J. Biol. Chem.*, 1927, **72**, 177) and (2) Sullivan (*J. Biol. Chem.*, 1927, **74**, xiv).

Tryptophan was determined by the methods of (1) Komm (*Z. Physiol. Chem.*, 1926, **156**, 202) using *p*-dimethylaminobenzaldehyde, and (2) Tillmans and Alt (*Biochem. Zeit.*, 1925, **164**, 135), using in both cases a solution of pure tryptophan as standard. It was however found difficult to compare colours by Komm's method, those developed by the proteins having always a shade of violet different from that

TABLE VI.

Distribution of Nitrogen in Concajanin, Cajanin and Albumin (Percentages of total nitrogen).

Form of Nitrogen	Concajanin			Cajanin			Albumin
	I	II	Average	I	II	Average	
Amide ...	9·05	8·97	9·01	10·42	10·20	10·31	9·05
Humin :— ...	2·0[illegible]	1·93	1·98	1·22	1·17	1·20	3·13
Insoluble ...	1·15	1·07	1·11	0·75	0·66	0·71	1·61
Soluble (adsorbed by lime)	0·88	0·86	0·87	0·47	0·51	0·49	1·52
Basic :— ...	29·43	28·51	28·97	24·45	25·31	24·88	24·96
Arginine ..	14·11	14·21	14·16	11·34	11·40	11·37	12·04
Cystine ...	1·01	1·23	1·12	0·28	0·34	0·31	1·31
Histidine ...	4·09	3·53	3·81	4·52	5·48	5·00	4·43
Lysine ...	10·22	9·54	9·88	8·31	8·09	8·20	7·18
Non-basic :— ...	59·42	59·88	59·65	64·81	63·33	64·07	62·84
Amino ...	55·64	57·22	56·43	62·69	61·65	62·17	59·26
Non-amino ...	3·78	2·66	3·22	2·12	1·68	1·90	3·58
Total ...	99·93	99·29	99·61	100·90	100·01	100·46	99·98

given by tryptophan. Since similar difficulties were also experienced in trials using egg and blood albumins the results obtained by Komm's method were not recorded. The method of Tillmans and Alt worked satisfactorily.

Tyrosine was estimated independently by the method of Folin and Ciocalteu (*J. Biol. Chem.*, 1927, **73**, 627) and together with tryptophan by that of Tillmans, Hirsch and Stoppel (*Biochem. Zeit.*, 1928, **198**, 379). The percentages of the essential amino-acids as determined by the different methods are given in Table VII.

TABLE VII.

Amino-acid	Albumin	Concajanin	Cajanin	Method
Lysine ...	5·54	7·90	6·93	Van Slyke
Arginine ...	5·53	6·75	5·72	,,
Histidine ...	2·42	2·16	2·99	,,
Cystine ...	2·73	2·24	1·41	Folin and Looney
,, ...	2·32	1·98	1·39	Sullivan
Tryptophan ...	2·05	1·48	0·21	Tillmans and Alt
,, ...	...	1·53	0·12	Tillmans, Hirsch and Stoppel
Tyrosine ...	...	3·45	3·16	Tillmans, Hirsch and Stoppel
,, ...	...	3·61	3·29	Folin and Ciocalteu

The figures for cystine by Sullivan's method are probably more trustworthy than those by that of Folin and Looney because the latter method is not specific for cystine (U. du Vigneaud, *J. Biol. Chem.*, 1927, **75**, 393). The methods of Tillmans and Alt for tryptophan and Folin and Ciocalteu for tyrosine gave more consistent results than the combined one of Tillmans, Hirsch and Stoppel; the figures obtained by the first two have therefore been taken as correct.

CAJANUS INDICUS FLAVUS (*tur tuar*)

The seeds of the above variety were from Thiruppattur in South India. The meal was prepared in the manner described already and on analysis was found to have the following percentage composition :— Moisture, 7·07 ; Ash, 3·57; Crude fat, 0·83 ; Protein (N × 6·25), 24·09 ; Crude fibre, 4·21 ; Carbohydrate (by difference) 60·24.

Saline 3·5 per cent. extracted 78·0 per cent. of the total nitrogen at $-2°$ and yielded on fractional precipitation with ammonium sulphate followed by repeptisation and dialysis in the manner described already the two globulins concajanin and cajanin. Pure specimens of the proteins were analysed for their sulphur and tryptophan content (Table VIII).

TABLE VIII.

Constituent and method	Percentage	
	Cajanin	Concajanin
Sulphur (Hoffman and Gortner, *loc. cit.*) ...	0·40	1·11
Tryptophan (Tillmans and Alt, *loc. cit.*) ...	0·32	1·61

The results approximate to those from peas of the *bicolor* variety.

DISCUSSION.

As observed in other plants of the natural order *leguminosæ*, the seeds of *Cajanus Indicus* contain among their reserve proteins two globulins which (1) appear to be characteristic of genera and independent of differences in species or varieties, (2) form the chief proteins of the seed and (3) differ from each other with regard to their sulphur contents and limits of precipitability with ammonium sulphate (compare Jones and Johns, *J. Biol. Chem.*, 1916–17, **28**, 67; Jones, Gersdorff, Johns and Finks, *J. Biol. Chem.*, 1923, **53**, 231).

The three proteins differ from each other in their general distribution of nitrogen, particularly in their amide and basic amino-acid contents. Cystine and tryptophan decrease in the order, albumin, concajanin and cajanin: lysine and arginine also vary, but follow a different order. Cystine appears to be the main sulphur-containing amino-acid in cajanin, while in concajanin and the albumin there appears to be also a large proportion of another sulphur-containing amino-acid of a lower molecular weight. The presence of identical proteins containing similar amounts of sulphur and tryptophan suggests that the peas of the *bicolor* and the *flavus* varieties are of almost equal nutritive value.

All the proteins in the pea are rich in tyrosine and contain the requisite amounts of cystine and the other essential diamino-acids;

but although the albumin and concajanin contain the necessary amounts of tryptophan, cajanin, the chief protein of the seed and representing over 58 per cent. of the total nitrogen, is deficient in that acid which is essential to growth and maintenance. Although this tends to reduce, to some extent, the independent nutritive quality of the pea, it does not lower its value in a mixed diet containing the essential amino-acids generally supplied by a variety of vegetable and animal proteins, so that deficiency in one is made up by adequate supply from another. In India where the peas are generally taken together with cereals it ought to be of high supplementary nutritive value, since it supplies useful amounts of the essential diamino-acids, particularly lysine, in which the latter are generally deficient.

SUMMARY.

1. The chief proteins of the peas of *Cajanus Indicus* of both the *bicolor* and *flavus* varieties were two globulins, cajanin and concajanin which (*a*) accounted for about 58 and 8 per cent. respectively of the total nitrogen and (*b*) differed from each other in their sulphur and tryptophan content. An albumin representing about 4 per cent. of the total nitrogen was also prepared and examined.

2. The three proteins contained requisite amounts of cystine, arginine and lysine : but since cajanin the chief protein of the seed was found to be deficient in tryptophan, the pea could not be said to make a complete protein food by itself. It should, however, make a valuable supplement to the cereal foods which are generally deficient in the essential diamino-acids.

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