

it is likely that White<sup>1</sup> describes the quantitative application of it in much the same form as is given above. The original paper not being at hand this is not positively known.

STANFORD UNIVERSITY, April 15, 1897.

## THE AMOUNT AND PROPERTIES OF THE PROTEIDS OF THE MAIZE KERNEL.<sup>2</sup>

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SOME time since Prof. R. H. Chittenden and the writer published the results of an extended investigation of the proteids of this seed.<sup>3</sup> In that paper no definite statements were made respecting the quantities of the various proteids found, nor were the properties of some of them as fully described as is now possible. For these reasons the results of some additional researches are here put on record.

The proteids of the maize kernel may be distinguished according to their solubilities as follows:

- a. Proteid, soluble in pure water, having some of the properties of proteose.
- b. Globulins, insoluble in pure water, but soluble in salt solutions.
- c. Proteid, insoluble in water and salt solutions, but soluble in alcohol of sixty to ninety-nine per cent.
- d. Proteid matter, insoluble in water, salt solutions and alcohol, but soluble in dilute alkalies and acids.

### a. PROTEID SOLUBLE IN WATER.

If the substance precipitated from an aqueous extract of yellow corn meal by saturating with ammonium sulphate, is dissolved in water and the resulting solution dialyzed, the globulins extracted from the meal by aid of the soluble mineral constituents of the seed are *largely* precipitated. If these globulins are next *completely* removed by heating the solution to 80° and the filtrate therefrom be precipitated by an excess of alcohol, a small quantity of proteid is obtained having many of the reactions characteristic of proteose. A recent determination showed

<sup>1</sup> *Chem. News*, 58, 229.

<sup>2</sup> From the Report of the Connecticut Agricultural Experiment Station for 1896.

<sup>3</sup> *Am. Chem. J.*, 13, 453, 529, and 14, 20. Abstract in Report of Conn. Agr. Expt. Station for 1891, p. 136.

the presence of only 0.06 per cent. of this body. The quantity found was too small for a satisfactory study of the properties of the substance, but the following observations were made. Dissolved in a little water, only a very small quantity of undissolved substance remained, showing the nearly complete removal of proteid coagulable by alcohol. The clear filtrate from this insoluble matter when diluted with an equal volume of distilled *water* gave a considerable coagulum on boiling, but when diluted with the same quantity of *ten per cent. salt solution* only an opalescence resulted on boiling. Nitric acid added to the aqueous solution gave a heavy precipitate which nearly all dissolved on warming, with the production of a strong yellow color, and reappeared on cooling. Saturation of its solution with sodium chloride gave a precipitate much increased by the addition of acetic acid, the filtrate from which was not further precipitated on adding nitric acid. The biuret reaction was violet, not rose red, as is usually given by proteoses. This color reaction, however, was probably modified by the color substance associated with the proteid. Sulphate of copper gave with solutions of this proteose only a turbidity. These reactions do not altogether agree with those given by the proteoses which result from the action of enzymes on native proteids. It is possible that future investigation will show that the so-called proteoses found in seeds belong to a different order of proteids from those usually formed by proteolysis.

In the paper already referred to, a substance is described as albumin which was obtained from solutions that were supposed to have been freed from globulin by prolonged dialysis, by adding thereto ten per cent. of sodium chloride and precipitating with very dilute hydrochloric acid. My recent experience in investigating plant proteids has shown that it is extremely difficult and in many cases impossible to completely precipitate all of the very soluble globulins by dialysis, and since the composition of the so-called albumin thus obtained agreed quite closely with that of a very soluble globulin separated from these solutions by prolonged dialysis, and also since the globulin and the so-called albumin coagulated at about the same temperature, I now feel convinced that the two substances are identical, the latter being a part of the globulin which was not precipitated by

dialysis. In the former paper attention was called to the fact that this body, in some respects, resembled a globulin more closely than an albumin.

In the former paper were also described, as albumin, coagula obtained by concentrating solutions supposed to have been freed from globulin by dialysis and heating to 100°. It was there suggested that these coagula were probably alteration products of the proteids in solution. Since then "proteoses" from many different seeds have been found to yield coagula under similar conditions. It seems therefore quite certain that no true albumin exists in maize kernel.

*b.* PROTEIDS SOLUBLE IN SALINE SOLUTIONS.

If an aqueous extract of yellow corn meal is dialyzed for some time, a proteid, having the properties of a globulin, is precipitated which was found to have the following composition :

MAYSIN.

Carbon .....	52.68
Hydrogen .....	7.02
Nitrogen .....	16.76
Sulphur.....	1.30
Oxygen .....	22.24
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	100.00

In our paper on the proteids of this seed, Prof. Chittenden and myself designated this globulin "maize myosin." Further study of plant proteids has shown that no sharp distinction can be drawn between plant myosin and plant vitellin, and I now propose to call this proteid *maysin*, in reference to the specific botanical name *mays*. This globulin readily loses its solubility in saline solutions after precipitation, and therefore the amount present in the seed was underestimated in our former paper. A recent determination in yellow corn meal gave 0.25 per cent.

This proteid is readily soluble in very dilute saline solutions so that it is completely extracted from corn meal by water. Dissolved in ten per cent. sodium chloride brine it is coagulated by heating to 70°.

After separating maysin from the extract of corn meal by dialysis, further prolonged dialysis throws down a small quantity of another globulin having the following composition :

## MAIZE GLOBULIN.

Carbon.....	52.38
Hydrogen.....	6.82
Nitrogen.....	15.25
Sulphur.....	1.26
Oxygen.....	24.29
	100.00

This is the globulin which seems to be identical with the "albumin" which was formerly obtained by precipitation with salt and acid. This proteid was found in very small amount, twenty-five kilos of fine meal yielding only four and one-tenth grams by dialysis and three and four-tenths grams by precipitation with salt and acid, that is, seven and five-tenths grams in all or 0.03 per cent. of the meal. This figure cannot be taken as representing quite accurately the total quantity present, for doubtless some was lost in consequence of the globulin becoming insoluble and some also through incomplete extraction. The total quantity, however, is exceedingly small, probably not more than 0.04 per cent.

Dissolved in ten per cent. sodium chloride solution, this globulin coagulates on heating to 62°.

If yellow corn meal, after thorough extraction with water, is treated with ten per cent. salt solution, a further quantity of globulin is extracted, which is readily precipitated by dialysis in well developed spheroids.

This globulin, formerly designated maize vitellin, agrees in composition and reactions and is, doubtless, identical with edestin which I have found in various seeds.

A recent determination of edestin in the seed of yellow corn showed the presence of but 0.06 per cent. The quantities obtained in the former investigations were 0.06, 0.10 and 0.10 per cent. The composition of this globulin is as follows:

## MAIZE EDESTIN.

Carbon.....	51.43
Hydrogen.....	6.86
Nitrogen.....	18.06
Sulphur.....	0.86
Oxygen.....	22.79
	100.00

Edestin is much less soluble in saline solutions than the two globulins previously described, and for this reason is readily precipitated by dialysis or dilution. In warm dilute salt solutions it dissolves freely, but on cooling, separates more or less completely, according to the temperature and the strength of the salt solution. Dissolved in ten per cent. sodium chloride brine, it is partly coagulated by heating above 90°, but even on boiling the coagulation is far from complete.

c. PROTEID SOLUBLE IN DILUTE ALCOHOL.

Finely ground maize meal when extracted by hot alcohol loses eight-tenths per cent. of nitrogen, equivalent to five per cent. of the characteristic proteid called maize-fibrin by Ritthausen, but first described by Prof. Gorham, of Harvard University, in 1821, and named by him zein. The composition of zein, as shown by the average of nine closely agreeing analyses of as many preparations, is the following :

ZEIN.	
Carbon .....	55.23
Hydrogen .....	7.26
Nitrogen .....	16.13
Sulphur .....	0.60
Oxygen .....	20.78
	100.00

Zein is in many ways a remarkable proteid. It dissolves abundantly in ethyl alcohol of 0.820 specific gravity, forming solutions which, on evaporation in thin layers, leave a perfectly transparent sheet of the proteid.

In *absolute* alcohol, as also in water, zein is wholly insoluble, but in mixtures of water and alcohol it dissolves to a greater or less extent, according to the proportions of the two liquids. It is most soluble in alcohol of from eighty-five to ninety-five per cent., and dissolves but little in alcohol of less than fifty per cent. Solutions of zein in diluted alcohol deposit the proteid on evaporation as the proportion of water in the solution increases. Strong alcoholic solutions of zein gradually coagulate to transparent jellies, which, on long standing, become hard and solid. In ninety-five per cent. methyl alcohol and in commercial propyl alcohol hydrate, zein dissolves readily.

In concentrated glycerol, zein is freely soluble on heating to

about 150° C., to solutions which, when much is dissolved, solidify on cooling to 20°. In such solutions zein can be heated to 200° C. without undergoing any apparent change, for, on pouring them into water, the zein separates as a pulverulent precipitate readily and completely soluble in dilute alcohol.

In crystallized phenol, zein is readily soluble on warming, yielding solutions which leave on evaporation clear films of unchanged zein. In glacial acetic acid, zein dissolves in large proportion and is left, by evaporating the acid on a boiling water-bath, in transparent films of apparently unchanged proteid, which readily dissolve in alcohol.

Strong solutions of zein in glacial acetic acid when poured rapidly into water give large coherent precipitates which retain all the original properties of the proteid; if the solution is dilute the zein is, to a greater or less extent, dissolved by the aqueous acetic acid.

In one-half per cent. sodium carbonate solution and in two-tenths per cent. hydrochloric acid, zein is wholly insoluble even when warmed for twenty-four hours at 40°.

In one-tenth to two per cent. caustic potash solution, zein is very readily dissolved and even by heating to 40° for twenty-four hours in such solutions is not converted into "alkali-albumin," for the precipitate obtained by neutralizing solutions so treated is completely soluble in alcohol.

Alcoholic solutions of zein are not precipitated by tannin, picric acid, trichloroacetic acid, lead acetate, silver nitrate, mercuric chloride, ferric chloride, or potassio-mercuric iodide. Clear solutions mixed with silver nitrate dissolved in alcohol leave clear films when evaporated on glass, which gradually turn deep red on exposure to sunlight. When hydrochloric acid in considerable quantity is added to a solution of zein in ethyl alcohol containing much silver nitrate, no precipitate is produced until the mixture has stood for some time, when a turbidity gradually develops which is affected but slowly by light. If the mixture of acid, zein and silver nitrate is boiled it becomes turbid at once.

Zein treated with an alcoholic solution of ferric chloride shows no visible change, but if tested with potassium ferricyanide a deep blue solution is formed, showing that the ferric chloride is reduced at once.

Potassium ferricyanide added to the zein solution is not reduced even after standing some hours.

On digestion with pepsin in hydrochloric acid, zein is converted into proteoses and peptones.<sup>1</sup>

According to J. G. C. T. Kjeldahl (*Bied. Centr.*, 1896, 25, 197, from Forhandl. Skand. Naturfors, 1892, 385-390) zein dissolved in seventy-five per cent. alcohol has a specific rotation of  $(\alpha)_D - 35^\circ$  and in glacial acetic acid  $(\alpha)_D - 28^\circ$ .

d. PROTEID MATTER SOLUBLE IN ALKALIES.

This was estimated as follows : One hundred grams of very finely ground maize meal which contained 1.54 grams of nitrogen were successively exhausted with two-tenths per cent. potash water and with alcohol. The dried residue weighed seventy-seven grams and contained 0.1645 gram of nitrogen. Accordingly, 1.3755 grams of nitrogen, including that of all the soluble proteids, had been extracted. This amount multiplied by the factor 6.25 gives the total quantity of soluble proteids, viz., 8.5969 grams. Subtracting therefrom the sum of the several proteids previously determined, viz., zein five grams, globulins 0.39 gram and proteose 0.06 gram, there remains 3.1469 grams of proteid insoluble in salt solutions and alcohol, but soluble in dilute potash water.

The alkaline extract obtained in estimating the quantity of this proteid was filtered perfectly clear, neutralized with acetic acid, the precipitate filtered out, washed thoroughly with water and extracted with hot alcohol to remove zein.

The proteid residue was then redissolved in dilute potash water, filtered clear and again precipitated by neutralization with acetic acid and thoroughly washed with water, with hot alcohol, and finally with ether. After drying at 110° the preparation was analyzed with the following results :

Carbon .....	51.26
Hydrogen .....	6.72
Nitrogen .....	15.82
Sulphur .....	0.90
Oxygen .....	25.30
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	100.00
Ash .....	2.38

<sup>1</sup> Chittenden : *Medical Record*, 45, 487.

The results of this analysis do not indicate that this substance has any relation to the other proteids already described. Owing to its insolubility in neutral fluids no characteristic reactions could be obtained, and accordingly nothing more was learned respecting it.

The foregoing statements show that 100 grams of the yellow corn meal contained approximately:

Proteid soluble in two-tenths per cent. potash....	}	3.15 grams containing 15.82 per cent. N. = 0.4983 gms.				
Zein .....		5.00	"	"	16.13	" " = 0.8065 "
Very soluble glob- ulin .....	0.04	"	"	15.25	" " = 0.0061 "	
Edestin .....	0.10	"	"	18.10	" " = 0.0181 "	
Maysin .....	0.25	"	"	16.70	" " = 0.0417 "	
Protease .....	0.06	"	"	17.00	" " = 0.0102 "	
					1.3809 "	
Nitrogen undissolved by dilute potash water .....					0.1645 "	
Total .....					1.5454 "	
Nitrogen in meal by analysis.....					1.5400 "	
Mean percentage of nitrogen in Maize Proteids .....					16.057	

## THE COMMERCIAL PREPARATION OF NITRONAPHTHALENES.

BY WILLIAM H. KRUG AND J. E. BLOMEN.

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THE manufacture of nitronaphthalenes has of recent years acquired considerable importance in the arts, and particularly in the explosive industry. In this industry it has been utilized in various ways and for different purposes. The late Nobel was the first to point out that the addition of nitronaphthalene to a nitroglycerol explosive, such as nitrogelatin, rendered this practically non-sensitive to concussion, and this property has been and is still widely applied to render the handling of nitroglycerol explosives more safe.

In the manufacture of nitro substitution powders, nitronaphthalene soon replaced the more expensive nitrobenzol as a basis, and a large number of patents were taken out for its use with oxidizing agents alone or with an admixture of sensitizing agents, such as nitroglycerol, picric acid, etc. A third use was found for nitronaphthalene when it was discovered that it seemingly ren-