KAFIRIN, AN ALCOHOL-SOLUBLE PROTEIN FROM KAFIR, ANDROPOGON SORGHUM.*

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Kafir has become an important crop in the United States during the last decade. In 1910 three million acres were under cultivation and the value of the crop was thirty million dollars. Hitherto, no study has been reported on the proteins of kafir. It has been known for some time that this cereal contains an alcohol-soluble protein. Osborne,[1] among others, states that he found it to contain such a protein, but he did not make a study of it. The seeds used in our experiments were grown in Kansas in 1915 and were of the variety known as dwarf kafir. These contained a strong red coloring substance soluble in alcohol, which accounts for the color of the alcoholic extracts described below.

Nitrogen determinations on the kafir meal showed that it contained 11.7 per cent of protein (N $\times$ 6.25). Of this, boiling 60 per cent alcohol extracted 7.9 per cent of protein, based on the nitrogen content of the extract. We were able to isolate 5.2 per cent of pure protein, dried at 110°C., by the use of alcohol ranging from 60 to 70 per cent by volume. As no account was taken of the losses occurring in the various manipulations during the preparation of the protein, this yield indicates that most of the nitrogen extracted was in the form of protein.

Since this is the first protein isolated from kafir and constitutes the greater part of the protein contained therein, we have named it *kafirin*.

* A preliminary paper was presented at the Urbana meeting of the American Chemical Society (*Science*, 1916, xliv, 217).

The kafir used in these experiments was obtained from the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture. It is known as Dwarf Blackhull kafir and is described by Ball, C. R., and Rothgeb, C., *U. S. Dept. Agric., Farmers' Bull. 552*, 1913, p. 8.

[1] Osborne, T. B., The Vegetable Proteins, London, 1909, 80.

In its ultimate composition kafirin closely resembles zein from maize, as is shown by a comparison of the following values.

	Kafirin.	Zein.*
C.	55.19	55.23
H.	7.36	7.26
N.	16.44	16.13
S.	0.60	0.60
O.	20.41	20.78

* Chittenden, R. H., and Osborne, T. B., *Am. Chem. J.*, 1891, xiii, 453; 1892, xiv, 20.

While zein is very soluble in 70 per cent alcohol at all temperatures, kafirin requires a large quantity of the same strength of alcohol to effect solution. Kafirin is much more soluble in hot alcohol than in cold and even rather dilute solutions will form a jelly on cooling. On this account it was necessary to use the large volumes of alcohol stated in the experimental part of this paper, and to filter the extracts while hot. Kafirin also coagulates easily, while an alcoholic solution of zein does not coagulate when heated.

A striking difference between kafirin and zein is found in the distribution of the nitrogen in these proteins as shown by the following figures.

N	Kafirin.	Zein.*
Humin	0.17	0.16
Amide	3.46	2.97
Basic	1.04	0.49
Non-basic	11.97	12.51
Total	16.64	16.13

* Osborne, T. B., and Harris, I. F., *J. Am. Chem. Soc.*, 1903, xxv, 323.

Apparently a further difference exists between kafirin and zein in the proportion of the diamino-acids which these proteins yield. The following figures give the percentages of these acids as shown by an analysis of kafirin by the Van Slyke method and an analysis of zein made by Osborne and Jones[2] by Kossel's direct method.

[2] Osborne, T. B., and Jones, D. B., *Am. J. Physiol.*, 1910, xxvi, 227.

In the analysis of kafirin 0.78 per cent of cystine was precipitated with the phosphotungstates of the other bases and a corresponding correction has been made in the figures.

	Kafirin.	Zein.
Arginine	1.58	1.55
Lysine	0.90	0.00
Histidine	1.00	0.82
Tryptophane	Present.	0.00

It is thus seen that kafirin contains lysine and tryptophane, both of which are lacking in zein, and which are necessary for animal nutrition.

Further investigations on the proteins of kafir are in progress.

EXPERIMENTAL.

Preparation of Kafir Meal.—The kafir seeds were ground to a meal. This was placed in stoppered bottles and used for the experiments described below.

Preparation of Kafirin.—The kafir meal was stirred into eight to ten parts of boiling 70 per cent ethyl alcohol (by volume). This mixture was heated for an hour in a double boiler made by placing a pail containing the extraction mixture in a somewhat larger pail containing water heated to about 80°C. To avoid coagulation of the protein, the mixture must be frequently stirred to prevent the meal from settling as a hard cake on the bottom of the pail and becoming overheated. If none of the kafirin had been coagulated the hot mixture was readily filtered on a Buchner funnel and the deep red filtrate again filtered through a folded filter. In this way a perfectly clear liquid was obtained. This was concentrated under diminished pressure to about one-half its original volume and poured into a large quantity of chilled, distilled water.

The kafirin first appeared as a milky suspension which showed no tendency to flock. The addition of sufficient sodium chloride solution while stirring caused the protein to settle as a bulky flocculent or granular precipitate, leaving the supernatant liquid almost clear. This precipitate was washed several times by decan-

tation and then filtered off on cheese-cloth, and the water held in the precipitate was pressed out as far as possible by wringing the cloth. The protein was disintegrated and suspended in absolute alcohol for 24 hours. The alcohol assumed a deep red color from the coloring substances carried down by the protein. To remove all the color it was necessary to renew the absolute alcohol two or three times. The kafirin was then filtered off and suspended in absolute ether for 24 hours. The ether was poured off and the kafirin squeezed out in a cloth and disintegrated in a meat chopper, which left it in a granular condition. It was then placed in a vacuum desiccator over sulfuric acid until freed from ether, and finally dried in the vacuum oven, the temperature being gradually raised to 110°C. The dried kafirin was then easily ground to a flour in a mill and obtained as a gray powder. Preparations 1 to 12, inclusive, were made in this way.

In order to determine whether the kafirin could be obtained in a purer state, Preparation 13, after precipitation with water and filtering, was redissolved in hot 70 per cent alcohol. A large volume of alcohol is necessary in redissolving the kafirin as coagulation occurs if too small a volume is used. The kafirin was reprecipitated by pouring into a large volume of water and was then treated as described above. Comparison of the analytical results makes it apparent that there is no advantage to be gained by purification in this manner, which at the same time greatly reduces the yield.

Analyses of Preparations 1 to 13, calculated on a moisture-free basis, gave the following results.

	Preparation 1.				Preparation 2.	
	I.	II.	Average.	Ash-free.		Ash-free.
C	55.22	55.01	55.12	55.20	55.36	55.52
H	7.31	7.25	7.28	7.29	7.29	7.31
N	16.40	16.47	16.43	16.46	16.20	16.25
S	0.63		0.63	0.63	0.55	0.56
O				20.42		20.36
Ash	0.17	0.17	0.17		0.26	

	Preparation 3.				Preparation 4.	
	I.	II.	Average.	Ash-free.		Ash-free.
C	55.07	55.35	55.21	55.38	55.07	55.21
H	7.20	7.25	7.22	7.26	7.24	7.26
N	16.30	16.15	16.22	16.28	16.37	16.41
S	0.71		0.71	0.71	0.66	0.66
O				20.37		20.46
Ash	0.33				0.28	

	Preparation 5.				Preparation 6.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
C	55.33	55.10	55.21	55.33	54.86	54.90	54.88	54.99
H	7.38	7.35	7.36	7.38	7.25	7.25	7.25	7.27
N	16.36	16.29	16.32	16.35	16.32	16.36	16.34	16.38
S	0.62		0.62	0.62	0.49	0.52	0.51	0.51
O				20.32				20.85
Ash	0.22				0.22			

	Preparation 7.				Preparation 8.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
C	55.38	55.42	55.40	55.60	55.02	54.88	54.95	55.07
H	7.35	7.32	7.34	7.36	7.47	7.44	7.45	7.47
N	16.31	16.46	16.38	16.43	16.41	16.55	16.48	16.53
S	0.56		0.56	0.56	0.64		0.64	0.64
O				20.05				20.29
Ash	0.31				0.20			

	Preparation 9.				Preparation 10.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
C	55.12	54.94	55.03	55.19	54.80	54.87	54.83	55.06
H	7.36	7.41	7.38	7.40	7.30	7.46	7.38	7.41
N	16.57	16.61	16.59	16.64	16.61	16.43	16.52	16.58
S	0.50		0.50	0.50	0.60		0.60	0.60
O				20.27				20.35
Ash	0.24				0.47			

	Preparation 11.				Preparation 12.			
	I.	II.	Average.	Ash-free.	I.	II.	Average.	Ash-free.
C	54.98	54.81	54.89	55.03	54.79	54.75	54.77	54.89
H	7.22	7.34	7.28	7.30	7.37	7.51	7.44	7.46
N	16.48	16.33	16.40	16.45	16.43	16.39	16.41	16.45
S	0.59			0.59	0.60		0.60	0.60
O				20.63				20.60
Ash	0.23				0.21		0.21	

	Preparation 13 (redissolved).			
	I.	II.	Average.	Ash-free.
C	54.92	54.90	54.91	55.04
H	7.41	7.42	7.41	7.43
N	16.54	16.40	16.47	16.51
S	0.65		0.65	0.65
O				20.37
Ash	0.20		0.20	

Summary of Analyses of Kafirin.

Preparation No.	1.	2.	3.	4.	5.	6.	7.
C	55.20	55.52	55.38	55.21	55.33	54.99	55.60
H	7.29	7.31	7.26	7.26	7.38	7.27	7.36
N	16.46	16.25	16.28	16.41	16.35	16.38	16.43
S	0.63	0.56	0.71	0.66	0.62	0.51	0.56
O	20.42	20.36	20.37	20.46	20.32	20.85	20.05

Preparation No.	8.	9.	10.	11.	12.	13.	Average.
C	55.07	55.19	55.06	55.03	54.89	55.04	55.19
H	7.47	7.40	7.41	7.30	7.46	7.43	7.36
N	16.53	16.64	16.58	16.45	16.45	16.51	16.44
S	0.64	0.50	0.60	0.59	0.60	0.65	0.60
O	20.29	20.27	20.35	20.63	20.60	20.37	20.41

Distribution of Nitrogen in Kafirin.—The distribution of nitrogen was obtained from an analysis made by the Van Slyke method. A sample of kafirin containing 16.64 per cent of nitrogen gave the

following results, from which it appears that this protein differs from zein in containing distinctly more amide nitrogen as well as basic nitrogen.

N	Kafirin.	Zein.*
Humin	0.17	0.16
Amide	3.46	2.97
Basic	1.04	0.49
Non-basic	11.97	12.51
Total	16.64	16.13

*Osborne and Harris, *J. Am. Chem. Soc.*, 1903, xxv, 323.

The percentage of diamino-acids in kafirin was also determined by the Van Slyke method. The results given below have been corrected for 0.78 per cent of cystine which was precipitated with the phosphotungstates of the other bases.

	Kafirin.	Zein.*
Arginine	1.58	1.55
Lysine	0.90	0.00
Histidine	1.00	0.82
Tryptophane	Present.	0.00

*See reference 2.

These results indicate that kafirin contains the lysine and tryptophane complexes, both of which are wholly lacking from zein.

SUMMARY.

A new alcohol-soluble protein has been isolated from kafir seeds, which constitutes more than one-half the protein in the seed. This protein has been named *kafirin*. Kafirin resembles zein in its ultimate composition, but is different in physical properties. It contains tryptophane and apparently lysine, both of which are lacking in zein. An analysis of kafirin has been made by the Van Slyke method. This shows quantitative differences between kafirin and zein.