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STUDIES IN NUTRITIVE VALUE OF INDIAN FOODSTUFFS II-AMINO ACID COMPOSITION OF CERTAIN LEAFY VEGETABLES AND MILLETS

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The information on the essential amino acid composition is of great help in assessing the nutritive value of proteins in foods and computing a balanced diet adequate in all the essential amino acids. Such knowledge also helps in improving the nutritive value of a foodstuff by supplementation with small amount of protein from other source or pure amino acids.

The amino acid composition of some of the common Indian foodstuffs have been reported.[2-7] However, there is still a gap in our knowledge regarding the amino acid composition of a number of foodstuffs, particularly, leafy vegetables. The leafy vegetables are valued in nutrition mainly for their vitamins and minerals. However, in recent years the nutritional importance of leafy vegetables as a source of dietary protein has been recognised and a few reports on the supplementary value of proteins of certain leafy vegetables to the proteins of cereals and legumes have been published.[8-10]

The present paper describes the results of investigation of six leafy vegetables and four millets in common use for their essential amino acid content by microbiological methods.

Experimental

1. *Samples*: The leafy vegetables such as *chakvat*, colocasia, *naval-kol*, safflower *shepu* and spinach were purchased from, the local market and cleaned with a piece of muslin cloth to remove the adhering soil. The leaves and tender stems (in case of colocasia, only leaves were taken) were then separated from the non-edible portion and cut into small pieces using stainless steel knife and plate. The samples were then spread out in small trays and dried in a current of air at 50°C and powdered in a grinder to pass through a 70 mesh sieve and stored in wide-mouth glass bottles until used for analysis.

The samples of the millets such as *banti*, *cheno*, *kodra* and *vari* were purchased from the local market. They were cleaned, powdered in a porcelain mortar to pass through a 70 mesh sieve and stored in tightly stoppered glass bottles until used for analysis.

2. *Microbiological procedure*:

(a) *Organisms and basal medium*: The microbiological assay of amino acids was carried out according to the method of Barton-Wright[11] using *L. arabinosus*, *L. mesenteroides P-60* and *Streptococcus faecalis R*. The uniform basal medium of Henderson and Snell[12] was used for the growth of the three organisms.

(b) *Preparation of hydrolysates*: Finely ground material corresponding to 60 mg. of nitrogen was hydrolysed by autoclaving for 8 to 10 hours at 15 lbs. steam pressure with 25 ml. of 2.5 N hydrochloric acid in a sealed tube. The hydrolysate was cooled and 2.0 ml. of 2.5 M sodium acetate solution was added to it. The pH of the solution was adjusted to 4.5 with dilute sodium hydroxide solution, diluted to 100 ml. and filtered. A portion (25 ml.) of the hydrolysate was extracted thrice with small portions of ethyl ether in a separating funnel and 20 ml. of the extracted aliquot was brought to pH 6.8 and diluted to 100 ml. (1 : 5 dilution). The other two dilutions 1 : 10 and 1 : 20 were also prepared and used directly for the assay of amino acids.

As tryptophan is completely destroyed by hydrolysis with strong acids, alkali hydrolysis was used for the liberation of this amino acid. A material corresponding to 60 mg. of nitrogen was hydrolysed by autoclaving for 7-8 hours at 15 lbs. steam pressure with 15 ml. of 5 N sodium hydroxide. The hydrolysate was cooled and 2 ml. of 2.5 M sodium acetate solution was added to it. The pH of the solution was

Table I. *The moisture, total nitrogen and minerals content of the foodstuffs*

Sl. No.	Name of the food-stuffs (English or Local)	Botanical name	Moisture (%)	Nitrogen (%)	Crude Protein (%) (N×6.25)	Minerals (%)
LEAFY VEGETABLES (Air dried)						
1.	Chakwat	*Chenopodium album*	7.45	4.44	27.75	20.96
2.	Colocasia	*Colocasia antiquorum*	6.78	5.27	32.94	12.20
3.	Naval-Kol	*Brassica oleracea caulorapa*	5.56	3.92	24.50	8.62
4.	Safflower	*Carthamus tinctorius*	7.06	3.38	21.12	14.96
5.	Shepu	*Peucedanum graveolens*	7.01	3.73	23.31	16.71
6.	Spinach	*Spinacia oleracea*	7.62	4·36	27.25	23.10
MILLETS						
7.	Banti (dehusked grain)	*Echinochloa Stagnana*	11.34	1.33	8.31	1.41
8.	Chenno (whole)	*Panicum repens.*	12.60	1.36	8.50	4.10
9.	Kodra (dehusked grain)	*Paspalum scorbiculatum*	12.25	1.32	8.25	1.45
10.	Vari (dehusked grain)	*Panicum miliaceum*	10.66	1.03	6.42	1.41

Table II. *Essential amino acid content of certain leafy vegetables and millets*

Sl. No.	Name of the food-stuffs (English or Local).	Total nitrogen in g/100 g. of edible sample	Essential amino acids in g. per 16g. of nitrogen							
			Leucine	Isoleucine	Valine	Lysine	Phenyl-alanine	Methionin	Tryptophan	Threonine
LEAFY VEGETABLES (air-dried)										
1	Chakwat	4.44	6.55	7.01	4 61	11.88	1.75	0.84	0.30	2.67
2.	Calocasia	5.27	5.82	4.94	4.46	4.15	2.88	1.06	0.65	2.41
3.	Naval-kol	3.92	8.06	5.32	4.97	8.61	2.62	0.73	0.59	2.16
4.	Safflower	3.38	11.14	8.57	6.57	9.71	2.63	0.88	0.43	2.74
5.	Shepu	3.73	4.60	5.63	4.47	7.13	1.89	0.30	0.41	1.97
6.	Spinach	4·36	9.79	6.12	4.89	7.76	2.52	0.62	0.58	2 49
MILLETS										
7.	Banti	1.33	16.64	8.73	6.46	2.93	3.21	1.90	0.51	2.29
8.	Cheno	1.36	17.75	11.99	7.91	3.51	3.81	1.89	0.38	2.80
9	Kodra	1.32	12.68	7.64	6.96	3.55	4.58	1.90	0.48	2.46
10.	Vari	1.03	13.49	6.26	6.93	4.24	5.02	2.80	1.00	1.32
	FAO Reference protein[13]	—	4.96	4.32	4.32	4.32	2.88	2.24	1.44	2.88
	Whole egg[14]	—	9.20	8.00	7.30	7.20	6.30	4.10	1.50	4.90

adjusted to 4.5 with dilute hydrochloric acid solution, diluted to 100 ml. and filtered. The rest of the procedure was the same as that described above. A complete racemization was assumed to have occurred of the amino acid during hydrolysis and dl-tryptophan was assumed to be exactly half as active as l-tryptophan. Thus, the values for the tryptophan content were doubled so as to make the correction for racemization.

(c) *Assay*: The standard and the hydrolysate (test solution) were pipetted in graded volumes from 0.1 to 1.0 ml. in test tubes (10 concentration levels) and in triplicate. One millilitre of basal medium was added to each tube and the final volume was made to 2 ml. with distilled water. The lactic acid produced was titrated with 0-02 N sodium hydroxide using bromothymol blue indicator. The results were calculated by reading the values corresponding to responses at different concentration levels of the hydrolysate directly from the standard curve.

The moisture, ash and total nitrogen

content of the foodstuffs were determined by the methods described by Ambegaokar *et al.*[1]

Results and Discussion

The moisture, crude protein (N x 6.25) and ash content of the foodstuffs analysed are shown in Taole I. The essential amino acids content of the foodstuffs calculated as g. of amino acid/16 g. nitrogen is shown in Table II. The values of the 'FAO reference protein'[13] and whole egg[14] have also been included in the table for comparison.

The leafy vegetables on dry weight basis contain fairly large amount of crude protein (Table I). The values vary from 21.12% (safflower) to 32.94% (colocasia), with an average of 26.14%. The values are comparable with those of pulse proteins. The protein content of the millets analysed varies from 6.42% (*vari*) to 8.50% (*cheno*) with an average of 7.87%.

The amino acids content of *cheno, banti* and *kodra* show more or less an uniform pattern. They contain fairly good amounts of leucine, isoleucine, valine and phenylalanine; but are deficient in tryptophan, lysine, methionine and threonine compared to the whole egg. Tryptophan is in the maximum deficit, and is followed by lysine, methionine and threonine, in the degree of deficiency. *Vari* is mainly deficient in threonine and the next in order of deficiency is lysine.

When the amino acid composition of the four millets is compared with the FAO reference protein, it will be seen that *benti, cheno* and *kodra* are deficient only in tryptophane and '*vari*' is deficient only in threonine.

The amino acid pattern of *kodra* (Bombay strain) reported by Ramachandran and Phansalkar[5] is in agreement with that shown in the present paper, with the exception that the values for phenylalanine and methionine obtained by them are higher. This may perhaps be due to the difference in the protein content of the two samples. The sample obtained by them from the Agriculture College, Poona, containd 4.78 g. % protein, while that purchased by us from the local market in Bombay City. contained 8.26% proteln.

The six leafy vegetables analysed contained more lysine than whole egg. They are fairly well balanced in respect of leucine, isoleucine, valine and threonine but are very poor in methionine, tryptophan and phenylalanine compared to the whole egg. Methionine is in the maximum deficit and is followed by tryptophan, and phenylalanine, in the degree of deficiency.

When the amino acid composition of these leafy vegetables is compared with FAO reference protein, it will be seen that they are deficient only in methionine and tryptophan.

Summary

The essential amino acids content of the four millets and six leafy vegetables were determined by microbiological methods.

Banti, cheno and *kodra* are deficient in tryptophan, lysine, methionine and threonine as compared to whole egg, tryptophan being the limiting amino acid. *Vari* is mainly deficient in threonine.

The leafy vegetables are rich in lysine content but are very poor in methionine and tryptophan.

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