

**Hydrolysis of the proteins of maize, Zea Mays / by Thomas B. Osborne and S.H. Clapp.**

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## HYDROLYSIS OF THE PROTEINS OF MAIZE, ZEA MAYS.<sup>1</sup>

By THOMAS B. OSBORNE AND S. H. CLAPP.

[From the Laboratory of the Connecticut Agricultural Experiment Station.]

THE seeds of maize or Indian corn, *Zea Mays*, like those of the other cereals, contain a very small proportion of protein soluble in water or neutral saline solutions, a relatively large amount of protein soluble in strong alcohol, and a considerable quantity insoluble in all neutral solvents but soluble in very dilute alkaline and acid solutions.

An investigation of the proportions of these several proteins was made some years ago in this laboratory,<sup>2</sup> and it was found that the sample of yellow corn meal then examined contained 5 per cent of zein soluble in strong alcohol, 3.15 per cent of protein soluble only in alkaline or acid solutions, and 0.45 per cent of globulins, albumins, and proteoses. The composition and properties of these different proteins have been extensively described by Chittenden and Osborne.<sup>3</sup>

Owing to the very small amount of globulins and albumins, no attempt was made to prepare any of these for hydrolysis, but the zein and alkali soluble proteins were prepared in quantity from freshly harvested, high-protein, white maize, which was kindly sent to us by Professor Hopkins of the University of Illinois. We wish to here express our high appreciation of Professor Hopkins' kindness in furnishing so large a quantity of these valuable seeds.

After the seeds were dried until they became hard, they were ground to a fine powder in the laboratory mill and then extracted

<sup>1</sup> The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

<sup>2</sup> OSBORNE, *Journal American Chemical Society*, 1897, xix, p. 525.

<sup>3</sup> CHITTENDEN and OSBORNE, *American chemical journal*, 1891, xiii, pp. 327 and 385; 1892, xiv, p. 20.



with cold 85 per cent (by volume) alcohol as long as anything was removed. The voluminous extract was filtered perfectly clear and concentrated under reduced pressure to a small volume. The clear syrup that remained, after cooling, was poured in a very fine stream into about eight volumes of distilled ice water, containing a very little sodium chloride in order to promote the separation of the zein. The zein, which separated as a flocculent precipitate, soon united to a coherent plastic mass. This was filtered out on cloth, washed superficially with water, and again dissolved in 95 per cent alcohol to a syrup. This solution was then shaken out repeatedly with petroleum benzine until all the yellow coloring matter was removed, together with most of the fat.

The perfectly water-clear solution of the zein was then poured into eight volumes of cold water, containing a very little sodium chloride. The zein that separated was then dried and ground, yielding a fine powder that was entirely colorless.

The meal that had been extracted with alcohol until nothing more could be obtained from it was then extracted with a large quantity of 0.20 per cent sodium hydroxide solution, the extract filtered perfectly clear and treated with very dilute hydrochloric acid until the dissolved protein separated as a flocculent precipitate. This was then filtered out, again dissolved in the dilute sodium hydroxide solution, the resulting solution filtered perfectly clear, and the protein precipitated as before. After washing with water, this precipitate was digested several successive times with fresh quantities of strong alcohol as long as any zein was removed, and when dehydrated with absolute alcohol and dried, formed a dusty powder entirely free from color.

This preparation represents the residual protein of the seed after all that is soluble in alcohol has been removed. Of its individuality as a protein substance we of course know nothing, for it has heretofore been impossible to obtain any considerable quantity of it in a state of even approximate purity as respects admixture with non-protein substances. This preparation contains more nitrogen than the five other preparations that we have previously made, and probably contains but a small amount of non-protein matter. As to whether it consists of a single protein substance or is a mixture, we can say nothing, for we have no evidence whatever on this point. We have, nevertheless, made this preparation for hydrolysis because it represents a large part of the protein of the seed and it is im-



portant to know how its products of hydrolysis compare with those of zein.

In addition to the preparations just described we have made similar ones from the "gluten-meal," a by-product of the glucose works. For this meal we wish to thank Mr. H. C. Humphrey of the Corn Products Refining Company. The material was sent to us in cold weather in a moist state, just as it came from the filter press. It was at once frozen solid and kept in that condition until we dried it rapidly at a low temperature.

This meal contains about 50 per cent of protein, a large part of which is zein. The method of preparation of the proteins from this meal was essentially the same as that described for the corn meal. The products obtained were used for some of the separate determinations to be described later, as the quantity of the preparation from the corn meal was insufficient for all. Where the "gluten" preparations were used special mention will be made of the fact, although we have no doubt that the two products are entirely alike.

#### HYDROLYSIS OF ZEIN.

Five hundred and sixty-five grams of zein, equal to 487.2 gm. moisture, ash, and fat free, were suspended in a mixture of 565 c.c. of water and 565 c.c. of hydrochloric acid of specific gravity 1.19, and heated for ten hours at 100°. The hydrolysis was then made complete by boiling the solution for sixteen hours in a bath of oil.

A preliminary removal of glutaminic acid hydrochloride, performed in the usual manner, yielded 69.80 gm. freed from ammonium chloride, or 55.92 gm. of free glutaminic acid, which is 10.9 per cent of the protein. The substance melted at about 202°–203°.

*Carbon and hydrogen*, 0.1607 gm. subst. gave 0.2413 gm. CO<sub>2</sub> and 0.0953 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . = C 40.95; H 6.58 " "

The filtrate from the glutaminic acid hydrochloride was then concentrated very sharply under strongly reduced pressure, and the residue esterified with alcohol and dry hydrochloric acid gas in the usual way.

After liberating the free esters with sodium hydroxide and potassium carbonate, as described by Emil Fischer in the original hydro-



lysis of casein, and shaking out with ether, the aqueous layer was made strongly acid with hydrochloric acid, freed from inorganic salts, and the esterification repeated. The combined esters were divided into the following fractions by distillation under diminished pressure:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	70°	12 mm.	11.24 gm.
II	92°	12 "	25.38 "
III	a	100°	12 "
	b	94°	2 "
	c	94°	1 "
	d	105°	0.92 "
IV	a	145°	0.92 "
	b	170°	0.92 "
	c	186°	0.92 "
Total			304.97 gm.

The undistilled residue weighed 84 gm.

**Fraction I** — From this fraction no glycocoll could be brought to separation as the hydrochloride of the ethyl ester. The fraction consisted essentially of alanine, of which 3.13 gm. were obtained.

**Fraction II.** — This fraction was saponified with boiling water, the solution of the amino acids evaporated to dryness under reduced pressure, and the proline extracted with boiling absolute alcohol. The part remaining undissolved was then examined for glycocoll,\* but no crystals of the ethyl ester hydrochloride could be obtained.<sup>4</sup>

By systematic fractionation of the free amino acids from water and from water and alcohol, there were obtained from this fraction 3.36 gm. of leucine and 5.52 gm. of alanine, while the presence of valine could not be established. The leucine gave the following analysis:

*Carbon and hydrogen*, 0.1105 gm. subst. gave 0.2224 gm. CO<sub>2</sub> and 0.0983 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 54.89; H 9.88 " "

The alanine decomposed at about 290° and gave the following analysis:

<sup>4</sup> We were, furthermore, unable to detect this substance in the ether distilled from the esters on the water bath.



Carbon and hydrogen, 0.1496 gm. subst. gave 0.2237 gm. CO<sub>2</sub> and 0.1066 gm. H<sub>2</sub>O.

Calculated for C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N = C 40.45 ; H 7.86 per cent.

Found . . . . = C 40.78 ; H 7.91 " "

**Fraction III.** — The esters of this fraction were boiled with five volumes of water for six hours, after which time the solution had ceased to react alkaline to litmus. The leucine that had separated was then filtered off, and the filtrate rapidly evaporated to dryness under strongly reduced pressure. The large quantity of proline present was then dissolved out with boiling absolute alcohol, and the insoluble portion dissolved in water and systematically fractionated. The yield of leucine was 87.75 gm.

Carbon and hydrogen, 0.1790 gm. subst. gave 0.3610 gm. CO<sub>2</sub> and 0.1593 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96 ; H 9.92 per cent.

Found . . . . = C 55.00 ; H 9.89 " "

The substance decomposed at about 298°.

In the filtrate from the leucine there were further isolated 2.04 gm. of alanine and 1.4 gm. of valine. To isolate the latter substance it was found necessary to have recourse to racemization. The fraction containing the valine was accordingly heated in the autoclave with excess of baryta for twenty-four hours at 175°. After removing the barium exactly with sulphuric acid, a preparation was readily obtained, which not only gave closely agreeing results on analysis, but which under the microscope looked perfectly homogeneous.

Carbon and hydrogen, 0.1444 gm. subst. gave 0.2721 gm. CO<sub>2</sub> and 0.1246 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>11</sub>O<sub>2</sub>N = C 51.28 ; H 9.40 per cent.

Found . . . . = C 51.38 ; H 9.58 " "

The remainder of the substance was then coupled with phenylisocyanate in alkaline solution and the hydantoic acid recrystallized from water. The substance separated in the characteristic plates, which, heated side by side with a similar preparation from phaseolin,<sup>5</sup> melted simultaneously with the latter at 161° (corr.).

Carbon and hydrogen, 0.1331 gm. subst. gave 0.2974 gm. CO<sub>2</sub> and 0.0820 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>16</sub>O<sub>3</sub>N<sub>2</sub> = C 61.02 ; H 6.78 per cent.

Found . . . . = C 60.94 ; H 6.84 " "

<sup>5</sup> OSBORNE and CLAPP: This journal, 1907, xviii, p. 301.



The phenyl-isocyanate derivative was then converted to the anhydride by dissolving in strong hydrochloric acid and concentrating on the water bath. The prisms of the hydantoine melted on recrystallizing from ether and petroleum ether at  $120.5^{\circ}$  (corr.), while the preparation from phaseolin<sup>6</sup> melted at  $122.5^{\circ}$  (corr.), whereas Slimmer<sup>7</sup> found, in the case of the synthetic  $\alpha$ -amino-iso-valerianic acid,  $163.5^{\circ}$  (corr.) for the phenyl-isocyanate derivative and  $124^{\circ}$ – $125^{\circ}$  (corr.) for the hydantoine.

The proline extracts of Fractions II and III were united. The solutions were evaporated to dryness under reduced pressure, and the residue taken up in boiling absolute alcohol. After prolonged standing 1.24 gm. of substance, melting at  $250^{\circ}$ , had separated, the identity of which was not established.

The filtrate was then slightly concentrated and the proline precipitated with ether, as a slightly colored crystalline mass, which dried in vacuum over sulphuric acid to constancy, weighed 31.99 gm.

On redissolving in absolute alcohol, the substance separated in the characteristic prisms melting at about  $203^{\circ}$ – $205^{\circ}$ .

*Carbon and hydrogen*, 0.2045 gm. subst. gave 0.3889 gm.  $\text{CO}_2$  and 0.1472 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_5\text{H}_9\text{O}_2\text{N} = \text{C } 52.18$ ;  $\text{H } 7.83$  per cent.

Found . . . . =  $\text{C } 51.86$ ;  $\text{H } 8.00$  " "

For the strict identification the proline was converted to the copper salt, and the latter substance separated into the lævo and racemic by boiling with absolute alcohol. The undissolved racemic salt separated from water in the characteristic plates.

*Water*, 0.1506 gm. subst. (air dry) lost 0.0164 gm.  $\text{H}_2\text{O}$  at  $110^{\circ}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2\text{Cu} \cdot 2 \text{H}_2\text{O} = \text{H}_2\text{O } 10.99$  per cent.

Found . . . . =  $\text{H}_2\text{O } 10.89$  " "

*Copper*, 0.1294 gm. subst. (dried at  $110^{\circ}$ ) gave 0.0351 gm.  $\text{CuO}$ .

Calculated for  $\text{C}_{10}\text{H}_{16}\text{O}_4\text{N}_2 \text{Cu} = \text{Cu } 21.81$  per cent.

Found . . . . =  $\text{Cu } 21.67$  " "

The amorphous copper salt of lævo proline was converted to the phenyl-hydantoine. The substance crystallized from water in the characteristic prisms melting at  $143^{\circ}$ .

<sup>6</sup> OSBORNE and CLAPP: This journal, 1907, xviii, p. 301.

<sup>7</sup> SLIMMER, MAX D.: Berichte der deutschen chemischen Gesellschaft, 1902, xxxv, p. 403.



*Nitrogen*, 0.1897 gm. subst. required 2.5 c.c. 5/7 N-HCl.

Calculated for  $C_{12}H_{12}O_2N_2 = N$  12.96 per cent.

Found . . . . . = N 13.17 " "

**Fraction IV.**— From this fraction phenylalanine was removed as the ester in the usual way. The yield of the hydrochloride of phenylalanine was 29.11 gm. The free phenylalanine decomposed at about  $270^\circ$  and gave the following analysis:

*Carbon and hydrogen*, 0.1150 gm. subst. gave 0.2773 gm. CO and 0.0699 gm.  $H_2O$ .

Calculated for  $C_9H_{11}O_2N = C$  65.45; H 6.66 per cent.

Found . . . . . = C 65.76; H 6.75 " "

The aqueous layer was saponified by warming with an excess of baryta on the water bath. The yield of aspartic acid isolated as the barium salt was 5 gm.

*Carbon and hydrogen*, 0.2571 gm. subst. gave 0.3426 gm.  $CO_2$  and 0.1252 gm.  $H_2O$ .

Calculated for  $C_4H_7O_4N = C$  36.09; H 5.26 per cent.

Found . . . . . = C 36.34; H 5.41 " "

The filtrate from the barium aspartate was freed quantitatively from barium, concentrated under reduced pressure, and saturated with hydrochloric acid gas. The yield of glutaminic acid, which separated after prolonged standing at  $0^\circ$ , was 20.37 gm. The free glutaminic acid decomposed at about  $201^\circ$ – $202^\circ$  with effervescence to a clear oil. From this fraction there were further isolated 3.94 gm. of air-dry copper aspartate, while no serine was obtained.

*Copper*, 0.1135 gm. subst. (air dry) gave 0.0330 gm. CuO.

*Nitrogen*, 0.5275 gm. subst. (air dry) required 2.7 c.c. 5/7 N-HCl.

Calculated for  $C_4H_5O_4NCu \cdot 4\frac{1}{2} H_2O = Cu$  23.07; N 5.08 per cent.

Found . . . . . = Cu 23.23; N 5.12 " "

#### THE RESIDUE AFTER DISTILLATION.

The residue remaining after distillation of the esters weighed 84 gm. It was saponified with baryta and the glutaminic acid separated as the hydrochloride in the usual manner. The yield of glutaminic acid hydrochloride was 14.32 gm., which makes the



total yield of free glutaminic acid obtained on this hydrolysis 83.71 gm., or 16.32 per cent of the protein, while by the direct method 18.28 per cent was obtained.

#### GLUTAMINIC ACID.

Osborne and Gilbert<sup>8</sup> found 16.87 per cent of glutaminic acid in zein, but as the amount of protein available for that determination was only 32 gm. we have repeated the determination with the following results: 100 gm. equal to 91.6 gm. moisture, ash, and fat free zein from the "gluten-meal" was heated on a water bath for two and a half hours with 200 c.c. of hydrochloric acid sp. gr. 1.19, and then boiled in an oil bath for twelve hours. After concentrating the hydrolysis solution to about two thirds its original volume it was saturated with hydrochloric acid gas and allowed to stand for some days on ice. There were thus obtained 20.9 gm. of glutaminic acid hydrochloride equal to 16.74 gm. glutaminic acid, or 18.28 per cent.

#### TYROSINE.

Kutscher<sup>9</sup> has stated that zein yields 10.06 per cent of tyrosine. We have made every effort to isolate all the tyrosine possible from our solutions, but have obtained barely one third as much as Kutscher. Although we are convinced that tyrosine still remained in our solutions, we have obtained no evidence that the amount that defied separation was more than relatively small in proportion to that which did separate.

As several unusual observations were made in the course of our efforts to separate the last traces of tyrosine, we will give a brief description of them.

Three hundred grams of the zein from the "gluten meal" were hydrolyzed with 900 gm. of sulphuric acid and 1800 gm. of water by heating on the water bath for ten hours and then boiling in an oil bath for ten hours longer. After removing the sulphuric acid with an equivalent quantity of baryta and thoroughly washing the barium sulphate, the filtrate and washings were concentrated to about 2500 c.c. and cooled. The substance that separated, when

<sup>8</sup> OSBORNE and GILBERT: This journal, 1906, xv, p. 333.

<sup>9</sup> KUTSCHER, *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 111.



washed with cold water and thoroughly dried at 100°, weighed 22.8 gm., I. The filtrate and washings were concentrated to about 1200 c.c. and similarly yielded 19.88 gm. of II. On further concentration 12 gm. of III were obtained and 16.27 gm. of IV. The filtrate from IV, when concentrated to a syrup, gave 27.75 gm. of V, from which the thick mother liquor could not be very thoroughly sucked out.

The total yield of substances that were thus separated in a solid state capable of removal by filtration was about one third of the hydrolyzed zein. Small parts of the five fractions were then treated with glacial acetic acid in which IV and V were completely soluble at the room temperature, II and III were mostly soluble, while I contained much that did not dissolve. The whole of Fractions I, II, and III were therefore treated with an abundant quantity of glacial acetic acid, the residue filtered out, washed with absolute alcohol, and when dried at 100° weighed 8.27 gm. (crude tyrosine A). The acetic acid solution filtered from A was freed from acetic acid as completely as possible by repeatedly evaporating under diminished pressure with water and with alcohol and then subjected to extensive fractional crystallization. The less soluble fractions, which closely resembled nearly pure leucine, when recrystallized gave no Millon's reaction, whereas, although the most soluble fractions gave strong Millon's reactions, no tyrosine could be obtained from them in characteristic crystals. All the fractions which gave Millon's reaction were set aside for further examination.

Fraction IV was dissolved in water, the solution treated with bone-black and subjected to fractional crystallization. There was obtained 9.35 gm. of the original 16.27 gm. which did not give Millon's reaction; but the second fraction, which was very soluble in water, gave a Millon's reaction, while the filtrate from this, on concentration to a syrup and adding alcohol, gave a gummy deposit which would not harden under alcohol and gave a strong Millon's reaction.

A similar fractional recrystallization of V yielded no fractions which did not give a strong Millon's reaction.

The thick syrup that had been separated from V was then treated with alcohol, and on long standing a product separated that could be sucked out but contained so much mother liquor that it could not be weighed. This was redissolved, its solution treated with bone-black and concentrated, and 8.8 gm. of substance obtained which gave a moderately strong Millon's reaction.



The fractions thus far obtained which gave a Millon's reaction were united, dissolved in a considerable quantity of 5 per cent sulphuric acid, and treated, as long as a precipitate formed, with a 20 per cent solution of phosphotungstic acid dissolved in 5 per cent sulphuric acid. After washing the precipitate with a dilute solution of phosphotungstic acid in 5 per cent sulphuric acid, these two acids were removed from the filtrate and washings with an excess of baryta and the excess of baryta with an equivalent quantity of sulphuric acid.

On concentrating and cooling the solution and subjecting it to fractional crystallization, 3.78 gm. of tyrosine were obtained in characteristic crystals suitable for weighing. Although long-continued and persistent effort was made to bring more tyrosine to separation, no more than traces could be obtained. Occasionally small fractions crystallized out in which a few needles that looked like tyrosine could be seen under the microscope, but no weighable quantity could be separated. As before, the tyrosine accumulated, so far as could be judged from the Millon's reaction, in the most soluble fractions. So extensive and thorough was this fractionation that two fractions were obtained, weighing 0.50 and 1.10 gm., equal to 0.57 per cent of the zein, which, after a single recrystallization, were found to be pure serine.

The crystals, which had the characteristic form and sweet taste of serine, browned at about  $215^{\circ}$  and melted with effervescence to a brownish mass at about  $240^{\circ}$ .

*Carbon and hydrogen*, 0.2644 gm. subst. gave 0.3317 gm.  $\text{CO}_2$  and 0.1623 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_3\text{H}_7\text{O}_3\text{N} = \text{C } 34.29$ ;  $\text{H } 6.67$  per cent.

Found . . . . =  $\text{C } 34.22$ ;  $\text{H } 6.82$  " "

Further in the filtrate from the serine 8.5 gm. substance was obtained which crystallized in needles, had the composition of alanine, and decomposed at about  $285^{\circ}$ .

The preparation gave a somewhat too high carbon determination.

*Carbon and hydrogen*, 0.2226 gm. subst. gave 0.3338 gm.  $\text{CO}_2$  and 0.1614 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_3\text{H}_7\text{O}_2\text{N} = \text{C } 40.45$ ;  $\text{H } 7.86$  per cent.

Found . . . . =  $\text{C } 40.89$ ;  $\text{H } 8.05$  " "

The crude tyrosine A, when recrystallized from water, yielded 5.47 gm. of nearly pure tyrosine. The substance in the filtrate,



which still contained some tyrosine, was added to the solution that was treated with phosphotungstic acid, as already described. The total quantity of tyrosine thus obtained in weighable form was 9.25 gm. As this was obtained from 274.77 gm. of moisture, ash, and fat free zein, it is equal to 3.37 per cent.

Another determination of tyrosine was made from the same preparation of zein with a similar result. A quantity weighing 100 gm., equal to 91.6 gm. moisture, ash, and fat free zein, from the "gluten-meal," were heated with a mixture of three times its weight of sulphuric acid and six times its weight of water for two hours in a water bath and boiled for twelve hours in an oil bath. The hydrolysis solution was then diluted with water, the sulphuric acid removed with an equivalent quantity of baryta, and the barium sulphate boiled out four times with water and washed very thoroughly. The filtrate and washings were then concentrated to about 500 c.c. under diminished pressure, with the addition of barium carbonate, filtered from the barium carbonate, and the latter boiled out with water and thoroughly exhausted with boiling water.

The filtrate and washings from the barium carbonate were concentrated to crystallization, and, after standing over night, the product that separated was filtered out, washed with cold water, and dried. It weighed 4.2 gm. This was dissolved in about 800 c.c. of boiling water, the solution decolorized with a little bone-black, the latter extracted thoroughly with boiling water, and the solution concentrated to crystallization. After twenty-four hours 2.95 gm. of tyrosine separated. The filtrate on further concentration yielded 0.3 gm. more, making 3.25 gm. of tyrosine, or 3.55 per cent.

#### ARGININE, HISTIDINE, AND LYSINE.

A quantity of the "gluten-meal" weighing 100 gm. was heated for three hours on the water bath with three times its weight of sulphuric acid and six times its weight of water and then boiled for twelve hours on an oil bath. The products of hydrolysis were worked up for bases according to the method of Kossel and Patten with the following results:

The solution containing the histidine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. sol. required 1.26 c.c.  $\frac{5}{7}$  N-HCl = 0.0126 gm. N = 0.0630 gm. N in 500 c.c. = 0.2322 gm. histidine, or 0.24 per cent.



The solution of the arginine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 50 c.c. sol. required 1.61 c.c. 5/7 N-HCl = 0.0161 gm. N = 0.1610 gm. in 500 c.c. = 0.5000 gm. arginine, or, allowing for the solubility of the arginine silver, 0.6180 gm., or 0.67 per cent.

The filtrate from the first silver precipitate of arginine and histidine was examined carefully for lysine according to the method of Kossel and Kutscher, but none was found.

As the results of these determinations were so low, we repeated them, using Kossel's later method for separating histidine from arginine, which depends on heating the neutral or slightly acid solution of the silver salt with barium carbonate.<sup>10</sup>

One hundred grams of zein from the "gluten-meal" were hydrolyzed as before, and, after removing the sulphuric acid, the solution containing histidine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. solution required 2.14 c.c. 5/7 N-HCl = 0.0214 gm. N = 0.1070 gm. N in 500 c.c. = 0.3589 gm. histidine = 0.43 per cent.

The solution containing the arginine was made up to 500 c.c. and nitrogen determined in it.

*Nitrogen*, 100 c.c. solution required 6.1 c.c. 5/7 N-HCl = 0.0610 gm. N = 0.305 gm. N in 500 c.c. = 0.9477 gm. arginine. Adding 0.1180 gm. for the solubility of the arginine silver gives 1.0657 gm., or 1.16 per cent.

These results are somewhat higher than those first obtained, but fall decidedly below those published by Kossel and Kutscher,<sup>11</sup> namely, histidine 0.81, arginine 1.85 per cent.

#### HYDROLYSIS OF THE ALKALI-SOLUBLE PROTEIN.

The preparation used for this hydrolysis was that made from the seeds ground in the laboratory. Unfortunately the amount of this material made it necessary to use much less of this protein for the hydrolysis than we would have used had our supply been greater.

<sup>10</sup> Cf. WEISS, *Zeitschrift für physiologische Chemie*, 1907, lii, p. 107.

<sup>11</sup> KOSSEL and KUTSCHER: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 165.



Two hundred and fifty grams of the protein (containing ash equal to 1.45 per cent, moisture equal to 10.63 per cent, ether-soluble substance equal to 0.81 per cent) were suspended in a mixture of 250 c.c. of water and 250 c.c. of hydrochloric acid of specific gravity 1.19 and warmed at 100° for about five hours. The hydrolysis solution was then boiled in the oil bath for eighteen and a half hours.

A preliminary removal of glutaminic acid yielded 23.66 gm. of the free acid, or 10.87 per cent of the protein.

*Carbon and hydrogen*, 0.1654 gm. subst. gave 0.2484 gm. CO<sub>2</sub> and 0.0916 gm. H<sub>2</sub>O.

Calculated for C<sub>5</sub>H<sub>9</sub>O<sub>4</sub>N = C 40.81; H 6.12 per cent.

Found . . . . = C 40.95; H 6.15 " "

The substance melted at about 202°.

The filtrate from glutaminic acid hydrochloride was then freed from water by evaporation under reduced pressure and the residue esterified precisely as in the case of zein. By distillation under diminished pressure the following fractions were obtained:

Fraction.	Temp. of bath up to	Pressure.	Weight.
I	85°	12.00 mm.	15.62 gm.
II	95°	5.00 "	19.47 "
III	80°	2.00 "	17.73 "
IV	110°	0.70 "	15.73 "
V	200°	0.36 "	37.19 "
Total . . . . .			105.74 gm.

The undistilled residue weighed 39 gm.

**Fraction I.** — This fraction yielded 1.00 gm. of the hydrochloride of glycocoll ethyl ester. The melting-point was 144°.

*Chlorine*, 0.2390 gm. subst. gave 0.2411 gm. AgCl.

Calculated for C<sub>4</sub>H<sub>10</sub>O<sub>2</sub> NCl = Cl 25.45 per cent.

Found . . . . . = Cl 24.94 " "

The filtrate from the glycocoll was added to the corresponding filtrate of Fraction II.

**Fraction II.** — From this fraction there were obtained 2.6 gm. of leucine, while the presence of neither glycocoll nor alanine could



be definitely established. The fraction further contained a not inappreciable quantity of proline which was worked up conjointly with that from Fractions III and IV. The leucine gave the following analysis:

*Carbon and hydrogen*, 0.1304 gm. subst. gave 0.2621 gm. CO<sub>2</sub> and 0.1180 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 54.82; H 10.05 " "

**Fraction III and IV.** — From Fractions III and IV there were further isolated 10.98 gm. of leucine.

*Carbon and hydrogen*, 0.1266 gm. subst. gave 0.2541 gm. CO<sub>2</sub> and 0.1167 gm. H<sub>2</sub>O.

Calculated for C<sub>6</sub>H<sub>13</sub>O<sub>2</sub>N = C 54.96; H 9.92 per cent.

Found . . . . = C 54.74; H 10.23 " "

The proline extracts from Fractions II, III, and IV were united. By concentrating somewhat under reduced pressure and precipitating with ether, the proline was obtained as a somewhat colored crystalline mass, which proved to be readily soluble in alcohol. After drying to constancy over sulphuric acid, it weighed 10.86 gm. For identification the phenyl-hydantoin of the lævo modification was employed.

*Carbon and hydrogen*, 0.1504 gm. subst. gave 0.3674 gm. CO<sub>2</sub> and 0.0792 gm. H<sub>2</sub>O.

Calculated for C<sub>12</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub> = C 66.67; H 5.56 per cent.

Found . . . . = C 66.62; H 5.85 " "

The melting-point was 143°.

**Fraction V.** — From this fraction there were isolated by the usual method 4.63 gm. of phenylalanine hydrochloride, 0.60 gm. of aspartic acid as the barium salt, and 1.58 gm. of air-dry copper aspartate, while no glutaminic acid hydrochloride was obtained. The phenylalanine gave, on boiling with dilute sulphuric acid and potassium bichromate, the characteristic odor of phenylacetaldehyde, but the analysis indicated a considerable admixture.

*Carbon and hydrogen*, 0.1667 gm. subst. gave 0.3922 gm. CO<sub>2</sub> and 0.1031 gm. H<sub>2</sub>O.

Calculated for C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N = C 65.45; H 6.66 per cent.

Found . . . . = C 64.16; H 6.87 " "



The aspartic acid reddened but did not decompose at  $300^{\circ}$ .

Carbon and hydrogen, 0.0966 gm. subst. gave 0.1288 gm.  $\text{CO}_2$  and 0.0515 gm.  $\text{H}_2\text{O}$ .

Calculated for  $\text{C}_4\text{H}_7\text{O}_4\text{N} = \text{C } 36.09$ ;  $\text{H } 5.26$  per cent.

Found . . . . =  $\text{C } 36.36$ ;  $\text{H } 5.92$  " "

#### TYROSINE.

Fifty grams of the protein, equal to 43.55 gm. moisture, ash, and fat free, were hydrolyzed by boiling in an oil bath for twelve hours with a mixture of 150 gm. sulphuric acid and 300 c.c. of water. After removing the sulphuric acid with baryta the hydrolysis solution was concentrated until crystallization began and then allowed to stand for twenty-four hours. The substance that had separated was filtered out, washed with cold water, and recrystallized. There were thus obtained 1.51 gm. of tyrosine in the characteristic needles, equal to 3.44 per cent. By similar treatment of a preparation from the "gluten-meal" there were obtained from 100 gm. equal to 86.4 gm. moisture, ash, and fat free, 3.30 gm. recrystallized tyrosine equal to 3.82 per cent.

Nitrogen, 0.2340 gm., dried at  $110^{\circ}$ , required 1.82 c.c.  $5/7 \text{ N-HCl}$ .

Calculated for  $\text{C}_9\text{H}_{11}\text{O}_3\text{N} = \text{N } 7.73$  per cent.

Found . . . . =  $\text{N } 7.78$  " "

#### HISTIDINE.

The solution, washings, and mother liquor, from which the tyrosine first described had separated, were concentrated and worked up for bases according to the method of Kossel and Patten. The solution of the histidine was made up to 500 c.c. and nitrogen determined in it.

Nitrogen, 100 c.c. solution, required 7.09 c.c.  $5/7 \text{ N-HCl} = 0.0709 \text{ gm. N} = 0.3545 \text{ gm. N in } 500 \text{ c.c.} = 1.3063 \text{ gm. histidine, or } 3.00 \text{ per cent.}$

The histidine was converted into the dichloride. The characteristic prisms decomposed at about  $230^{\circ}$  and gave on warming a pronounced biuret reaction.



## ARGININE.

The solution of the arginine was made up to 1000 c.c. and nitrogen determined in it.

*Nitrogen*, 50 c.c. solution, required 4.84 c.c. 5/7 N-HCl = 0.0484 gm. N = 0.968 gm. in 1000 c.c. = 3.0047 gm. arginine. Adding 0.072 gm. for the solubility of silver arginine gives 3.0767 gm., or 7.06 per cent.

The arginine was identified as the copper nitrate double salt.

*Water*, 0.2893 gm. subst., air dry, lost 0.0296 gm. H<sub>2</sub>O at 110°.

Calculated for C<sub>12</sub>H<sub>28</sub>O<sub>4</sub>N<sub>8</sub>Cu (NO<sub>3</sub>)<sub>2</sub> 3 H<sub>2</sub>O = H<sub>2</sub>O 9.16 per cent.

Found . . . . . = H<sub>2</sub>O 10.23 " "

*Copper*, 0.1822 gm. subst., dried at 110°, gave 0.0267 gm. CuO.

Calculated for C<sub>12</sub>H<sub>28</sub>O<sub>4</sub>N<sub>8</sub>Cu (NO<sub>3</sub>)<sub>2</sub> = Cu 11.87 per cent.

Found . . . . . = Cu 11.71 " "

## LYSINE.

The lysine was isolated as the picrate, of which there were obtained 3.27 gm. equal to 1.2730 gm. of lysine, or 2.93 per cent. The lysine picrate gave the following determination of nitrogen:

*Nitrogen*, 0.0980 gm. subst. gave 16.8 c.c. moist N<sub>2</sub> at 28° and 756.6 mm.

Calculated for C<sub>6</sub>H<sub>14</sub>O<sub>2</sub>N<sub>2</sub> · C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>N<sub>3</sub> = N 18.67 per cent.

Found . . . . . = N 18.77 " "

## GLUTAMINIC ACID.

Fifty grams, equal to 43.09 gm. ash, moisture, and fat free protein, were hydrolyzed with 200 c.c. of hydrochloric acid sp. gr. 1.11 by boiling on an oil bath for fifteen hours. The solution was concentrated to about 75 c.c., saturated with hydrochloric acid gas, and after standing on ice for some time the substance that had separated was filtered out and recrystallized after decolorizing with animal charcoal. The weight of the glutaminic acid hydrochloride thus obtained was 6.85 gm. after deducting the ammonium chloride which it contained. This is equal to 5.48 gm. glutaminic acid, or 12.72 per cent.

## TRYPTOPHANE.

A qualitative test for tryptophane with glyoxylic acid gave a strong reaction for this substance.



## AMMONIA.

One gram protein equal to 0.8711 gm. moisture, ash, and fat free substance was hydrolyzed by boiling for eight hours with 50 c.c. hydrochloric acid, during which time the solution was finally concentrated to about 3 c.c. The residual solution was then taken up in about 300 c.c. water and distilled with magnesium oxide. The ammonia that was liberated was equivalent to 1.52 c.c. 5/7 N—HCl equal to 0.0152 gm. N, or 2.12 per cent of ammonia.

The results of these hydrolyses are given in the following table:

	Zein. per cent.	Alkali soluble Protein. per cent.		Zein. per cent.	Alkali soluble Protein. per cent.
Glycocoll . . .	0.00	0.25	Serine . . . .	0.57	not isolated
Alanine . . . .	2.23	not isolated	Tyrosine . . . .	3.55	3.78
Valine . . . .	0.29	not isolated	Arginine . . . .	1.16	7.06
Leucine . . . .	18.60	6.22	Histidine . . . .	0.43	3.00
Proline . . . .	6.53	4.99	Lysine . . . .	0.00	2.93
Phenylalanine . .	4.87	1.74	Ammonia . . . .	3.61 <sup>12</sup>	2.12
Aspartic acid . .	1.41	0.63	Tryptophane . .	0.00 <sup>18</sup>	present
Glutaminic acid .	18.28	12.72	Total . . . .	61.53	45.44

These figures show that zein, like the other alcohol soluble proteins, is characterized by yielding a very small percentage of arginine and histidine, no lysine, and much ammonia and proline. The proportion of glutaminic acid is much less than that found in the other alcohol soluble proteins, hordein and gliadin, while the proportion of leucine is very much greater. Unfortunately the amount of the alkali soluble protein which was available for hydrolysis was too small to enable us to obtain satisfactory results for quantitative comparison. It is interesting, however, to note that those amino acids which are lacking in zein are all present in notable proportions in this protein, so that the mixture of the proteins as they occur in this seed yields all of the amino acids usually obtained from protein substances.

<sup>12</sup> OSBORNE and HARRIS: Journal American Chemical Society, 1903, xxv, p. 323.

<sup>18</sup> OSBORNE and HARRIS, *Ibid.*, 1903, xxv, p. 853.











