II.—STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART I.—THE PROTEINS OF RAGI (ELEUSINE CORACANA): ELEUSININ, THE ALCOHOL-SOLUBLE PROTEIN.

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Ragi (*Eleusine coracana*), the staple cereal crop of the Mysore State, forms the greater part of the diet of the lower classes in that province. Its food-value is popularly supposed to equal that of wheat and to be much higher than that of rice. The proteins of this grain, hitherto uninvestigated, therefore appeared to merit study. Ragi contains from 1·1 to 1·7 per cent. of nitrogen or 6 to 11 per cent. crude protein (N × 6·25), typical analyses of the dry grain being given below expressed as percentages.

TABLE I.

Analysis of Ragi: dried at 100°.

...	Ash	Ether extractives	Crude protein* (N × 6·25)	Crude fibre	Carbohydrates (by difference)
I	2·78	1·82	6·90	3·27	85·23
II	2·52	1·61	9·83	3·54	82·50

In common with other cereal grains a considerable proportion of the protein consists of a prolamine, or alcohol-soluble protein which may be given the name eleusinin. In the present paper a preliminary account is given of an examination of this substance.

Separation of Eleusinin.

The grain was finely ground and passed through a 30 mesh sieve. The flour was then treated with three times its weight of 70 per cent. alcohol and vigorously stirred by mechanical means for six to ten hours. The extract was then filtered, yielding a clear, yellow liquor.

* True protein, 6·78 and 9·75.

In the earlier experiments the prolamine was separated by concentrating the extract at a temperature not exceeding 45° under reduced pressure. When the volume is reduced by about one-third precipitation of the protein begins and is complete when about half the liquid has been evaporated. Separated in this way, however, the protein is tough and leathery; after being washed, dried, powdered and extracted with ether to remove fatty material it was found to contain only 13·9 per cent. of nitrogen, and was therefore obviously impure. Moreover, probably owing to denaturation, it is no longer soluble in 70 per cent. alcohol and its further purification becomes a matter of difficulty.

A more convenient method of separation is to concentrate the alcoholic extract under reduced pressure until the first signs of precipitation are evident. The liquid is then filtered and poured slowly with constant stirring into ten times its volume of distilled water. A milky fluid results from which, on addition of a small quantity of an electrolyte such as sodium chloride, the protein is readily precipitated in flakes and being then easily soluble in 70 per cent. alcohol can be purified by repeated solution and reprecipitation. The precipitated protein is finally washed on a filter-funnel with large quantities of water to remove the sodium chloride and dried in a desiccator over sulphuric acid. The dry powder is then extracted in a Soxhlet with ether to remove traces of fat and again dried, when the protein is obtained as a greyish white powder containing on an average 15·9 per cent. of nitrogen. After treatment in the above manner it is no longer soluble in dilute alcohol. It gives positive reactions with the usual protein tests and contains both tyrosine and tryptophan. It contains no phosphorus however, and cystine is probably absent; but this point is receiving further investigation.

It is advisable not to prolong the preliminary extraction with 70 per cent. alcohol for more than ten hours: Otherwise denaturation takes place and the protein is always precipitated in a leather-like mass insoluble in dilute alcohol and therefore incapable of further purification. For comparative purposes slight variations were made from time to time in the method of preparation, e.g.,

(*a*) Direct extraction of the flour with cold 70 per cent. alcohol.

(*b*) Extraction at 50–60°.

(*c*) A preliminary extraction with 4 per cent. sodium chloride for 12 hours followed by extraction with cold 70 per cent. alcohol.

(*d*) As in (*c*) but with the alcoholic extraction carried out at 50–60°.

All four methods have given a final product with much the same degree of purity, the figures obtained by elementary analysis being as follows :—

TABLE II.

Composition of Eleusinin prepared by Different Methods.

Method of Preparation	*a*	*b*	*c*	*d*	Mean Result
C ...	52·83	53·87	53·56	52·92	53·29
H ...	7·14	7·60	7·73	7·34	7·35
N ...	16·10	15·98	15·91	15·65	15·91
O ...	23·93	22·53	22·79	24·09	23·34

The nitrogen distribution was next determined in a mixed sample by the Van Slyke method (*J. Biol. Chem.*, 1911, **10**, 15) as modified by Plimmer (*Biochem. J.* 1925, **19**, 1004). Hydrolysis was effected by boiling 3–4 grams of the protein with 100 c.c. of 20 per cent. HCl under a reflux condenser for 30 to 36 hours. Even after a prolonged digestion an insoluble residue is invariably left containing nitrogen equivalent to about 0·8–0·9 per cent. of the total nitrogen.

TABLE III.

Nitrogen Distribution in Eleusinin.

(Results expressed as percentages of total nitrogen).

Sample	I		II		III		Average	
Humin								
Insoluble	0·85		0·88		0·89		0·87	
Soluble	0·25		0·29		0·31		0·28	
Amide	19·72		20·97		20·87		20·52	
Basic, comprising	5·71		6·23		5·87		5·93	
Arginine		2·37		2·90		2·54		2·60
Histidine		2·79		2·52		2·76		2·69
Lysine		0·55		0·81		0·57		0·64
Non-basic, comprising ...	71·86		70·52		71·11		71·16	
Amino		69·31		68·93		68·85		69·03
Non-amino		2·55		1·59		2·26		2·13
Total ...	98·39		98·89		99·05		98·76	

TABLE IV.

*Comparison of Eleusinin with other Prolamines.**

(Results expressed as percentages of total nitrogen).

...	Gliadin from *Triticum vulgare*	Secalin from *Secale cereale*	Sativin from *Avena sativa*	Hordein from *Hordium vulgare*	Zein from *Zea mais*	Kaffirin from *Andropogon sorghum*	Sorghumin from *Sorghum vulgare*	Teozein from *Euchlaena Mexicana*	Eleusinin from *Eleusine coracana*
Amide	25·52	22·18	22·20	23·38	18·06	20·76	18·96	18·99	20·52
Humin	0·87	1·17	1·25	1·44	1·11	1·35	3·08	1·19	1·17
Basic, comprising									
Arginine	6·38	6·80	8·93	6·22	3·92	3·92	4·83	3·90	2·60
Histidine	5·41	7·21	2·31	10·36	2·45	1·71	1·19	3·58	2·69
Cystine	1·68	1·44	1·74	1·38	0·98	1·23	0·92	0·80	? ?
Lysine	0·57	0·42	1·20	3·02	0·89	2·48	3·45	2·57	0·64
Non-basic, comprising									
Amino	53·49	50·14	54·60	50·41	66·08	68·85	60·01	64·44	69·03
Non-amino ...	6·14	9·77	5·86	3·65	5·90	0·32	5·33	3·62	2·13
Total ...	99·77	99·13	98·09	100·26	99·40	100·62	97·77	97·09	98·76

* Cf. Hoffman and Gortner, Colloid Symposium Monograph, Vol. II, Chemical Catalog Co., New York, 1925.

The essential difference in nitrogen distribution between eleusinin and most of the other prolamines examined is, therefore, the lower content of basic nitrogen including the possible absence of cystine and a correspondingly high percentage of amino-nitrogen. The work is being extended and a complete estimation of the constituent amino-acids is being attempted.

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