THE PROLAMIN OF COIX LACRYMA L.

By

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The alcohol-soluble protein of Hato-mugi, *Coix lacryma L.*, has already been studied by S. Yoshimura and N. Sagara (1919). They prepared the protein, extracting the meal with 70% alcohol at 60° and determined the percentage of amino-acids in the crude protein by the usual method.

For this prolamin contains a relatively high percentage of leucine and tyrosine compared with the gliadin of other cereals; they have distinguished it by calling it coicin.

In their experiments, however, finding that the actual percentage of the basic amino-acids is not in harmony with the calculated value from nitrogen distribution in the protein, the authors has undertaken the present experiment to ascertain the true nature of the pure protein.

The material used for this experiment consists of Hato-mugi kernels and was bought in market overt in Osaka.

The kernel entirely free from bran, was greenish white, and the meal was prepared by one of the authors. The composition of this meal (A) thus obtained together with the results (B) of another sample prepared from seeds grown at Yodo near Kyoto, in a wild state, gathered by one of the authors at the end of October, are given below:

	(A)	(B)
Water	11.52	12.33
Ash	1.84	7.06
Protein N	(2.89)	(2.92)

Crude protein	18.49	18.68
Crude starch	57.33	50.66
Soluble non-nitrogenous matter	8.61	4.93
Crude fat	2.47	4.69
Crude fibre	7.77	7.42
Total	108.03	105.77

The following experiments were carried out with fat-free material extracted with petroleum ether as usual.

The authors, first tried to extract a protein from 100 gm. of the meal by shaking it for 3 hours in water, 10% sodium chloride and 5% barium hydroxide solutions successively, but a nitrogen determination by Kjeldahl's method showed that only traces of protein were thus extracted as shown in the following analysis:

Water extract:	0.8 gm. protein (N×6.45)
10% NaCl extract:	0.25 gm. „ („)
5% $Ba(OH)_2$ extract:	0.5 gm. „ („)

From these experimental results, the authors came to the conclusion that the meal contains small quantity of proteins other than prolamin.

Preparation of Protein soluble in dilute Alcohol, Coicin.

One part of the meal was extracted with 15 parts of 85% (I), 80% (II) and 70% (III) alcohol at various temperatures for 5 hours and the supernatant liquid was decanted and the residue squeezed nearly dry. The nitrogen content in the extracts after filtering perfectly clear, was determined by Kjeldahl's method:

	Sample		Nitrogen gm.	%	Protein gm.	%	Total protein
(I)	90 gm. (ordin. temp.)	extract:	0.038	0.1	0.56	0.62	16.13%
		residue:	2.181		13.99		
	90 gm. (50°—60°)	extract:	0.152	0.17	0.97	1.08	15.39
		residue:	2.012		12.88		
	90 gm. (75°—80°)	extract:	0.731	0.81	4.68	5.2	15.83
		residue:	1.491		9.57		

(II)	350 gm. (78°)	extract: 2.54 residue: 5.75	0.72	16.22 36.82	4.6	}	15.2
(III)	300 gm. (78°)	extract: 1.72 residue: 5.32	0.57	11.01 34.05	3.7	}	15.02

The most successful preparation of coicin as seen in the foregoing table, was obtained by extracting the meal with 80% alcohol below 80°, precipitating the protein after concentrating the solution under reduced pressure, pouring into water; redissolving it in 80% alcohol at 70°, filtering through silk cloth, concentrating and then reprecipitating with water. After repeating the reprecipitation 4 times, the protein dissolved in 80% alcohol, and the concentrated solution was poured into absolute alcohol.

The pure protein, thus obtained, was digested with absolute alcohol for 2 months and then treated with absolute ether. After filtering off the ether, the prolamin was placed in a vacuum desiccator over paraffin to remove the ether and dried at 100° in a vacuum tube.

The pure protein, thus prepared, was a yellowish brown powder, containing 0.6% of ash.

1) 0.1448 gm. (ash free) sub. gave 0.2792 gm. CO_2 & 0.085 gm. H_2O
2) 0.0847 „ 0.1655 „ 0.052 „
3) 0.1117 „ 0.213 „ 0.0672 „
4) 0.5912 „ 0.0613 gm. $BaSO_4$
5) 0.4255 „ 0.0251 gm. $BaSO_4$
6) 0.0972 „ 0.01558 gm. N (Kjeldahl's method)
7) 0.0805 „ 0.01345 „
8) 0.0688 0.01177 „

	I	II	III	IV	V	VI	VII	VIII	mean
C	52.59	53.29	52.01						52.63
H	6.65	6.87	6.73						6.75
N						16.03	16.71	17.10	16.61
S				1.44	0.81				1.12
O									22.89

Distribution of Nitrogen.

To determine the distribution of the nitrogen in the pure prolamin by T. B. Osborne and J. F. Harris' method (1903), about 1 gm. protein was heated with 50 cc. of 20% hydrochloric

acid for 10 hours. The results together with those published by Yoshimura and Sagara (Y-S) are given below:

	Authors		Y–S
	In percentage of Protein	total Nitrogen	In percentage of total N
Humine N	0.1		
Ammonia N	3 32	19.82	21.26
Basic N	0.94	4.44	8.18
Non-basic N	0.23	1.39	
Other N (Mono amino N (by van Slyke's method))	5.50	32.81	62.56
Other N (Imino N (by difference))	6.95	41.54	
Total	16.84	100.00	100.00

Contents of some Amino Acids.

Analysis of *basic amino acids* and *tyrosine* of pure coicin was made by the usual method, 8.6455 gm. of protein being taken.

The determination of nitrogen of basic amino acids was made by both the van Slyke and Kjeldahl's methods:

Amino acids	Authors				Y—S	
	Van Slyke's m.		Kjeldahl's m.			
		%		%		
Arginine N	0.0301	2.12	0.0141	3.07	1.70	86.00
Histidine N	0.0062	0.43	0.0056	0.39	0.15	11.31
Lysine N	0.0072	0.50	0.0126	0.88	—	2.96
Total		3.05		4.34		100.00

2.91 gm. of crude tyrosine was obtained from which 1.458 gm. pure compound was isolated.

Quantities of *glutamic acid* and *leucine* were determined as usual, using 4.5828 gm. of the pure protein; and 1.181 gm. pure glutamic acid hydrochloride (0.9462 gm. glutamic acid) and 0.6028 gm. esters of amino-acids, boiling at 35—125°, 10—11 mm. Hg., were obtained.

0.1877 gm. pure leucine, which gave 0.01975 gm. N. by Kjedahl's method, 10.52% N, (theoretical value for $C_6H_{13}NO_2$: 10.65%), was isolated from 0.4558 gm. free amino acid mixture prepared by saponifying the ester above described.

0.1343 gm. of glutamic acid hydrochloride gave 0.0107 gm. N. by Kjeldahl's method, 7.98% N, (theoretical value for $C_5H_{10}NO_4Cl$: 7.63%).

The percentage composition of amino-acids in Hato-mugi is as follows:

Amino-acids	
Glutamic acid	20.65%
Leucine	4.10
Tyrosine	1.46
Arginine	0.20
Histidine	1.88
Lysine	0.76

Summary.

1. The prolamin of Hato-mugi has been isolated in pure state and analysed.

2. Results indicate that it contains glutamic acid, leucine, tyrosine and the basic amino-acids, arginine, histidine and lysine and that it resembles the prolamin of oats.

References.

Osborne, T. B. and Harris, J. F. (1903): J. A. C. S., **22**, 323.
Yoshimura, S. and Sagara, N. (1919): Tokyo Kagaku-Kaishi, **40**, 483.