PHYSIOLOGICAL STUDIES ON CEREALS. III. THE OCCURRENCE OF POLYPEPTIDES AND AMINO ACIDS IN THE UNGERMINATED MAIZE KERNEL[1]

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INTRODUCTION

Up to comparatively recent years the generally accepted idea was that the ungerminated kernel of cereals contains the nitrogen practically only in the form of proteins and nucleoproteins. Having shown in previous papers that the ungerminated kernel of wheat (*Triticum vulgare*) (*8*)[3] and of oats (*Avena sativa*) (*9*) contains in addition to proteins also polypeptides and free amino acids, it was of considerable interest to find out whether or not this holds good for the maize kernel also. This work was prompted not only by the fact that corn (*Zea mays*) is the most important crop in the United States both in acreage and in value (*11*), but also by the consideration that few polypeptides have ever been shown to occur in plant and animal materials and by the further consideration that the nutritive and physiological significance of foodstuffs and feedstuffs will not be fully understood until all their constituents have been determined. It is, therefore, believed that this paper fills a gap in our knowledge concerning the nitrogen compounds met with in the maize kernel.

So far as proteins are concerned, Chittenden and Osborne (*2*) found that the corn kernel contains albumins, globulins, an alcohol-soluble protein (zein), and proteose. The latter, which is found in the extracts of corn meal, may, however, be an artificial product formed by hydrolysis of the above-mentioned proteins. In addition to these proteins, the presence in the maize kernel of glutelin (a protein soluble in dilute alkalis or acids) was later reported by Osborne (*12*). According to Osborne and Clapp (*13*), the proportions in which the proteins occurred in yellow corn meal were as follows: Globulins, albumins, and proteoses, 0.45 per cent; zein (soluble in alcohol), 5 per cent; and glutelin (soluble in alkaline or acid solutions), 3.15 per cent. Similar data have been reported by Johns, Finks, and Paul (*10*). Knowledge is very meager concerning the nonproteins, to say nothing of the complete absence of data with regard to their presence in the different varieties of corn. Owing to the work of Schulze and Castoro (*15*) it is known that the seed of maize contains 0.90 per cent of nonproteins calculated on the basis of the oven-dried seed, or 4.9 per cent if calculated on the total nitrogen content. It is also known from the work of Schulze (*14*) that maize seed contain 0.25 per cent of lecithin. According to Czapek (*3, p. 157*), the proportion of lecithin in the yellow maize seed is 0.25 per cent, while in the white seed it is 0.28 per cent, calculated on the oven-dried seed. In this paper it is shown that the ungerminated corn kernel contains amino acids and polypeptides. While it is true that the proportions in which they occur in the corn kernel are small, they seem nevertheless to be of considerable significance because amino acids are the bricks out of which the great protein structures are built, and for the further reason that the amino acids, being the most reactive material of the cells, are capable of performing important vital functions in the plant and animal organism (*1, p. 61–62*). The polypeptides, too, standing closely to the proteins, no doubt offer the immediate material for the synthesis of proteins. While the maize seed contains great quantities of proteins, the latter are not diffusible. On the other hand, the amino acids, being soluble and diffusible, represent the best material for translocation of the nitrogen to the growing parts of the young seedling, before the proteolytic enzymes in the seed have come into play.

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[3] Reference is made by number (italic) to "Literature cited," p. 592.

EXPERIMENTAL DATA

MATERIAL USED

Three corn varieties of commercial and agricultural importance were used in the experiments reported in this paper.

The Four County corn variety, which was started with different strains of Silver King, has been bred since 1910 or 1912 by the Four County Grain Improvement Association at Ackley, Iowa, under the direction of the Iowa Agricultural Experiment Station. About 100,000 acres are grown each year in north-central Iowa.

United States selection No. 77, which is a White Dent variety, selected and bred by the United States Department of Agriculture from Woodburn's White Dent, is grown in southern Ohio and Indiana.

United States selection No. 193, which is an 8-rowed yellow flint, selected and developed by the United States Department of Agriculture from Hall Gold Nugget, is grown in the Hudson River Valley in southeastern New York. The corn samples were ground in an electric buhr-mill and passed through a 40-mesh sieve.

DESCRIPTION OF METHODS

(1) The total nitrogen content was estimated according to Kjeldahl's method.

(2) The protein nitrogen was estimated according to Stutzer's method (*17*).

(3) The nitrogen of amino acids was determined according to Sörensen's method (*16*), as applied by the writer elsewhere (*5*, *6*, *7*).

Other methods and their details will subsequently be described in this paper.

From the experimental data presented in Table I it will be seen that whereas the Four County corn is characterized by the highest percentage of total and of protein nitrogen, of dry substance and of ash, Hall Gold Nugget is characterized by the lowest percentage of the corresponding constituents; the figures for United States selection No. 77 are between these two, with the exception of the dry substance, which is lower in United States selection No. 77 variety than in Hall Gold Nugget.

TABLE I.—*Percentage of dry substance, of ash, and of total, protein, and nonprotein nitrogen in the ungerminated maize kernel*

Variety of corn	Where and when grown	Dry substance	Ash	Total nitrogen	Protein nitrogen		Nonprotein nitrogen	
		Air-dry corn	Oven-dried corn	Oven-dried corn	Oven-dried corn	Total nitrogen	Oven-dried corn	Total nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Four County corn	At Iowa Agricultural Experiment Station, Ames, Iowa, in 1921.	91.34	1.40	1.74	1.62	95.22	0.081	4.78
	do	91.51	1.39	1.67	1.62	95.31	0.080	4.69
	do	91.41	1.40	1.66	1.64	96.58	0.058	3.42
	do	91.51	1.40	1.71				
Average	do	91.44	1.40	1.70	1.63	95.70	0.073	4.30
United States selection No. 77.	At Piketon, Ohio, in 1919.	90.05	1.32	1.59	1.51	94.96	0.080	5.04
	do	89.95	1.32	1.58	1.52	95.85	0.066	4.15
	do	90.52	1.30	1.59	1.52	95.85	0.066	4.15
	do	89.50	1.33	1.59	1.53	96.14	0.061	3.86
	do		1.34	1.60				
	do		1.33	1.60				
	do		1.32	1.55				
	do			1.61				
Average	do	90.00	1.33	1.59	1.52	95.69	0.068	4.31
Hall Gold Nugget selection No. 193.	At Rhinebeck, N. Y., in 1921.	91.40	1.34	1.46	1.40	96.19	0.056	3.81
	do	91.37	1.33	1.45	1.39	95.26	0.069	4.74
	do	91.37	1.33	1.49	1.39	95.46	0.066	4.54
	do	91.38	1.31	1.46	1.39	95.46	0.066	4.54
	do			1.47				
	do			1.49				
	do			1.42				
	do			1.45				
Average	do	91.38	1.33	1.46	1.39	95.59	0.064	4.41

Experiments conducted with the three corn varieties in order to ascertain how best to extract the nitrogen of the corn flour have shown that the nitrogen extracted by water at room temperature in one hour is as great as that obtained in two and four hours, respectively. In this connection it was found, as would be expected, that a cold aqueous extract of the flour is absolutely free from starch and is very convenient to handle, while a hot aqueous extract contains great quantities of starch and is considerably harder to handle. Since the data concerning the extraction of the flour with water are quite uniform, they are omitted here to save space. It may suffice to mention that in the experiments reported subsequently definite amounts of flour with measured quantities of water were agitated by means of a shaking machine for one hour at room temperature, an antiseptic being used to prevent bacterial action.

The extract obtained was freed from starch, proteins and other insoluble substances by filtration, the filtrate concentrated *in vacuo*, any precipitates formed during concentration were removed by centrifugation, the supernatant liquid evaporated to dryness, and the residue extracted with 80 per cent alcohol, filtered and the alcohol distilled off. The residual yellow sirup was dissolved in hot water, heated to boiling, acidified with acetic acid, boiled, and filtered, the filtrate treated with freshly made lead hydroxide and some lead acetate, and again boiled and filtered. This filtrate was now concentrated under reduced pressure to a small volume.

It should be borne in mind, however, that a water-extract of seeds, prepared in the cold, usually contains proteolytic enzymes which may in part hydrolyze the proteins and proteoses present in the maize kernel. For this reason it seemed necessary to carry out parallel experiments under conditions which destroy the enzymes completely, in order definitely to decide whether or not the amino acids and polypeptides are preformed in the maize kernel. Hence weighed quantities of flour were treated in flasks with boiling hot ammonia-free water and kept on the water bath for about half an hour, whereupon their contents were contrifuged. The solid residue was then treated in the same way once more. The hot water-extracts thus obtained were then treated essentially as described above. The concentrated purified extracts obtained were applied to the estimation of the acid amides, amino acids, and polypeptides.

In order to determine the nitrogen of acid amides, the purified extract corresponding to a definite quantity of flour was made up to 100 c. c., of which two portions of 20 c. c., each were oxidized according to Kjeldahl's method to ascertain the amount of nitrogen present. To 50 c. c. of the remaining solution hydrochloric acid was added to a concentration of 20 per cent and boiled for 30 minutes using a reflux condenser. The hydrolysate was evaporated on the water bath to dryness, the residue transferred quantitatively to a Kjeldahl flask and distilled with magnesium oxide, the ammonia thus obtained being titrated with standard acid.

For the estimation of the nitrogen of amino acids and of polypeptides a sufficient quantity of the purified extract, at least twice the amount used for the acid amide determination, was made up to 200 c. c., in two 10 c. c. portions of which the nitrogen was estimated according to the Kjeldahl method. The remaining 180 c. c. were made up to 200 c. c. and divided into 100 c. c. portions *a* and *b*. In portion *a*, freed from carbon dioxide, phosphoric acid, and coloring matter, the amino nitrogen was determined by the formol-titration method (*16*), while portion *b* was employed for the estimation of the peptide nitrogen. Enough hydrochloric acid was added to portion *b* to make a 20 per cent solution and boiled under a reflux condenser for 12 hours, in accordance with the observations of Fischer (*4, p. 53*). The hydrolyzed material was then evaporated on the water bath to dryness, transferred to a Kjeldahl flask, to which magnesium oxide was added and the ammonia expelled by distillation. The residue was then thoroughly extracted with boiling hot ammonia-free water and the filtered extract concentrated to 100 c. c. of which two portions of 20 c. c. each were oxidized according to the Kjeldahl method, while 50 c. c. of the remaining liquid were used for formol-titration. The result obtained here by titration with formaldehyde diminished by the amino nitrogen secured prior *to* the hydrolysis yields the nitrogen of the polypeptides. The data are recorded in Table II.

Examination of Table II shows that the highest proportion of the nitrogen of acid amides and polypeptides is found in Four County corn, while the highest proportion of amino nitrogen is contained in Hall Gold Nugget selection 193, when the latter is referred to the oven-dried kernel and to its total nitrogen. The figures for United States selection 77 fluctuate, some higher and some lower than for

TABLE II.—*Partition of the nonprotein nitrogen in the ungerminated maize kernel*

Variety of corn	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen	Remarks
Data expressed in percentage of the oven-dried maize kernel:	*Per cent*	*Per cent*	*Per cent*	
Four County corn	0.034	0.044	0.070	Hot-water extract.
United States selection No. 77	.018	.042	.052	Cold-water extract.
Hall Gold Nugget selection No. 193	.021	.052	.035	Hot-water extract.
Do	.022	.050	.039	Cold-water extract.
Data expressed in percentage of the total nitrogen of the maize kernel:				
Four County corn	2.00	2.59	4.12	Hot-water extract.
United States selection No. 77	1.13	2.64	3.27	Cold-water extract.
Hall Gold Nugget selection No. 193	1.44	3.56	2.40	Hot-water extract.
Do	1.51	3.43	2.67	Cold-water extract.
Data expressed in percentage of the water-soluble nitrogen of the maize kernel:				
Four County corn	16.58	21.68	34.36	Hot-water extract.
United States selection No. 77	11.64	28.11	34.11	Cold-water extract.
Hall Gold Nugget selection No. 193	11.15	27.57	18.55	Hot-water extract.
Do	11.32	26.51	20.66	Cold-water extract.

the last named variety. The outstanding feature of Table II, in which each figure ordinarily represents the average of at least four individual estimations, is the fact that each of the three varieties contains acid amides, amino acids, and polypeptides in not inconsiderable quantities. From the circumstance that the results obtained with the hot-water extracts do not essentially differ from the data secured with the cold-water extracts it follows that under the conditions of the work, extraction with cold water for but one hour and rapid evaporation of the extracts *in vacuo*, no noticeable hydrolysis of the proteins under the influence of enzymes took place. It further follows that amino acids and polypeptides are preformed in the corn kernel.

In the experiments described, the purification of the aqueous extracts was effected by means of acetic acid, lead hydroxide, and lead acetate. This treatment removes completely the proteins but does not remove the proteose quantitatively. Since the latter is present in the water extracts of corn, though in very small quantity, the idea suggested itself that the hydrolysis of the polypeptides might be accompanied by the hydrolysis of the proteose, in which case the results reported for the polypeptides might be somewhat too high (see the fourth column of Table II). Hence, it seemed necessary to remove quantitatively the proteose along with the proteins before hydrolysis takes place. This was accomplished by the use of phosphotungstic acid. The dry residue of the alcoholic extract of a known quantity of flour was dissolved in water and made up to a definite volume, usually to 100 c. c. or its multiple. After determining the nitrogen by the Kjeldahl method in a small portion, the bulk of the solution was treated with 5 gm. of sulphuric acid mixed with 30 c. c. of a solution containing 20 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. In each case it was ascertained that a slight excess of the precipitant was used. The precipitates formed were filtered, after 24 hours, and washed with a solution made up of 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. The quantity of nitrogen present in the phosphotungstic precipitate was estimated by the Kjeldahl method. The filtrate from phosphotungstic acid precipitate was treated with calcium hydroxide to slight acidity, then with barium hydroxide to slight alkalinity, whereupon it was saturated with carbon dioxide, the whole heated to boiling, filtered, and thoroughly washed with hot ammonia-free water. The filtrate and washings from the phosphotungstate, sulphate, and carbonate of calcium and barium were evaporated in a vacuum, made up to a definite volume, and the amino nitrogen estimated by formol titration, while the peptide nitrogen was determined, on hydrolysis, as already outlined. The results secured are summarized in Table III.

A glance at Table III shows that the prevalent quantity of nitrogen is present in the phsophotungstic acid precipitate which contains the proteins, proteose, and any basic compounds occurring in maize, while the nitrogen proportions of the polypeptides, amino acids, and acid amides follow in the order named, with the exception of Hall Gold Nugget selection 193, in which the proportion of amino acids is

TABLE III.—*Partition of the nonprotein nitrogen in the ungerminated maize kernel (hot-water extraction and phosphotungstic-acid method)*

Variety of corn	Nitrogen in phosphotungstic precipitate	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Data expressed in percentage of the oven-dried maize kernel:	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Four County corn	0.076	0.032	0.045	0.069
United States selection No. 77	.061	.019	.040	.050
Hall Gold Nugget, selection No. 193	.076	.021	.051	.036
Data expressed in percentage of the total nitrogen of the maize kernel:				
Four County corn	4.47	1.88	2.65	4.06
United States selection No. 77	3.84	1.19	2.52	3.14
Hall Gold Nugget selection No. 193	5.21	1.44	3.49	2.47
Data expressed in percentage of the water-soluble nitrogen of the maize kernel:				
Four County corn	37.35	15.61	21.97	34.07
United States selection No. 77	40.11	12.18	26.67	32.79
Hall Gold Nugget selection No. 193	39.57	11.20	26.95	19.17

higher than that of the polypeptides. From the fact that the percentage of acid amides, amino acids, and polypeptides obtained by the phosphotungstic acid method is not essentially different from the results secured by the first method (without phosphotungstic acid treatment), it follows that under the conditions outlined the amount of proteose in the aqueous extract of the maize kernel is quite insignificant.

It seemed of certain interest to compare the results at hand with those found for the wheat kernel. The average figures of Table IV were calculated from the Tables II, III, and IV of the paper on wheat (*8*).

TABLE IV.—*Partition of the nonprotein nitrogen in the ungerminated wheat kernel.*

Variety of wheat	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Data expressed in percentage of the oven-dried wheat kernel:	*Per cent*	*Per cent*	*Per cent*
Fultz	0.026	0.032	0.084
Kanred	.053	.067	.111
Kubanka	.052	.041	.155
Marquis	.058	.054	.151
Data expressed in percentage of the total nitrogen of the wheat kernel:			
Fultz	1.46	1.77	4.67
Kanred	1.88	2.35	3.89
Kubanka	1.72	1.35	3.13
Marquis	1.91	1.77	4.98
Data expressed in percentage of the water-soluble nitrogen of the wheat kernel:			
Fultz	8.76	10.66	28.09
Kanred	12.99	16.25	26.86
Kubanka	12.61	9.91	37.76
Marquis	12.33	11.46	32.20

When Tables III and IV are examined it is readily seen that, with but few exceptions, the nitrogen of amino acids is higher in the maize kernel than in the wheat kernel, while the reverse is true of the peptide nitrogen. As to nitrogen of acid amides the figures are rather fluctuating, the total difference between the maize and the wheat kernel being not very considerable.

CONCLUSIONS

Polypeptides and free amino acids, which have been shown in this paper to be present in the ungerminated maize kernel, are performed in it.

The amino nitrogen in the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193, makes up, respectively, 0.045, 0.040, and 0.051 per cent calculated on the basis of the oven-dried kernel, and 2.65, 2.52, and 3.49 per cent calculated on the basis of the total nitrogen.

The peptide nitrogen in the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193 makes up, respectively, 0.069, 0.050, and 0.036 per cent calculated to the oven-dried kernel, and 4.06, 3.14, and 2.47 per cent calculated to its total nitrogen.

The proportions of acid amide nitrogen in the ungerminated kernel of the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193 are, respectively, 0.032, 0.019, and 0.021 per cent calculated on the oven-dried kernel, and 1.88, 1.19, and 1.44 per cent calculated on its total nitrogen.

The varieties Four County corn and Hall Gold Nugget selection 193 are, respectively, characterized by the highest and the lowest percentage of their total nitrogen, as well as of their protein nitrogen.

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