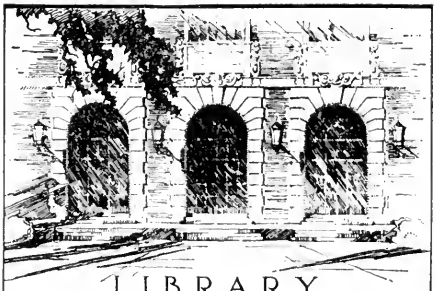

AGRICULTURE

UNIVERSITY OF ILLINOIS

Agricultural Experiment Station.

URBANA, JULY, 1898.

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THE CHEMISTRY OF THE CORN KERNEL[1].

INTRODUCTION.—The object of these studies on the chemisty of corn[2] is to trace its historical development, to bring together from many sources the existing knowledge of the subject, and, if possible, to add thereto in certain lines where our present knowledge seems most deficient, omitting fields wherein other investigators are known to be engaged. With the single purpose of being faithful to the history of the subject, I have felt equally free to point out misconceptions, erroneous conclusions, or real advances of past investigations. The subject has naturally divided itself into two parts:

1st. The proximate composition of corn, which has a very practical significance as indicating its value as food for man and domestic animals and as raw material for various manufacturing purposes.

2nd. The complete and exact composition of the different groups of substances found by proximate analysis, a matter of more purely scientific interest, though not without phases of economic importance.

ACKNOWLEDGMENTS.—I acknowledge with pleasure and gratitude my indebtedness to the Department of Chemistry of Cornell University for the opportunities and privileges which have been freely accorded to me. I am especially grateful to Professor G. C. Caldwell, under whose direction these studies have been carried on, and who has been to me a constant source of counsel and encouragement.

[1] Presented to the Faculty of Cornell University as a thesis for the degree of Doctor of Philosophy, June, 1898.

[2] Indian corn, maize; Ger. *Wälschkorn, Mais;* Fr. *maïs;* Sp. *maiz;* from Haytian *mahis.* (Zea Mays L.)

Bizio found corn to contain oil, which had not been discovered by Gorham. The substance, *hordein*, was so called by Bizio because of its similarity to the substance which had been obtained from barley by Proust[1] and so named by him; which, however, was afterward shown by Guibourt[2] to be merely a mixture of hulls and cellular tissue; and the hordein as found by Bizio was doubtless a mixture of these fibrous substances with considerable amounts of adhering starch and protein.

Probably the first work from the record of which the total amount of nitrogenous matter can be very approximately calculated was that of Bousingault, published[3] in 1836 upon the total nitrogen content of corn. By combustion with copper oxid .617 grammes of corn (containing 18 per cent. of water) were found to yield 10.3 cubic centimeters of nitrogen gas measured at 9 degrees and 738 millimeters. By computation I find this to be equivalent to 2.39 per cent. of nitrogen in the dry matter, and by using the factor 6.25, this gives 14.9 per cent. of protein.

In 1846 Horsford reported[4] a complete ultimate organic analysis of corn and then by an ingenious use of the formula which had been worked[5] out for the average composition of several proteid bodies, as egg-albumen, gluten (*Kleber*) of wheat, rye, etc., he calculated the ultimate composition not only of the nitrogenous matter, but also of the nitrogen-free organic matter. Using the factor 6.375 for converting nitrogen into protein, and having determined the percentage of mineral matter he gives corn the following composition:

Nitrogenous matter.....	14.66	Carbon.....	8.07
		Hydrogen.....	1.00
		Nitrogen.....	2.30
		Sulfur..	.16
		Oxygen.....	3.13
Non-nitrogenous organic matter.	84.52	Carbon	37.38
		Hydrogen.....	5.61
		Oxygen.....	41.53
Mineral matter.....	1.92		1.92

[1] Annales de Chimie et de Physique (1817), [1] **5**, 337.

[2] Jahresbericht [Berzelius] über die Fortschritte der physischen Wissenschaften (1831) **10**, 202.

[3] Annales de Chimie et de Physique (1836) [2] **63**, 239.

[4] Annalen der Chemie und Pharmacie (1846) **58**, 182.

[5] Scherer, ibid. (1841) **40**, 1; Jones, ibid. (1841) **40**, 65; Heldt, ibid. (1843) **45**, 198.

A very extended article by J. H. Salisbury on the general subject of corn was published[1] in 1848. It included a report of considerable chemical work, done by such imperfect methods as nearly to deprive it of permanent value, as will appear from the following analysis of two samples of corn kernels :

	1.	2.
Albumen	9.29	4.64
Zein	6.73	3.98
Casein	1.44	.09
Dextrine or gum	5.94	3.53
Fiber	12.09	.96
Matter separated from fiber by weak potash solution,	7.80	6.48
Sugar and extract	13.27	14.42
Starch	38.28	60.92
Oil	5.18	4.98

The methods employed by Salisbury were in the main similar to those of the earlier investigators and are briefly indicated as follows :

The powdered corn was washed with water which was decanted. The residue extracted with alcohol and dilute potash water gave the fiber. The matter held in suspension in the water was collected, washed with alcohol and noted as starch, the residue from the evaporation of the alcohol became a portion of the "sugar and extract." The turbid water from the starch determination was heated and the coagulated matter called albumen. In one portion of the filtrate the "casein" was precipitated by acetic acid, and the "dextrine or gum" by alcohol after partial evaporation. In a second portion the "casein" and "dextrine or gum" were together removed by alcohol and another portion of "sugar and extract" obtained by evaporating the filtrate to dryness.

The zein and oil were extracted from the corn by alcohol and separated by ether after evaporation of the alcohol.

Following Salisbury's work proximate analyses were reported by Polson[2], Poggiale[3], Stepf[4], Payen[4], and also by the renowned and but recently deceased R. Fresenius[5].

[1] Transactions of the New York State Agricultural Society (1848) **8**, 678; American Journal of Science and Arts (1849) [2] **8**, 307.

[2] Chimic. Gazette (1855) 211 ; Journal für praktische Chemie (1855) **66**, 320.

[3] Jahresbericht [Leibig und Kopp] über die Fortschritte der Chemie (1856) 809.

[4] Journal für praktische Chemie (1859) **76**, 88,

[5] Landwirtschaftliche Versuchs-Stationen (1859) **1**, 179; Jahresbericht [Hoffmann] uber die Fortschritte auf dem Gesammtgebiete der Agricultur-Chemie (1859) **2**, 76.

The following will serve as illustrations of the results:

	Polson.		Poggiale.		Fresenius.	
Water	11.8	dry	13.5	dry	13.46	dry
Ash	1.8	2.04	1.4	1.62	1.58	1.83
Protein	8.9	10.09	9.9	11.44	10.04	11.60
Oil	4.4	4.99	6.7	7.75	5.11	5.90
Fiber	15.9	18.03	4.0[2]	4.62	1.58	1.83
Sugar	2.9[1]	3.29			2.33[3]	2.69
Starch	54.3	61.56	64.5	74.57	65.90	76.15

In 1869, Atwater reported[4] the following results from a study[5] of the proximate composition of corn:

	Early Dutton.	Common yellow.	King Philip.
Ash	1.66	1.46	1.77
Protein	10.46	10.86	13.16
Fat	6.16	4.94	4.93
Fiber	2.74	2.68	2.45
Sugar	3.26	5.34	3.38
Gum	4.59	2.64	5.32
Starch	71.13	72.08	68.99

The protein was estimated by multiplying the total nitrogen by the factor **6**.25, a method which had come into general use, and which has already been referred to under Horsford's work. Sugar was estimated by Fehling's method from the aqueous extract, and the gum is the difference between the sugar and the dried aqueous extract. The oil is the ether extract. Fiber was determined by extracting with dilute acid and alkali, essentially the method employed by Gorham nearly eighty years ago, and in general use among agricultural chemists of to-day, having been known under various names, as Peligot's[6], Henneberg's, or the Weende[7] method, the last being common at the present time. Starch was estimated by difference.

Closely following Atwater's work numerous analyses were reported by European chemists. In the group of carbohydrates only the fiber was determined, the remainder being estimated by difference and reported under the negative and indefinite heading "nitrogen-free extract" for which I have recently proposed[8] to substitute the more definite and logical term *carbohydrate extract.*

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[1] and gum.

[2] and loss.

[3] dextrine.

[4] W. O. Atwater—The proximate composition of several varieties of American maize—Thesis for the degree of Doctor of Philosophy, Yale College (1869); American Journal of Science and Arts (1869) [2] **48**, 352.

[5] The analysis of a sample of sweet corn also reported is omitted.

[6] Journal für praktische Chemie (1850) **50**, 261,

[7] Landwirtschaftliche Versuchs-Stationen (1864) **6**, 497.

[8] University of Illinois Agr. Exp. Station Bulletin (1896) **43**.

The following table gives a number of the results obtained, all being reduced to the basis of dry matter :

Analyst[1],	Ash.	Protein.	Fat.	Fiber.	Carbohydrate extract.
Dietrich	3.19	13.88	5.59	2.86	74.48
Nessler	4.53	8.81	5.87	6.24	74.55
Nessler	3.20	6.41	6.17	6.54	77.68
Nessler	3.98	10.01	6.25	5.35	74.41
Kreuzhage	1.70	13.03	4.79	1.74	78.74
Honig und Brimmer	1.50	9.00	4.16	1.58	83.76
Honig und Brimmer	1.42	10.35	4.36	1.55	82.32

In 1883 Richardson[2] made a compilation of analyses of corn grown in various parts of the United States during the years 1877 to 1882. The following table shows the number of samples analyzed and the averages of the analyses from each state represented. All dry matter other than ash, protein, and oil I have grouped under the general term *carbohydrates*. This is done for several reasons. 1. We are considering not complete but proximate analysis. 2. Ash, protein, fat, and carbohydrates constitute distinctly different groups with well known individual properties or characteristics as to use, value, etc. 3. The amount of fiber in corn is too small to warrant its determination ordinarily, even if it were known that its value differs slightly from that of other carbohydrates, the pentosans, for example. 4. The limit of error in fiber determination is wide and not only appears in the fiber itself but also in the carbohydrate extract (so called nitrogen-free extract.) 5. These data become more readily comparable with my own analyses which are herein reported without fiber determinations.

Samples.	Ash.	Protein.	Fat.	Carbohydrates.
New Hampshire 11	1.76	12.98	6.10	79.16
Vermont 1	1.59	11.10	6.16	81.15
Connecticut 13	1.73	11.75	5.27	81.25
Pennsylvania 5	1.55	9.65	5.67	83.13
North Carolina 2	1.50	12.03	5.43	81.04
Kentucky 1	1.62	10.62	5.77	81.99
Tennessee 1	1.33	10.05	5.51	83.11
Indiana 1	1.44	11.84	5.49	81.23
Michigan 12	1.67	12.83	5.70	79.80
Missouri 26	1.83	11.48	5.75	80.94
Kansas 6	1.69	11.53	5.53	81.25
Colorado 1	1.68	10.95	6.32	81.05
Texas 20	1.59	11.61	6.09	80.71
Oregon 1	1.61	8.68	7.80	81.91
Washington 1	1.67	9.36	6.39	82.58
Mexico 3	1.75	11.44	6.06	80.75
General average	1.69	11.63	5.78	80.90

[1] Jahresbericht [Hoffmann] über die Agricultur-Chemie (1872) **15**, 10; (1876) **19**, 7.

[2] U. S. Dept. of Agr., Division of Chemistry Bulletin (1883) **1**.

The following are some of the conclusions which Richardson draws from his data:

"There is apparently the same average amount of ash, oil, and albuminoids [protein] in a corn wherever it grows, with the exception of the Pacific Slope, where, as with wheat, there seems to be no facility for obtaining or assimilating nitrogen.

"Corn is, then, an entirely different grain from wheat. It maintains about the same percentage of albuminoids under all circumstances, and is not affected by its surroundings in this respect.

"Only two analyses have been made from the Pacific Slope and more are needed for confirmation, but as the two analyses, like those of the wheats grown there, are low in albuminoids, it may safely be assumed to be a characteristic of that portion of the country."

These conclusions scarcely appear to be warranted from the data. By computation from the 114 analyses of corn, I find the total variation in protein to be 63.6 per cent. of the average amount determined; while from the 260 analyses of wheat referred[1] to by him it is only necessary to exclude 5 analyses to bring the total variation in protein to 60.1 per cent. of the average amount determined. Or if we take the averages of the 10 highest and the 10 lowest results on the protein of 114 samples of corn, 12.34 per cent. and 8.19 per cent., respectively, we find the difference, 4.15 per cent., to be 40 per cent. of the general average; while with the averages of the 25 highest and the 25 lowest results on the protein of 260 samples of wheat, 14.97 per cent., and 9.28 per cent., respectively, the difference is 5.69 per cent. or 48 per cent. of the general average (11.95 per cent.). In other words the variation in the corn is only one-sixth less than that in the wheat. It may be noted that if we include the analyses of sweet corn (all varieties of wheat are considered) the variations in the protein content of corn exceed those in wheat. Jenkins and Winton's compilation[2] shows the protein content to vary more in 208 samples of corn than Richardson found in 260 samples of wheat.

As to the assumption regarding the Pacific Slope it may be pointed out that the table of analyses from the different States shows the average of 5 analyses of Pennsylvania corn to agree well in percentage of protein with the single analyses from Oregon and Washington. The average of 12 analyses of corn from California reported in 1884 by Richardson[3] shows practically the same percentage of protein as the general average for the United States.

In 1886 Flechig[4] made analyses of 14 different varieties of corn,

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[1] U. S. Dept. of Agr., Division of Chemistry Bulletin (1883), **1**.

[2] U. S. Dept. Agr., Exp. Station Bulletin (1892) **11**, 100.

[3] U. S. Dept. Agr., Division of Chemistry Bulletin (1884) **4**.

[4] Landwirtschaftliche Versuchs-Stationen (1886) **32**, 17.

all grown under uniform conditions of weather, soil, and fertilization. If we omit a variety of sweet corn[1] the following are his results:[2]

Variety.	Ash.	Protein.	Oil.	Carbohyrates.
Jaune Hâtif d'Antonina	1.29	12.63	5.40	80.68
Rother Hühnermais	1.43	11.06	5.80	80.71
Weisser steirischer	1.51	10.50	5.32	82.67
Weisser ungarischer	1.63	9.88	6.21	82.28
Canquatino	1.48	9.88	5.52	83.12
Türkischer vierzigtägiger	1.73	9.69	5.88	82.70
Canadischer aus Ungarn	1.58	9.50	6.00	82.92
Bunter Augustmais	1.44	9.50	5.02	84.04
Früh. Amerik. Bernsteinmais	1.42	9.19	5.75	83.64
Früher Badischer	1.46	9.06	5.43	84.05
Blanc hâtif des Landes	1.60	9.00	6.22	83.18
Improved King Philip	1.54	8.95	5.43	84.08
Papageienmais	1.35	8.69	5.88	84.08

In view of the fact that reference has already been made to the wide limit of error in fiber determinations, it may be noted here that the total variation on the final results for fiber as reported by Flechig on the 13 samples of corn is from 1.23 per cent. to 1.86 per cent., while the variation in the separate determinations made on a single sample is from 1.26 per cent. to 1.83 per cent. It is also observed that Flechig's results indicate protein as the most variable constituent of corn grown under uniform conditions.

Since the establishment of the experiment stations in the United States the number of proximate analyses of corn has been greatly increased[3], but in the main the analyses have been made for special purposes (as in feeding experiments) other than a study of the corn itself, and upon samples whose history was unknown or unnecessary for the object in view. Only one series of these analyses will be discussed in this connection.

In 1893 the Connecticut Experiment Station published[4] the analyses of 90 samples of corn grown in 1892 in various parts of the state from about 75 differently named varieties, and under exceedingly varying conditions of weather, soil, cultivation, fertilization, etc. If we omit one sample of sweet corn, and one sample which was injured by hail before maturing, the following are the five highest and the five lowest results from all determinations of each constituent; also the general average of all analyses:

[1] Sucre ridé.

[2] A few errors were found in Flechig's summary which I have corrected from his analytical data. Fiber is included in the column headed carbohyrates.

[3] Especially by U. S. Dept. of Agr. and Stations of Conn., Mass., Ill., Vt. and N. J.

[4] Conn. Agr. Exp. Station Annual Report (1893).

	Ash.	Protein.	Fat.	Carbohydrates.
1st highest	2.10	14.53	6.39	85.93
2nd "	1.90	14.04	5.97	85.14
3rd "	1.86	13.86	5.95	85.07
4th "	1.80	13.33	5.95	84.67
5th "	1.79	13.29	5.95	84.63
1st lowest	.91	8.33	3.15	78.56
2nd "	.98	8.69	3.55	78.85
3rd "	1.00	8.79	4.21	78.99
4th "	1.01	8.82	4.28	79.26
5th "	1.04	8.25	4.31	79.85
General average	1.39	11.63	5.27	81.71

The compilation[1] of Jenkins and Winton gives the average composition of dent and flint corn as follows:

	Samples.	Ash.	Protein.	Fat.	Carbohydrates.
Dent	86	1.7	11.5	5.6	81.2
Flint	68	1.7	11.8	5.6	80.9
General average	154	1.7	11.6	5.6	81.1

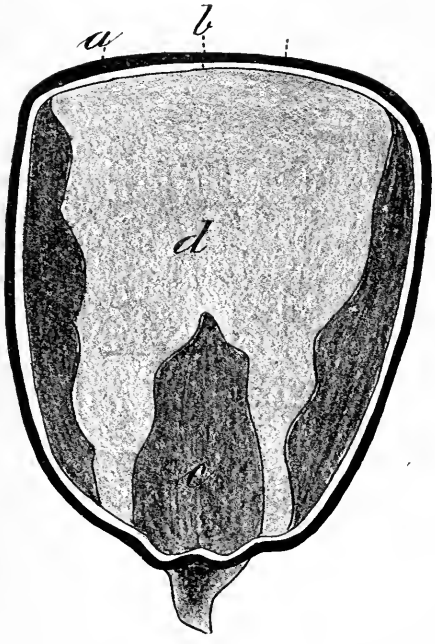

FIG. 1.

By mechanical means the corn kernel has been separated into four different parts. These may be designated (fig. 1[2]) as (a) the coat, or hull, of the kernel, (b) the hard glutenous layer underneath the hull, much thicker at the sides than at the crown, (c) the chit, or germ, and (d) the starchy matter constituting the chief body of the kernel. It has never been found possible to make such a separation with even approximate accuracy, the separation of the glutenous layer from the starchy portion being especially difficult. On this basis Salisbury[3] gives the following percentage composition of the kernel with the proximate composition of the different parts reduced to the dry basis:

[1] U. S. Dept. of Agr., Exp. Station Bulletin (1892) **11**.

[2] I am indebted to Director Voorhees, N. J. Agr. Exp. Station, for the use of this cut.

[3] Trans. N. Y. State Agr. Soc. (1848) **8**, 678.

	Hulls.	Glutenous layer.	Starchy portion.	Germs.
Per cent	4.30	66.63	18.04	11.03
Ash	4.56	.43	.61	14.05
Protein[1]		7.65	2.74	21.39
Oil		2.87	3.07	30.26
Carbohydrates[2]		89.05	93.58	34.30

In a microscopic study of the corn kernel Haberlandt[3] observed that the germ contained a large amount of oil while in the remaining portions of the kernel no oil was apparent. Acting upon this Lenz[3] undertook an analytical investigation of these portions. The germs were carefully removed from the kernels by mechanical means and the oil and protein in the two portions determined. His results on a sample of American white flint corn are as follows:

	Kernels less germs.	Germs.
Per cent	88.18	11.82
Per cent Oil[4]	1.57	32.83
" " Protein[4]	13.09	19.93

Lenz expressed the opinion that the small quantity of oil found in the kernel after the germ had been removed was really due to particles of the germ which had not been removed or to traces of oil deposited on the remainder of the kernel during the mechanical process of removing the germ.

This was further investigated by Atwater[5] who removed the germ together with a considerable portion of the kernel immediately surrounding the germ in order to insure the separation of all oil properly belonging to the germ. Following are his results:

	Outer portion free from germ.	Inner portion including germ.
Per cent	76.43	23.57
Per cent. Oil[4]	1.63	

Recently Voorhees[6] and Balland[7] have published the following results:

Hulls.	Glutenous layer and starchy portion.	Germs.	
5.56	84.27	10.17	Voorhees.
12.40	74.10	13.50	Balland.

[1] This is given here as the sum of the zein, albumen, and casein reported by Salisbury.

[2] By difference.

[3] Allgemeine land- und forstwirtschaftliche Zeitung (1866) 257; Jahresbericht [Hoffmann] über die Agricultur-Chemie (1866) **9**, 106.

[4] In the dry matter.

[5] Thesis, Yale College (1869); American Journal of Science and Arts (1869) [2] **48**, 352.

[6] New Jersey Agr. Exp. Station Bulletin (1894) **105**.

[7] Comptes rendus des Scéances (1896) **122**, 1004.

The following table shows the composition[1] of the separate parts (dry).

	Ash.	Protein.	Oil.	Fiber.	Carbohydrate extract.	
Hulls.	1.25	6.52	1.57	16.24	74.42	Voorhees.
	1.44	8.20	2.33	11.25	76.78	Balland.
Glutenous layer and starchy portion.	.68	12.15	1.53	.65	84.99	Voorhees.
	.68	8.53	1.08	.40	89.31	Balland.
Germs.	10.02	19.54	26.65	2.59	41.20	Voorhees.
	7.87	15.32	39.85	1.99	34.97	Balland.

These data confirm the earlier results, showing the germ, which constitutes only about 12 per cent. of the kernel, to contain nearly twice as much mineral matter and three or four times as much oil as all of the remaining portions of the kernel. It is also rich in protein. Voorhees states that the portion richest in protein is the glutenous layer.

In the manufacture of starch and glucose-sugar from corn these different portions of the kernel are separated much more perfectly than it is possible to do by hand although their original composition is somewhat altered. Various methods[2] have been employed, but the following will indicate briefly a common process :

The corn is steeped in warm water containing a little sulfurous acid and then reduced to a coarse powder. The germs together with a part of the hulls are recovered by floating and separated after drying. The material remaining in the water in suspension is passed through sieves and the remainder of the hulls and some other coarse matter can thus be separated from the starch and the more finely divided gluten. The starch is finally allowed to settle and then the water containing the larger part of the gluten is run off. After further purification the starch is sold as such or is manufactured into other products, as glucose-sugar. The by-products, hulls, "gluten," and germs, separate or mixed, are sold as food stuffs, the larger part of the oil usually having been expressed from the germs. The mineral matter is, of course, largely removed from these products by the solvent action of the water.

The analyses of corn oil cake was reported[3] by Moser as early as 1867 with the following results :

Ash.	Protein.	Fat.	Fiber.	Carbohydrate extract.
8.07	17.19	12.58	11.41	50.75

The following is believed to fairly represent the composition (dry) of the several individual products, not as usually found on the market but in their purest condition :

[1] As published Voorhees' results are evidently given on the basis of ash-free organic matter. They are here calculated to the basis of total dry matter.

[2] Journal Society Chemical Industry (1887) **6**, 84.

[3] Jahresbericht (Hoffmann) über die Agricultur-Chemie (1867) **10**, 259. Cf. ibid. (1872) **15**, 21 ; (1874) **17**, 15 ; (1876) **19**, 15.

	Ash.	Protein.	Fat.	Fiber.	Carbohydrate extract.
Hulls[1]	1.02	11.18	4.13	11.98	71.69
Gluten[2]	1.14	44.03	7.69	2.26	44.88
Germ cake[1]	2.58	27.23	14.84	7.41	47.94
Starch[3]	0.30	.....	....	.....	99.70[4]

The correctness of Voorhees' statement that the portion of the corn kernel richest in protein is the glutenous layer is plainly apparent.

Richards[5] has recently made proximate analyses to determine the heating value of the corn kernel. Calorimetric determinations were also made, being reported in terms of the British thermal unit[6]. Following are the results :

	Moisture.	Volatile matter.	Fixed carbon.	Ash.	Fuel value.
Yellow dent	8.45	78.10	12.18	1.27	8202.
White dent	8.88	77.22	12.90	1.00	8338.

EXPERIMENTAL.

In the following work on the proximate composition of corn the total dry matter, the ash, the nitrogen, and the fat were determined directly. The protein was estimated by multiplying the total nitrogen by 6.25 and the carbohydrates by subtracting the sum of the ash, protein, and fat from the total dry matter. In each single determination of the several constituents 2 gms. of air-dry substance were regularly taken.

PREPARATION OF SAMPLE.—All samples were air-dried, ground to pass through a sieve with circular perforations 1 millimeter in diameter, and then preserved in air-tight vessels, being thoroughly mixed just before being analyzed.

DETERMINATION OF DRY MATTER.—The air-dry substance was placed in a glass tube 10 cm. long and 2 cm. in diameter over one end of which a piece of hardened filter paper had been firmly tied with nickel wire, the tube with paper bottom having been dried and weighed in weighing tubes before being charged with the substance. The substance was dried with the tube lying in a horizontal position in a current of dry hydrogen at a temperature of 105°, maintained by a boiling aqueous solution of glycerol in a double-wall bath provided with a return condenser. The gas entered the bath at one end near the top and passed out at the bottom near the opposite end.

[1] N. J. Agr. Exp. Station Bul. (1894) **105**.
[2] Conn. Agr. Exp. Station Report (1895) 231.
[3] Journal Society Chemical Industry (1887) **6**, 84.
[4] Starch.
[5] U. S. Dept. of Agr., Exp. Station Bulletin (1898) **49**, 95.
[6] Heat required to raise one pound of water from 50° to 51° F.

To determine the error in obtaining the weight of the empty tubes with the paper bottoms, 10 tubes were dried for one hour, cooled in desiccators and weighed in weighing tubes, then dried again for two hours and again weighed, with the following results :

	First weight.	Second weight.	Decrease.
1	47.7552	47.7550	.0002
2	49.0332	49.0328	.0004
3	46.1074	46.1074	.0000
4	48.9842	48.9843	—.0001
5	48.6642	48.6641	.0001
6	45.4501	45.4500	.0001
7	48.5461	48.5455	.0006
8	47.8516	47.8518	—.0002
9	44.8934	44.8930	.0004
10	46.2726	46.2727	—.0001

To determine the length of time required under the conditions mentioned to reduce the substance practically to a constant weight the following data were obtained, 2 gms. of air-dry substance being taken from 12 different samples :

	Weight of substance after drying			Difference in weight between drying	
	4 hours.	8 hours.	16 hours.	4 or 8 hours.	8 or 16 hours.
1	1.7759	1.7639	1.7600	.0120	.0039
2	1.7662	1.7545	1.7512	.0117	.0033
3	1.7569	1.7454	1.7413	.0115	.0041
4	1.7638	1.7525	1.7489	.0113	.0036
5	1.7662	1.7550	1.7513	.0112	.0037
6	1.7635	1.7520	1.7483	.0115	.0037
7	1.7589	1.7476	1.7435	.0113	.0041
8	1.7651	1.7541	1.7503	.0110	.0038
9	1.7738	1.7625	1.7580	.0113	.0045
10	1.7536	1.7422	1.7387	.0114	.0035
11	1.7623	1.7505	1.7457	.0118	.0048
12	1.7556	1.7450	1.7411	.0106	.0039

After drying 4 hours the average decrease in weight for four hours more is 0.0114 gms. or 0.6 per cent. of the amount determined, and then for 8 hours more it is 0.0039 gms. or 0.2 per cent. of the amount determined. This is a much narrower limit of error than can be maintained in the determination of the constituent groups of the dry matter, and all dry matter determinations which follow were made by drying the substance 8 hours. It is noteworthy that during the second and third periods of drying all of the samples lost weight and in very nearly equal amounts, showing that for comparative results a very high degree of accuracy is attained.

The following work was done to test the agreement of duplicate determinations on the same sample. Twelve different samples were selected, and the 24 portions of 2 gms. each were all dried together:

	Weight of dry matter.		Variation.		Weight of dry matter.		Variation.
1	1.8276	1.8273	.0003	8	1.8240	1 8242	.0002
2	1.8230	1.8238	.0008	9	1.8243	1.8240	.0003
3	1.8218	1.8222	.0004	10	1.8202	1.8209	.0007
4	1.8319	1.8314	.0005	11	1.8176	1.8186	.0010
5	1.8244	1.8249	.0005	12	1.8150	1.8155	.0005
6	1.8198	1.8194	.0004				
7	1.8264	1.8267	.0003		Average		.0005

From these results and those preceding it is seen that determinations made in the same bath and at the same time show a remarkable degree of accuracy when compared only with themselves, and among themselves they are strictly comparable.

To determine the variation which might be caused by unavoidable differences in temperature, hydrogen current, etc., the following 36 duplicate determinations of dry matter were made, in every case the duplicate determinations being made at different times, *i. e.*, the first determination on each sample was made one or more days previous to the second, or duplicate, determination:

	Weight of dry matter.		Variation.		Weight of dry matter.		Variation.
1	1.7456	1.7489	.0033	20	1.7566	1.7520	.0046
2	1.7493	1.7527	.0034	21	1.7730	1.7719	.0011
3	1.7444	1.7441	.0003	22	1.7795	1.7734	.0061
4	1.7362	1.7360	.0002	23	1.7668	1.7593	.0075
5	1.7200	1.7238	.0038	24	1.7584	1.7502	.0082
6	1.7514	1.7541	.0027	25	1.7560	1.7534	.0026
7	1.7675	1.7689	.0014	26	1.7431	1.7435	.0004
8	1.7628	1.7637	.0009	27	1.7526	1.7540	.0014
9	1.7659	1.7673	.0014	28	1.7540	1.7539	.0001
10	1.7540	1.7588	.0048	29	1.7590	1.7599	.0009
11	1.7522	1.7547	.0025	30	1.7494	1.7512	.0018
12	1.7554	1.7592	.0038	31	1.7489	1.7465	.0024
13	1.7698	1.7739	.0041	32	1.7498	1.7497	.0001
14	1.7546	1.7586	.0040	33	1.7552	1.7559	.0007
15	1 7691	1.7741	.0050	34	1.7925	1.7928	.0003
16	1.7552	1.7573	.0021	35	1.7515	1.7481	.0034
17	1.7723	1.7689	.0034	36	1.7451	1.7408	.0043
18	1.7736	1.7696	.0040				
19	1.7760	1.7742	.0018		Average		.0027

The maximum variation 0.0082 is 0.5 per cent. of the average amount determined; and is very much greater than when the duplicates were made at the same time. However, the agreement still appears very satisfactory. In all subsequent work herein reported the duplicate determinations of dry matter were made at different times in order that the results may show the widest variations possible with the method employed.

DETERMINATION OF ASH.—The air-dry substance was placed in a

porcelain crucible and burned to constant weight in a muffle at a low red heat, at a temperature below that at which portions of the ash would become fused and attached to the crucible.

DETERMINATION OF NITROGEN.—This was made by the ordinary Kjeldahl method. The metallic mercury used in the digestion was measured in a capillary tube, one end of which is doubly bent so as to form a loop, the short arm of which is turned back upon itself near the end while the long arm serves as a handle. The loop is made sufficiently narrow to pass into the mercury bottle, and of sufficient length to retain when raised above the liquid the exact quantity of mercury required for a single determination. By blowing in the longer arm the mercury is emptied into the digestion flask.

Heavy copper flasks were used in the distillation with much satisfaction, the sodium hydroxid solution (containing the necessary amount of potassium sulfid) being added in sufficient excess to "bump" before the contents may become dry, thus serving as a signal that the distillation has gone far enough.

Two common sources of error in the nitrogen determination were found and investigated. In titrating an acid solution in an open vessel with standard ammonia solution a very appreciable error is introduced by the volatility[1] of the ammonia, although the only possible loss is from the tip of the burette and from the falling drops.

In the following work ammonia of about one-sixth normal strength was used, the hydrochloric acid being of such strength that 3 cc. were equivalent to approximately 4 cc. of ammonia. The hydrochloric acid was measured from an automatic overflow pipette of 15 cc. capacity, and the ammonia from an automatic overflow burette graduated to 0.05 cc. and drawn to a fine tip at the outlet. The pipette and burette were each provided with three way stopcocks through which the standard solutions were drawn from the stock bottles by means of syphons. Perfectly neutral water free from ammonia and carbon dioxid was used for diluting. Lacmoid served as the indicator and gave an exceedingly sharp end reaction.

By titrating in beaker flasks with the tip of the ammonia burette well below the top of the flask the following results were obtained, the length of time taken in making the titration being also given:

1.....	15 cc. HCl	required	20.10 cc. NH_3,	—time =	1 minute.
2.....	15 cc. HCl	"	20.08 cc. NH_3,	— " =	1 "
3.....	15 cc. HCl	"	20.12 cc. NH_3,	— " =	1 "
4.....	15 cc. HCl	"	20.30 cc. NH_3,	— " =	2 "
5.....	15 cc. HCl	"	20.25 cc. NH_3,	— " =	2 "
6.....	15 cc. HCl	"	20.40 cc. NH_3,	— " =	3 "

[1] Rempel has already shown that dilute ammonia solution drawn into beakers or evaporating dishes and then titrated suffers marked loss.—Zeitschrift für angewandte Chemie (1889) 331.

By titrating in an Erlenmeyer flask attached to the burette by means of a rubber stopper[1], provided with a capillary tube for relieving the pressure, the following results were obtained :

1	15 cc. HCl required	19.82 cc. NH_3,	time = 1 minute.
2	15 cc. HCl "	19.83 cc. NH_3,	" = 1 "
3	15 cc. HCl "	19.81 cc. NH_3,	" = 3 "
4	15 cc. HCl "	19.81 cc. NH_3,	" = 5 "

As from 3 to 5 minutes are taken to make a titration when the amount of ammonia required is not known, as in ordinary nitrogen determinations, the error[2] from titrating in open vessels becomes an important factor, the total variation in the two series of experiments above noted amounting to 0.6 cc. or 3 per cent. of the ammonia required. The fact that the density of ammonia gas is but little more than half that of air explains its rapid upward diffusion from an open vessel.

Another error in nitrogen determinations may occur in the distillation by loss of ammonia from the receiving flask in case there is not sufficient acid *above the end of the delivery tube* to neutralize all of the ammonia distilled over.

In the following work a quantity of a very dilute solution of ammonium chlorid was prepared by exactly neutralizing standard hydrochloric acid with standard ammonia and diluting with ammonia-free water. A quantity of this solution equivalent to 12 cc. of standard ammonia was placed in a distillation flask with an excess of sodium hydroxid and distilled into 15cc. of standard hydrochloric acid diluted to about 40 cc., the end of the delivery tube from the condenser dipping *well into the acid solution.* The relation of the standard acid and ammonia solutions was such that 15 cc. HCl were equivalent to 19.82 cc. NH_3. Six distillations were made, in each case ammonium chlorid equivalent to 12 cc. of standard ammonia solution being taken. Following are the amounts of standard ammonia solution required to neutralize the excess of acid :

	Required.	Calculated.	Error.
1	8.20 cc.	7.82 cc.	0.38 cc.
2	7.85 cc.	7.82 cc.	0.03 cc.
3	7.93 cc.	7.82 cc.	0.11 cc.
4	8.60 cc.	7.82 cc.	0.78 cc.
5	7.84 cc.	7.82 cc.	0.02 cc.
6	7.95 cc.	7.82 cc.	0.13 cc.

Two of these are practically exact, the other four showing errors varying from 0.11 cc. to 0.78 cc. of standard ammonia.

This work was repeated with the distillation from quantities of ammonium chlorid equivalent to 15 cc. of standard ammonia solution, the

[1]By using a stopper which has been bored nearly through from the small end by a large borer, the flask may easily be given a free rotary motion.

[2]Confirmed by recent (unpublished) work of Dr. F. L. Kortright.

other conditions being as before. Following are the amounts of standard ammonia solution required to neutralize the excess of acid :

	Required.	Calculated.	Error.
1	6.10 cc.	4.82 cc.	1.28 cc.
2	5.40 cc.	4.82 cc.	0.58 cc.
3	5.95 cc.	4.82 cc.	1.13 cc.
4	6.20 cc.	4.82 cc.	1.38 cc.
5	5.65 cc.	4.82 cc.	0.83 cc.
6	5.18 cc.	4.82 cc.	0.36 cc.

Diluting the residues in the distillation flasks with ammonia-free water, and distilling, gave no further addition of ammonia in any case.

It was observed that in both trials the greatest errors occurred with Nos. 1 and 4. A careful inspection of the apparatus showed all connections to be perfect. It was observed, however, that the delivery tubes from Nos. 1 and 4 did not reach as far into the acid solution as most of the others.

With the thought that possibly ammonia escaped from the receiving flasks, the following six distillations were made, in each the quantity of ammonium chlorid employed being equivalent to 19.32 cc. of standard ammonia solution; thus, exactly 0.50 cc. of standard ammonia should have been required to neutralize the excess of acid. Some lacmoid indicator was added to the acid solutions in receiving flasks Nos. 1, 3, and 5; strips of moistened red litmus paper were also hung in the necks of these flasks. During the process of distillation, receiving flasks 2, 4, and 6 were agitated to keep their contents thoroughly mixed.

It was observed that, during the process of distillation, in receiving flasks 1, 3, and 5 the liquid above the end of the delivery tube turned blue, while a layer of liquid below this remained red; also that the moistened red litmus paper hung in the necks of these flasks turned blue.

In titrating the excess of acid the amounts of standard ammonia required were as follows :

	Required.	Calculated.	Error.
1	2.60 cc.	0.50 cc.	2.10 cc.
2	0.50 cc.	0.50 cc.	0.00 cc.
3	2.27 cc.	0.50 cc.	1.77 cc.
4	0.53 cc.	0.50 cc.	0.03 cc.
5	1.99 cc.	0.50 cc.	1.49 cc.
6	0.50 cc.	0.50 cc.	0.00 cc.

The explanation for the separation of the liquid in the receiving flasks into two layers as described is to be found in the different densities of aqueous solutions of ammonia and hydrochloric acid.

In subsequent work I have used delivery tubes reaching to the very bottom of the receiving flasks, and contracted at the end to an aperture of but 4 or 5 mm. diameter. This insures considerable agitation of the

content of the receiving flask produced by irregularities in the boiling of the liquid in the distillation flask.

This loss of ammonia shown to have taken place from the very dilute solution in the receiving flask after cooling by an efficient condenser emphasizes the results of the preceding work on titration and the importance of avoiding a common error in that process.

DETERMINATION OF FAT.—The glass tube with the bottom of hardened filter paper (previously described) containing the dry matter from 2 gms. of air-dry substance was placed in a Soxhlet tube and the fat extracted, the solvent passing through the substance and being filtered by the paper bottom. This arrangement is for several reasons preferred to the use of tubes made entirely of filter paper. 1. The determination of dry matter and the extraction of fat are done in the same tube without transferring the substance. 2. The solvent *must* pass through the substance. 3. The hardened paper can be removed from the tube (after taking off the wire ligature), spread out in the side of a funnel and the fat-free substance easily and completely removed from both paper and tube, by washing with the hot dilute sulfuric acid to be used in case a fiber determination is desired.

The ether used in the extraction was kept over metallic sodium in the form of wire, and redistilled before being used. The upper end of the condenser was protected by a calcium chlorid tube.

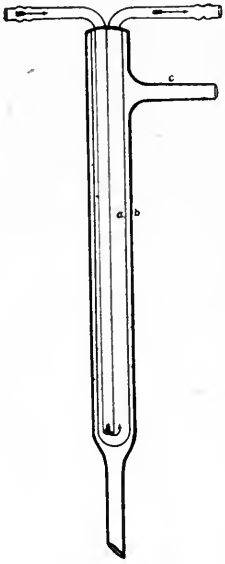

Fig. 2.

Mainly to avoid the constant trouble of having atmospheric moisture condense upon the outer surface of a Liebig or Allihn condenser and run down over the extraction apparatus, the following form of condenser (fig. 2) was designed:

This condenser is made entirely of glass, and consists of a *thin* glass tube (a) 25 mm. *outside* diameter and 25 cm. long, provided with two glass tubes about 6 mm. in diameter, one reaching to near the bottom of (a), sealed in for water inlet and outlet. The tube (a) is surrounded by a stronger glass tube (b) of 30 mm. *inside* diameter sealed on at the top and narrowed at the lower end to a 10 mm. tube which extends 8 mm. below and is ground off obliquely at the end. About 3 cm. from the top of tube (b) a side tube (c) is provided; it is 5 cm. long and 12 mm. inner diameter, and is widened, as indicated in the figure, where it is sealed into (b). The water tubes are cut off at a length of 5 cm., being blown as indicated to hold a rubber tube.

The outer tube of this condenser is not cooled to a temperature at

which atmospheric moisture will condense upon it. This is its chief advantage over the ordinary form in fat extraction with anhydrous ether. The side tube serves to connect with a drying tube.[1]

In making the proximate analyses which are reported herein the fat was always heated in a current of dry hydrogen for 3 hours at 105°; the flask allowed to cool in the air and then to stand in the balance case until the weight became constant. The flasks used in the work were of Erlenmeyer's pattern with about 100 cc. capacity and weighed 25 to 30 gms. each. Differences of barometric pressure and of humidity of the atmosphere of the laboratory may easily produce slight changes in weight.

To cool the flasks in desiccators before weighing was found unsatisfactory on account of the fact that the perfectly dry air of the desiccator is considerably heavier than the moist air of the laboratory, and after the flask is removed from the desiccator its weight does not become constant until the dry air is replaced by that of the laboratory and the condensation of moisture upon the surface of the glass ceases.

In all of my analyses herein reported to determine the proximate composition of corn, two complete single analyses were made; the computations were made separately with no averages, and the results are reported separately. Furthermore the two analyses were made at different times, and the differences between the duplicates certainly fairly represent the experimental error. The computations were made by logarithms and directly to the percentage composition of the dry matter The logarithm of 6.25 was included in the proper factor logarithm for calculating the protein equivalent from cubic centimeters of standard ammonia solution. In no case has the percentage of nitrogen or the percentage composition of the air-dry substance been calculated. If desired the former can be determined exactly by dividing the percentage

[1] A few other important points may be noted. The condenser may be used in ordinary distillation by passing the vapor in through the side tube. The ordinary condenser frequently breaks in consequence of the extreme differences in the temperature of the inner tube just above and below the surface of the surrounding water. The new form is free from this objection. The water tubes are both at the top and very convenient for joining up a series of condensers. These condensers are more compact and yet much more effective than the ordinary form, the vapor being distributed in a *thin* layer over a very large condensing surface, the outer tube also acting as an "air condenser."

These condensers have been in almost constant use during the past year in the chemical laboratories of the University of Illinois and have given excellent satisfaction.

There are several condensers which have the water tube inside, but I have found none suited to the purpose for which this was especially designed except that recently described by Sudborough and Feilmann (Jour. Soc. Chem. Ind. (1897) **16**, 979), which is certainly to be preferred to the ordinary form as a return condenser, though it cannot be used safely in distillation.

of protein by 6.25. The fact that the moisture content of air-dry corn merely depends upon the weather and is just as changeable is deemed sufficient reason for ignoring the percentage composition of the air-dry substance in this study.

COLLECTING SAMPLES OF CORN.—To determine the accuracy of taking samples of corn a bushel or more of shelled corn from each of ten different lots was thoroughly mixed, and then two samples of one pint each were taken for analysis, a single analysis being made of each sample. Following are the results obtained:

	Ash.	Protein.	Fat.	Carbohydrates.		Ash.	Protein.	Fat.	Carbohydrates.
1	1.42	10.07	4.71	83.80	6	1.48	11.04	4.66	82.82
	1.42	10.19	4.73	83.66		1.45	10.81	4.63	83.11
2	1.41	10.85	4.43	83.31	7	1.50	11.33	4.79	82.38
	1.41	10.78	4.40	83.41		1.49	11.43	4.77	82.31
3	1.43	10.72	4.24	83.61	8	1.51	11.35	5.14	82.00
	1.43	10.66	4.25	83.66		1.54	11.42	5.15	81.89
4	1.50	11.40	4.44	82.66	9	1.43	11.11	4.76	82.70
	1.53	11.42	4.49	82.56		1.43	11.09	4.81	82.67
5	1.48	11.24	4.73	82.55	10	1.49	11.09	4.73	82.69
	1 47	11.04	4.73	82.76		1.48	11.02	4.73	82.77

These results show the method of sampling to be satisfactory. The variations between results on duplicate samples are scarcely greater than the experimental error in making duplicate analyses of a single sample[1], although variations among the different lots amount to very much more. This is especially marked in the fat column where, although the average amount determined is less than 5 per cent., there is a difference among the lots of from 4.25 to 5.15 or 0.90 per cent. and between duplicate samples of only 0.05 per cent.

ANALYSES OF ONE VARIETY.[2]—The following ten duplicate analyses were made to determine the possible variation in a single variety of corn which had been grown under conditions as nearly uniform as possible. From each of ten different tenth-acre plots lying in the same field several bushels of corn were taken. The corn was shelled, thoroughly mixed, and a pint sample taken from each lot for anaylsis. Following are the results obtained:

	Ash.	Protein.	Fat.	Carbohydrates.		Ash.	Protein.	Fat.	Carbohydrates.
1	1.39	11.24	4.43	82.94	3	1.33	11.19	4.27	83.21
	1.41	11.17	4.41	83.01		1.36	11.08	4.27	83.29
2	1.42	11.54	4.45	82.59	4	1.49	11.47	4.38	82.66
	1.43	11.50	4.47	82.60		1.50	11.41	4.30	82.79

[1] See the following table.

[2] A variety of white dent corn well known in Illinois as Burr's White. This corn has been grown in large quantities for several years upon the University of Illinois Agricultural Experiment Station fields, and special precautions have been taken to keep it pure and distinct.

	Ash.	Protein.	Fat.	Carbohydrates.
5	1.34	11.26	4.49	82.91
	1.34	11.24	4.47	82.95
6	1.38	11.62	4.44	82.56
	1.42	11.70	4.41	82.47
7	1.41	11.42	4.36	82.81
	1.38	11.33	4.39	82.90
8	1.42	11.49	4.26	82.83
	1.41	11.44	4.30	82.85
9	1.39	11.56	4.47	82.58
	1.39	11.51	4.55	82.55
10	1.42	11.47	4.42	82.69
	1.42	11.45	4.48	82.65

These results show a marked degree of uniformity, seen more clearly from the following maxima and minima of all determinations:

	Ash.	Protein.	Fat.	Carbohydrates.
Maximum	1.50	11.70	4.55	83.29
Minimum	1.33	11.08	4.26	82.47
Difference	0.17	0.62	0.29	0.82

By referring to Flechig's experiment (page 137) it is seen that with thirteen different varieties of corn grown under uniform conditions he obtained results showing the following variations:

	Ash.	Protein.	Fat.	Carbohydrates.
Maximum	1.73	12.63	6.22	84.08
Minimum	1.29	9.00	5.02	80.68
Difference	0.44	4.63	1.20	3.40

ANALYSES OF DIFFERENT EARS.—In order to investigate more fully the question of variation or uniformity in a single variety 50 separate ears of Burr's White corn from the same field as that used in the preceding experiment were carefully selected from a number of bushels which had been especially picked out for seed corn. The 50 ears were all well formed and well matured, and had been grown in a field which had been selected because of its uniform soil conditions. Duplicate analyses were made of the corn from each ear. Following are the results obtained:

	Ash.	Protein.	Fat.	Carbohydrates.
1	1.44	10.79	5.66	82.11
	1.46	10.86	5.65	82.03
2	1.60	12.77	5.19	80.44
	1.60	12.84	5.22	80.34
3	1.32	10.77	4.16	83.75
	1.29	10.76	4.11	83.84
4	1.26	10.49	4.53	83.72
	1.26	10.46	4.54	83.74
5	1.09	9.33	4.35	85.23
	1.10	9.27	4.41	85.22
6	1.34	9.11	4.06	85.49
	1.32	9.13	4.13	85.42
7	1.30	10.41	4.19	84.10
	1.28	10.41	4.15	84.16
8	1.11	8.41	4.86	85.62
	1.10	8.35	4.90	85.65
9	1.41	9.91	4.22	84.46
	1.42	10.00	4.24	84.34
10	1.44	11.46	5.01	82.09
	1.43	11.35	5.02	82.20
11	1.54	12.40	4.61	81.45
	1.56	12.36	4.62	81.46
12	1.39	9.99	4.41	84.21
	1.38	9.96	4.42	84.24
13	1.37	10.12	4.80	83.71
	1.36	10.05	4.85	83.74
14	1.36	10.31	5.24	83.09
	1.36	10.31	5.26	83.07

	Ash.	Protein.	Fat.	Carbohydrates.
15	1.34	9.70	4.01	84.95
	1.33	9.65	4.01	85.01
16	1.45	11.88	4.62	82.05
	1.44	11.86	4.60	82.10
17	1.35	10.79	4.52	83.34
	1.34	10.67	4.54	83.45
18	1.48	13.88	5.71	78.93
	1.50	13.85	5.73	78.92
19	1.43	11.55	4.33	82.69
	1.43	11.52	4.29	82.76
20	1.32	11.63	4.56	82.49
	1.33	11.64	4.57	82.46
21	1.36	11.30	4.15	83.19
	1.37	11.19	4.17	83.27
22	1.35	11.81	4.97	81.87
	1.34	11.91	5.03	81.72
23	1.40	10.22	6.02	82.36
	1.40	10.13	6.02	82.45
24	1.48	11.14	5.11	82.27
	1.46	11.16	5.15	82.23
25	1.61	11.46	5.19	81.74
	1.59	11.38	5.20	81.83
26	1.70	10.03	4.77	83.50
	1.70	10.07	4.76	83.47
27	1.43	10.38	5.22	82.97
	1.46	10.44	5.25	82.85
28	1.55	10.95	4.86	82.64
	1.54	11.06	4.92	82.48
29	1.62	10.82	4.86	82.70
	1.62	10.95	4.89	82.54
30	1.63	11.45	4.56	82.36
	1.62	11.54	4.59	82.25
31	1.45	11.49	4.26	82.80
	1.48	11.48	4.25	82.79
32	1.38	11.78	4.84	82.00
	1.40	11.77	4.82	82.01
33	1.16	9.01	4.04	85.79
	1.17	9 13	4.06	85.64
34	1.50	12.72	4.24	81.54
	1.52	12.86	4.26	81.36
35	1.45	11.83	4 93	81.79
	1.46	11.73	4.93	81.88
36	1.48	12.07	4.60	81.85
	1.50	12.06	4.62	81.82
37	1.58	12.35	4.76	81.31
	1.60	12.44	4.72	81.24
38	1.33	9.38	4.86	84.43
	1.36	9.06	4.82	84.76
39	1.62	10.72	4.69	82.97
	1.60	10.71	4.71	82.98
40	1.54	9.85	4.95	83.66
	1.55	9.95	4.99	83.51
41	1.55	10.69	4.92	82.84
	1.57	10.67	4.90	82.86
42	1.47	12.98	3.98	81.57
	1.45	12.94	3.95	81.66
43	1.47	11.79	4.80	81.94
	1.48	11.81	4.79	81.92
44	1.74	11.91	4.55	81.80
	1.73	11.88	4.54	81.85
45	1.55	10.53	5.50	82.42
	1.54	10.46	5.52	82.48
46	1.60	11.06	4.38	82.96
	1.60	11.13	4.39	82.88
47	1.60	11.85	4.93	81.62
	1.58	11.82	4.98	81.62
48	1.38	10.21	5.47	82.94
	1.40	10.26	5.54	82.80
49	1.42	8.36	4.87	85.35
	1.42	8.43	4.94	85.21
50	1.65	12.28	4.76	81.31
	1.65	12.28	4.75	81.32

It must be admitted that these results are far from being uniform. Indeed, they are quite the opposite, and seem to bring out and clearly to establish the fact that there are extreme variations in the chemical composition of corn grown from the purest seed of a single variety and under markedly uniform field conditions. Then the results given in the experiment preceding this are to be considered merely as averages from a large number of small samples of widely varying composition.

Following are the maxima and minima of all constituents as shown by the 50 duplicate analyses:

	Ash.	Protein.	Fat.	Carbohydrates.
Maximum	1.74	13.88	6.02	85.79
Minimum	1.09	8.35	3.95	78.92
Difference	0.65	5.53	2.07	6.87

With every constituent the variation is greater than Flechig found with 13 different varieties, and it is nearly as great as found by the Connecticut Experiment Station with about 75 different varieties of corn grown under 90 presumably different conditions.

This comparison is facilitated by the following table which gives the number of samples containing the different constituents in amounts above and below certain specified percentages; columns I. and II. give the numbers of such samples[1] from my results and those of the Connecticut Station, respectively:

		Per cent.	I	II.		Per cent.	I.	II.
Ash	above	1.70	1	5	below	1.10	1	9
Protein	"	13.75	1	3	"	9.00	2	4
Fat	"	6.00	1	1	"	4.00	1	2
Carbohyrates	"	85.00	5	3	"	79.00	1	4

It is observed that the number of samples with percentages of ash outside of these extremes is 2 with my results and 14 with the Connecticut experiments. This is in accord with the well known fact that the amount of ash constituents taken up by plants varies largely with the amount of soluble mineral matter in the soil, somewhat regardless of the needs of the plant; and it indicates wide variations in Connecticut soils in this regard, as we should expect to be the case. By reference to page 138 it is seen that the percentages of ash in the 90 samples varied from 0.91 to 2.10.

If we omit the ash, the number of percentages of all constituents which fall outside the limits given above is 11 with my results from 50 samples and 16 with the Connecticut results from 90 samples.

ANALYSES OF PARTS OF THE EAR.—In studying this question 30 duplicate analyses were first made on different parts of ears. Five ears were divided lengthwise into 3 samples each in the following manner: If the ear were 12-rowed, 3 samples of 4 consecutive rows each were made; if 16-rowed, 3 samples of 5 consecutive rows each were made, one row being left, etc., etc.

Duplicate analyses of 15 samples thus prepared from 5 different ears gave the following results. The different ears are distinguished by the letters (a), (b), (c), (d), and (e):

[1]Not single determinations.

	Ash.	Protein.	Fat.	Carbohydrates.
1 (a)	1.42	10.79	4.57	83.22
	1.43	10.75	4.58	83.24
2 (a)	1.48	10.97	4.54	83.01
	1.47	10.94	4.51	83.08
3 (a)	1.50	10.66	4.53	83.31
	1.51	10.72	4.55	83.22
4 (b)	1.51	12.00	4.60	81.89
	1.52	11.98	4.59	81.91
5 (b)	1.49	12.01	4.57	81.93
	1.48	12.05	4.57	81.90
6 (b)	1.48	12.19	4.85	81.48
	1.47	12.08	4.80	81.65
7 (c)	1.37	10.09	5.24	83.30
	1.37	10.10	5.17	83.36
8 (c)	1.31	10.14	5.08	83.47
	1.34	10.18	5.18	83.30
9 (c)	1.36	10.15	5.20	83.29
	1.37	10.20	5.17	83.26
10 (d)	1.39	10.46	4.28	83.87
	1.38	10.46	4.29	83.87
11 (d)	1.43	10.25	4.22	84.10
	1.42	10.27	4.20	84.11
12 (d)	1.43	10.09	4.16	84.32
	1.45	10.06	4.15	84.34
13 (e)	1.34	11.19	4.80	82.67
	1.36	11.20	4.78	82.66
14 (e)	1.30	10.66	4.91	83.13
	1.28	10.62	4.89	83.21
15 (e)	1.36	10.81	4.83	83.00
	1.36	10.92	4.79	82.93

These results indicate uniformity in the composition of the different parts of the ear. The following shows the greatest total variation in the 6 single determinations of each constituent in any one ear; and also the total variation between the different ears:

	Ash.	Protein.	Fat.	Carbohydrates.
In any single ear.....	.09	.58	.28	.55
In five ears	.24	2.13	1.09	2.86

Another lot of five ears was selected and each of these was divided crosswise into 3 samples of approximately equal amounts, which for convenience are designated "tip," "middle," and "butt," the ears being lettered (f), (g), (h), (i), and (j).

The duplicate analyses follow:

	Ash.	Protein.	Fat.	Carbohydrates.
16 (f)	1.58	11.78	5.09	81.55
Tip	1.59	11.76	5.10	81.55
17 (f)	1.58	12.22	5.13	81.07
Middle	1.57	12.26	5.03	81.14
18 (f)	1.56	12.36	5.04	81.04
Butt	1.58	12.42	5.03	80.97
19 (g)	1.49	11.99	4.86	81.66
Tip	1.49	11.97	4.84	81.70
20 (g)	1.51	12.49	4.77	81.23
Middle	1.51	12 49	4.76	81.24
21 (g)	1.50	13.02	4.57	80.91
Butt	1.51	13.10	4.59	80.80
22 (h)	1.37	9.72	3.90	85.01
Tip	1.35	9.67	3.93	85.05
23 (h)	1.37	10.07	3.98	84.58
Middle	1.35	10.08	3.97	84.60
24 (h)	1.51	10.49	4.01	83.99
Butt	1.49	10.46	4.00	84.05
25 (i)	1.47	10.58	4.58	83.37
Tip	1.48	10.61	4.60	83.31
26 (i)	1.45	11.05	4.56	82.96
Middle	1.44	11.03	4.60	82.93
27 (i)	1.47	11.03	4.48	83.02
Butt	1.48	10.96	4.46	83.10
28 (j)	1.77	10.87	4.36	83.00
Tip	1.74	10.78	4.37	83.11
29 (j)	1.65	11.35	4.56	82.44
Middle	1.62	11.31	4.58	82 49
30 (j)	1.71	11.32	4.28	82.69
Butt	1.72	11.28	4.29	82.71

These results are similar to those in the preceding experiment. The following shows the total variation:

	Ash.	Protein.	Fat.	Carbohydrates.
In any single ear.....	.16	1.13	.30	1.06
In five ears.....	.42	3.43	1.23	4.25

It is observed that in every case the tip is lowest in protein and that usually the middle is lower than the butt, the average total difference in the ear being 0.73 per cent. and the widest 1.13 per cent. as shown above[1]. The variation in ash and fat is small and shows no such peculiarity. The carbohydrates, being estimated by difference, appear, of course, as the complement to the sum of the other substances and show in the opposite direction approximately the variation of the most variable determinable constituent.

PARTIAL ANALYSES OF SINGLE KERNELS.—From 1009 separate determinations Richardson[2] has found the average weight of 100 kernels of air-dry corn to be 36.7 gms. Allowing 10 per cent. for moisture, gives 0.330 gms. as the average weight of the dry kernel. This weight is too small for a very exact single determination of a single constituent, and, of course, no attempt has been made to do more than that.

The ash determination was made by incinerating the whole kernel without grinding, the weight of the dry matter having been previously taken after drying the kernel for 8 hours in a current of hydrogen at 105°; and the nitrogen determination was made on the whole kernel after drying and without grinding, the digestion proceeding as satisfactorily as with ground corn. No satisfactory method was found for the determination of the fat in a single kernel.

The ash determinations in 10 single kernels taken from as many different places on an ear gave the following results :

Kernel, weight.	Ash, weight.	Ash, per cent.	Kernel, weight.	Ash, weight.	Ash, per cent.
1.....0.3579	0.0048	1.34	6.....0.3953	0.0053	1.34
2.....0.2947	0.0042	1.43	7.....0.4507	0.0066	1.46
3.....0.3985	0.0052	1.30	8.....0.4589	0.0064	1.39
4.....0.3585	0.0046	1.28	9.....0.4211	0.0062	1.47
5.....0.3936	0.0054	1.37	10.....0.5072	0.0070	1.38

For further work on the ash content several ears of corn were selected, and from each a sample of corn, consisting of a number of rows and believed to fairly represent the ear, was taken and its percentage of ash in the dry matter determined. Then for the special investigation of the ash content of single kernels four ears from the lot were chosen, of which two were high and two were low, comparatively, in the

[1] It will be seen that later work on single kernels tends to confirm and establish this as a characteristic of the ear of corn.

[2] U. S. Dept. of Agr., Div. of Chem Bul. (1884) **4**, 82.

percentage of ash as previously determined. From each ear 10 kernels were selected at approximately equal distances apart throughout the length of the ear, the kernels being numbered from 1 to 10 and the order running from tip to butt. The data from the ash determinations in the single kernels and also the percentage of ash in the large sample from the same ear are given below:

Ear No. 1.—Ash = 1.73 per cent.

Kernel, weight.	Ash, weight.	Ash, per cent.
1.....0.3334	0.0050	1.50
2.....0.3367	0.0053	1.57
3 ....0.3662	0.0059	1.61
4.....0.3901	0.0061	1.56
5.....0.3417	0.0057	1.67
6.....0.3614	0.0061	1.69
7.... 0.3798	0.0065	1.71
8.....0.4030	0.0066	1.64
9.....0.4446	0.0073	1.64
10.....0.4176	0.0071	1.74

Ear No. 2.—Ash = 1.65 per cent.

Kernel, weight.	Ash, weight.	Ash, per cent.
1.....0.2933	0.0048	1.64
2.....0.2797	0.0046	1.64
3.....0.2945	0.0048	1.63
4.....0.2551	0.0042	1.65
5.....0.3207	0.0051	1.59
6.....0.3005	0.0049	1.63
7.....0.3340	0.0056	1.68
8.....0.3144	0.0052	1.65
9.....0.3463	0.0059	1.70
10.....0.3627	0.0058	1.60

Ear No. 3.—Ash = 1.10 per cent.

Kernel, weight.	Ash, weight.	Ash, per cent.
1.....0.2639	0.0029	1.10
2.....0.2591	0.0028	1.08
3.....0.2655	0.0029	1.09
4.....0.2887	0.0031	1.10
5.....0.3077	0.0033	1.07
6.....0.3216	0.0035	1.09
7.....0.3363	0.0036	1.07
8.....0.3476	0.0038	1.10
9.....0.3467	0.0042	1.21
10.....0.4042	0.0045	1.11

Ear No. 4.—Ash = 1.11 per cent.

Kernel, weight.	Ash, weight.	Ash, per cent.
1.....0.3080	0.0035	1.14
2.....0.3499	0.0043	1.23
3.....0.3351	0.0038	1.13
4.....0.3422	0.0040	1.17
5.....0.3970	0.0045	1.13
6.....0.3514	0.0043	1.22
7.....0.3767	0.0047	1.25
8.....0.4186	0.0050	1.19
9.....0.4331	0.0048	1.11
10.....0.4638	0.0051	1.10

These results confirm those of the previous experiments in indicating uniformity in the composition of the ear in all parts, although slight variations are found, of course. It may be noted, however, that the variation from the average percentage is rarely equivalent to more than three-tenths of a milligramme in the weight of the ash.

In the work on the protein content of single kernels, 5 ears, 3 of which were high and two relatively low, in protein were selected from a number of ears in a manner analogous to that described in the previous experiment.

As duplicate determinations were not made with single kernels the complete analytical data of this work are reported.

The water used in making up reagents and standard hydrochloric acid and in the analytical process where needed had been twice distilled, once with sulfuric acid, to free it from ammonia, and once with calcium

hydroxid to remove carbon dioxid and volatile acids. In standardizing the hydrochloric acid and ammonia solutions the same automatic pipette and burette were employed as in the subsequent analyses[1]. The hydrochloric acid was standardized by means of silver nitrate, a method whose details I have previously investigated[2] and found to be exceedingly accurate. Lacmoid indicator was used in standardizing the ammonia, and chemically pure cane sugar was employed in making "blank" determinations to find the "correction" for reagents. Following are these data :

Standardizing hydrochloric acid.
35 cc.[3] HCl gave 1.4103 and 1.4104 gms. AgCl.

Standardizing ammonia.
17.5 cc. HCl required 27.55 and 27.55 cc. NH_3.

Blank determinations with sugar.
17.5 cc. of standard hydrochloric acid were taken and to neutralize the excess of acid required
27.47, 27.45, and 27.47 cc. of standard ammonia solution.

The atomic weights[4] used are : Cl = 35.453; Ag = 107.938; N = 14.041. The factor, 6.25, was used to obtain the protein equivalent.

These data give 194933 as the logarithm (mantissa) for the weight of protein equivalent to one cubic centimeter of standard ammonia.

In the following work 17.5 cc. of standard hydrochloric acid were taken in each determination, and the volume of standard ammonia required to neutralize the excess of acid is given in the tables in cubic centimeters :

Ear No. 1.—Protein = 13.06 per cent.

	Kernel, weight.	Ammonia to neutralize.	Protein, per cent.
1	0.2945	25.12	12.46
2	0.3127	24.96	12.54
3	0.2893	25.16	12.44
4	0.2991	25.07	12.50
5	0.3147	24.99	12.30
6	0.3162	24.94	12.49
7	0.3544	24.63	12.50
8	0.3302	24.90	12.14
9	0.3601	24.67	12.14
10	0.3368	24.73	12.71

Ear No. 2.—Protein = 13.87 per cent.

	Kernel, weight.	Ammonia to neutralize.	Protein, per cent.
1	0.3206	24.97	12.17
2	0.3207	24.81	12.94
3	0.3094	24.99	12.51
4	0.2841	24.97	13.42
5	0.3475	24.55	13.12
6	0.2899	24.76	14.59
7	0.2835	25.07	13.21
8	0.3475	24.48	13.43
9	0.3179	24.79	13.16
10	0.3301	24.50	14.05

[1] If this precaution is observed, if the full measure of acid is always taken, and if the graduation of the automatic ammonia burette is strictly uniform, there is no special necessity for the apparatus to read absolute values.

[2] Methods of Standardizing Reagents.—Master of Science Thesis, Cornell University, 1894.

[3] Twice the volume of the automatic pipette.

[4] Ostwald, Grundriss der allgemeinen Chemie (1890) 31.

Ear No. 3.—Protein = 12.96 per cent.

Kernel, weight.	Ammonia to neutralize.	Protein, per cent.
1.....0.3626	24.79	11.53
2.....0.3039	25.07	12.32
3.....0.3353	24.85	12.19
4.....0.3048	25.02	12.54
5.. ..0.3225	24.96	12.14
6.....0.3013	24.97	12.95
7.....0.2635	25.30	12.84
8.....0.3204	Lost by accident.	
9.....0.3254	24.96	12.04
10.....0.3195	24.86	12.75

Ear No. 4.—Protein = 7.59 per cent.

Kernel. weight.	Ammonia to neutralize.	Protein per cent.
1 ....0.2503	26.27	7.45
2... .0.2432	26.29	7.54
3.....0.2383	26.29	7.69
4.....0.2118	26.45	7.47
5.....0.2752	26.10	7.74
6.....0.2719	25.95	8.70
7 ....0.2758	25.97	8.46
8.....0.2703	25.96	8.69
9.....0.2809	25.87	8.86
10.....0.3133	25.84	8.10

Ear No. 5 —Protein = 8.40 per cent.

Kernel, weight.	Ammonia to neutralize.	Protein per cent.
1.....0.2819	26.07	7.72
2.....0.2682	26.02	8.41
3.....0.2378	26.19	8.37
4.....0.2641	26.06	8.31
5.....0.2891	25.98	8.02
6.....0.3002	25.78	8.76
7.....0.2730	25.91	8.89
8.....0.2830	25.83	9.02
9.....0.2973	25.76	8.96
10.....0.2821	25.86	8.89

The concordant evidence of 30 duplicate analyses of parts of ears, of 50 ash determinations, and of 50 protein determinations in single kernels would seem to warrant the conclusion and to establish the fact that the composition of the ear is approximately uniform throughout.

Extended investigations, based upon the facts brought out in these studies of the proximate composition of corn, are being continued by the writer.

PART II.—THE COMPLETE COMPOSITION OF CORN.

HISTORICAL.

THE ASH OF THE CORN KERNEL.—The earliest analysis on record of the ash of corn is evidently that made by De Saussure[1] reported in 1804. Following are his results:

Potash	14.00
Phosphate of potash	47.50
Chlorid of potash	0.25
Sulfate of potash	0.25
Earthy phosphates	36.00
Silica	1.00
Metallic oxids	0.12
Loss	0.88

[1]Researches Chimiques sur la Vegetation, by Theod. De Saussure (1804) 351; Trans. N. Y. State Agr. Soc. (1848) **8**, 727.

Subsequently Letellier[1] reported the following analysis:

Magnesia	17.00
Lime	1.30
Phosphoric acid	50.10
Silica	0.80
Sulfuric acid	Trace
Potash, soda, and loss	30.80

As the later investigations will show, the analysis of Letellier gives very approximately the true composition of corn ash. Much less approximate are the analyses of Salisbury, of which he reported[2] several similar to the following:

Silica	1.45	2.65
SO_3	0.21	0.13
P_2O_5	50.96	49.31
Iron phosphate	4.35	0.75
Lime	0.15	0.45
Magnesia	16.52	15.49
Potash	8.29	5.19
Soda	10.91	19.18
NaCl	0.25	0.90
Cl	.....	0.10
Organic acids	3.10	3.45
Coal	.....	1.75

Later analyses by Liebig and Kopp[3], Stepf[4], Way and Ogston[5], and Bibra[6] gave the following results:

	Liebig and Kopp.	Stepf.	Way and Ogston.	Bibra.	Bibra.
K_2O	30.74	28.80	28.37	24.33	26.75
Na_2O	.....	3.50	1.74	1.50	3.85
MgO	14.72	14.90	13.60	16.00	15.24
CaO	3.06	6.32	0.57	3.16	2.56
Fe_2O_3	0.84	1.51[7]	0.47	1.88[8]	2.00[8]
P_2O_5	44.50	44.97	53.69	49.36	47.47
SO_3	4.13	.....	Trace.	1.00	1.20
SiO_2	1.78	.....	1.55	2.77	1.93
Cl	0.50	.....	.....	.....	.....

In 1880 Wolff[9] gave the following as the average of 15 analyses of the ash of corn:

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[1] Annalen der Chemie und Pharmacie (1844) **50**, 403.

[2] Trans. N. Y. State Agr. Soc. (1848) **8**, 678.

[3] Jahresbericht über die Fortschritte der Chemie (1856) 815.

[4] Journal für praktische Chemie (1859) **76**, 88.

[5] Liebig's die Chemie in ihre Anwendung auf Agricultur (1865) **1**, 384.

[6] Same reference.

[7] And SO_3 and loss.

[8] And loss.

[9] Wolff's Aschen Analysen (1880); Thorp's Dictionary of Applied Chemistry (1890) **1**, 497.

K_2O.	Na_2O.	MgO.	CaO.	Fe_2O_3.	P_2O_5	SO_3	SiO_2	Cl.
29.8	1.1	15.5	2.2	0.8	45.6	0.8	2.1	0.9

Quite recently Scovell and Peter have reported[1] a somewhat extended investigation of the ash of corn with reference to its content of fertilizing elements. Following are the percentages of potassium oxid and phosphoric oxid in the pure ash as found in 8 samples:

K_2O.	P_2O_5.	K_2O.	P_2O_5.
28.38	48.52	29.66	52.14
28.98	51.85	29.95	53.03
29.41	52.45	29.27	53.10
29.38	52.75	28.18	51.42

It seems evident that as a rule the ash of corn contains at least 95 per cent. of the phosphates of potassium and magnesium, about twice as much potash as magnesia being present.

THE PROTEIDS OF THE CORN KERNEL.—Zein, the most important proteid in corn was discovered and named by Gorham in 1821 (see page 130), although he concluded from his investigations that it was not a nitrogenous body. The zein was obtained by extracting with alcohol the residue of powdered corn insoluble in water, 3.30 per cent. of zein being found. By subsequent extraction of the corn with dilute acid and alkali 2.75 per cent. of what was thought to be albumen were obtained.

Soon after the publication of Gorham's work Bizio[2] reported an investigation of corn in which he claimed to have discovered the alcohol soluble proteid, and, curiously enough, he states that he had named it *zein*, from the Greek word meaning "nourishing substance" because of the fact that it was a nitrogenous body. He points out several differences between his zein and that which Gorham had found, and mentions especially that in 1820 Configliachi[3] had obtained ammonia from zein by dry distillation. By means of ether Bizio extracted oil from zein and then found that the residue was but partially soluble in alcohol. These two portions, the one soluble and the other insoluble in alcohol, he thought to be two different substances and to be identical with the gliadin and zymom which Taddei[4] had found in the gluten of wheat. He gives the alcoholic extract the following composition:

Oil, soluble in ether	20.0 per cent.
Gliadin, soluble in alcohol	43.4 "
Zymom, insoluble in alcohol	36.6 "

[1] Kentucky Agr. Exp. Station Report (1891) 16.
[2] Journal für Chemie und Physik (1823) **37**, 377.
[3] Ibid. (1823) **37**, 383.
[4] Ibid. (1820) **29**, 514.

Salisbury[1] obtained "albumen" from corn by extracting with water and coagulating by heat, and "casein" from the filtrate by precipitating with acetic acid. He extracted zein and oil by means of alcohol and separated them by evaporating the alcohol and extracting the oil with ether.

Evidently because Berzelius[2] in commenting on Gorham's results, had expressed the opinion that the zein of corn and the gluten of wheat were identical, Stepf[3] assumed and stated *incorrectly* that Gorham claimed to have obtained zein by kneading corn meal with water, in the same manner as gluten may be obtained from wheat; and he tried repeatedly but in vain to accomplish such result. By extracting corn with alcohol and purifying the extract by treating it with water and with ether to remove sugar and oil, he states that he obtained pure zein very similar to that obtained by Gorham. It was easily soluble in alcohol, but by repeated solution and evaporation of the alcohol the zein was partially changed into a modification insoluble in alcohol. Stepf called the two modifications plant glue (*Pflanzenleim*) and plant casein, substances already known.

Albumen was also obtained from an aqueous extract of corn by coagulating with heat. The dry matter of corn was found to contain 0.7 per cent. of albumen and 7.5 per cent. of zein. Stepf further states that from four closely agreeing determinations he found pure zein to contain 15.6 per cent. of nitrogen.

In 1869 Ritthausen reported[4] an investigation of the proteids of the corn kernel. Misled by Stepf's erroneous assumption, Ritthausen vainly endeavored to obtain a cohering glutenous mass by kneading corn meal with water.

Zein was obtained to the amount of 5 per cent. by extracting powdered corn with alcohol and (A) by evaporating the alcohol and extracting the residue with ether, or (B) by precipitating the zein in the alcoholic extract by the addition of much ether. Zein was further purified (C) by repeated treatment with alcohol and ether, and (D) by dissolving in 0.1 to 0.15 per cent. potassium hydroxid solution, precipitating with dilute acetic acid, redissolving completely[5] in alcohol, and precipitating with much water.

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[1] Trans. N. Y. State Agr. Soc. (1848) **8**, 727.

[2] Jahresbericht über die Fortschritte der physischen Wissenschaften (1823) **2**, 124.

[3] Journal für praktische Chemie (1859) **76**, 88.

[4] Journal für praktische Chemie (1869) **106**, 471.

[5] Ritthausen points out that this action shows zein to not consist in part of casein, which would have formed an "alkali albuminate" insoluble in alcohol.

Ultimate organic analyses of these four preparations gave the following results:

	A.	B.	C.	D.	Average.
Carbon	54.66	54.71	54.76	54.66	54.69
Hydrogen	7.45	7.50	7.57	7.51	7.51
Nitrogen	15.50	15.53	15.45	15.85	15.58
Sulfur	0.69			0.65	0.69[1]
Oxygen	21.70	22.16[2]	22.22	21.33	21.53

The fact may be noted that these results were not corrected for the ash content of the zein, which it is stated was insignificant; and also the more important fact that the nitrogen determinations of both Stepf and Ritthausen were made by the method of Varrentrap and Will[3] employing the old atomic weights of platinum (197.2) and nitrogen (14). I have recalculated their results using the revised atomic weights (Pt=194.8; N=14.041)[4] and find Stepf's average of four determinations to be 15.84 per cent. nitrogen and the average of Ritthausen's results[5] to be 15.82 per cent. nitrogen, in zein, while preparation (D) alone gives 16.10 per cent. nitrogen.

By repeated solution in alcohol and evaporation of the solvent, Ritthausen obtained zein which was insoluble in alcohol "dilute or strong, warm or cold." He states positively that zein (or *Maisfibrin*, as he prefers to call it) is not a mixture of proteid bodies but a single homogeneous substance.

After the alcoholic extraction of the corn was complete, the residue was extracted with 0.25 per cent. potassium hydroxid solution, and the extracted proteids precipitated by acetic acid. About 0.5 per cent. of substance was thus obtained from corn, which Ritthausen has since referred[6] to as globulin. He gives the following as the composition of the ash-free substance:

Carbon	51.41
Hydrogen	7.19
Nitrogen	17.72
Oxygen and Sulfur	23.68

[1] Sulfur determination in (D) was not considered trustworthy.

[2] Should be 22.26 evidently.

[3] Annalen der Chemie und Pharmacie (1841) **39**, 257.

[4] Ostwald, Grundriss der allgemeinen Chemie (1890) 31.

[5] I have checked this recalculation from the weight of zein employed and of platinum found as reported in Ritthausen's analytical data, and find that he used atomic weights as stated above.

[6] Landwirtschaftliche Versuchs-Stationen (1896) **47**, 391.

In 1877 Weyl[1] pointed out that a 10 per cent. solution of sodium chlorid extracted from the powdered corn kernel a globulin proteid which coagulates at 75°.

The corn proteids soluble in sodium chlorid solution have been very thoroughly investigated by Chittenden and Osborne[2] and the previous work on zein, the alcohol-soluble proteid, was carefully repeated.

With 10 per cent. sodium chlorid solution they extracted from powdered corn about 0.5 per cent. of proteid matter from which they were able to separate at least four different bodies now known[3] as (1) proteose, (2) very soluble globulin, (3) *maysin* (globulin), and (4) *edestin* (globulin). As the salt is removed from the solution by dialysis, the maysin and edestin precipitate, the other bodies remaining in solution. By long continued dialysis a part of the very soluble globulin is precipitated, the remainder (originally thought to be albumen by Chittenden and Osborne) being precipitated by hydrochloric acid. Of the proteose, a part (also first called albumen) was obtained by coagulating with heat, and the remainder was precipitated with alcohol. After redissolving in salt solution the mixture of the two precipitated globulins, maysin was separated from edestin by coagulating with heat, the edestin being finally precipitated as the salt was removed by dialysis. Other methods were also employed to separate these two globulins, based upon the fact that maysin is readily soluble in extremely dilute salt solutions, while edestin requires greater concentration of salt for solution.

The averages of all analyses of each of these four proteids follow:

Proteose.	Very soluble globulin.	Maysin.	Edestin.
Carbon.....51.30	52.84	52.68	51.71
Hydrogen . . 6.71	6.82	7.02	6.85
Nitrogen 16.35	15.38	16.78	18.12
Sulfur..... 2.00	1.37	1.30	0.86
Oxygen.....23.64	23.59	22.22	22.46

The different preparations of proteose and of the very soluble globulin show some wide differences in composition which, it is believed, are "simply due to their alteration by the process made use of" in their separation. It was found "that these soluble bodies are exceedingly prone to change." By the long continued action of water and salt solutions an insoluble modification of variable composition was produced from maysin and the very soluble globulin.

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[1] Zeitschrift für physiologische Chemie (1877) **1**, 84.

[2] American Chemical Journal (1891) **13**, 453, 529; (1892) **14**, 20.

[3] Osborne, Conn. Agr. Exp. Station Report (1896) **20**, 391. To avoid confusion these terms are here used instead of *myosin*, *vitellin*, etc.

Following are the maxima and minima of the several constituents determined in all analyses of proteose, very soluble globulin, and the insoluble modification:

	Proteose.	Very soluble globulin.	Insoluble modification.
Carbon	52.06 to 50.07	53.53 to 52.36	53.95 to 51.97
Hydrogen	6.91 " 6.54	6.90 " 6.74	7.05 " 6.90
Nitrogen	17.28 " 15.78	15.69 " 15.16	16.82 " 15.87
Sulfur	2.37 " 1.62	1.48 " 1.26	1.16 " 1.12

The several analyses of both maysin and edestin agree within narrow limits.

After the extraction with salt-solution was completed, zein, the most abundant proteid in the corn kernel, was obtained by extracting with 75 per cent. alcohol at about 50°, and highly purified by repeated solution in alcohol and precipitation with water, the last traces of oil being removed by final extraction with ether.

By warming with water or very dilute alcohol zein was readily changed into the insoluble modification.

Following is the composition of zein as shown by the averages of several closely agreeing analyses of both the soluble and the insoluble modifications:

	Soluble zein.	Insoluble zein.
Carbon	55.28	55.15
Hydrogen	7.27	7.24
Nitrogen	16.09	16.22
Sulfur	0.59	0 62
Oxygen	20.77	20.77

The statement is made that "corn meal, after thorough extraction with salt solution and warm dilute alcohol, yields little proteid matter to dilute solutions of potassium hydroxid (0.2 per cent.)."

Osborne's more recent investigations[1] have shown this assumption to be very erroneous; and he now estimates such treatment to yield 3.15 per cent. of proteid soluble in 0.2 per cent. potassium hydroxid solution. It is noteworthy that this quantity is seven times the total amount of the several proteids extracted by salt-solution. Analyses of the purified preparation gave the following results:

Carbon	51.26
Hydrogen	6.72
Nitrogen	15.82
Sulfur	0.90
Oxygen	25.30

[1] Conn. Agr. Exp. Station Report (1896) **20**, 391.

The quantities of the different proteids in the corn kernel are estimated as follows :

1. Proteose, soluble in pure water .....0.06 per cent.
2. Very soluble globulin.....0.04 " "
3. Maysin, soluble in extremely dilute salt-solutions.....0.25 " "
4. Edestin, soluble in more concentrated salt-solutions...0.10 " "
5. Zein, soluble in alcohol .....5.00 " "
6. Proteid matter, soluble in dilute alkalies.....3.15 " "
7. Proteid matter[1] insoluble in any of these solvents ....1.03 " "

Osborne has calculated the mean percentage of nitrogen in corn proteids to be 16.057.

In a review of the percentages of nitrogen in the proteids of various vegetable substances, Ritthausen[2] places corn in the class with proteids containing 16.67 per cent. of nitrogen, and uses the factor 6.00 for calculating protein from the percentage of total nitrogen. It is observed, however, that Ritthausen has misquoted his own results on the composition of zein, as will be seen from the following :

	Original[3].	As quoted.
Carbon.....	54.69	54.69
Hydrogen	7.51	7.56
Nitrogen	15.58	16.33
Sulfur.....	0.69	0.69
Oxygen	21.53	21.53

An error of 0.05 appears in the hydrogen and of 0.75 in the nitrogen, and furthermore the total is 100.80, clearly showing that the analysis is misquoted. His analysis of globulin is quoted correctly.

In this connection it is interesting to note that, if we take Ritthausen's determinations of zein (containing 15.58 per cent. of nitrogen) as 5.00 per cent. of the corn, and globulin (containing 17.72 per cent. of nitrogen) as 0.50 per cent. of the corn, and recalculate the nitrogen according to the revised atomic weights of platinum and nitrogen, which show zein to contain 15.82 per cent. and globulin 17.99 per cent. of nitrogen, we then find the mean percentage of nitrogen in the proteids to be 16.02, which is practically identical with Osborne's result, and proves conclusively that with our present knowledge we are to use 6.25 as the factor for estimating protein from the total nitrogen content of corn.

THE CARBOHYDRATES OF CORN.—Gorham and Bizio, to whose work reference has already been made, separated sugar, gum, fiber, and

[1] Nitrogen in residue from 100 parts of corn multiplied by the factor 6.25.

[2] Landwirtschaftliche Versuchs-Stationen (1896) **47**, 391.

[3] Journal für praktische Chemie (1869) **106**, 483.

starch in the carbohydrate group, with the following results, the starch being estimated by difference :

Carbohydrates.	Gorham.	Bizio.
Sugar	1.59	0.90
Gum	1.92	2.29
Fiber	3.30	7.71
Starch	84.60	80.91

In connection with his researches upon the starch content of many vegetable substances including corn, Krocker[1] showed the absence of appreciable amounts of sugar or dextrine in the ripe seeds of cereals. Mitscherlich is quoted as having reached the same conclusion. Krocker's method for determining starch was by hydrolysis and fermentation, the amount of starch being calculated from the weight of carbon dioxid liberated. In modern chemistry the relations are expressed by the following equations, in which the starch first takes up water and is converted into glucose-sugar by the catalytic action of acids:

$$C_6H_{10}O_5+H_2O=C_6H_{12}O_6$$

and then the sugar is decomposed into alcohol and carbon dioxid by yeast,

$$C_6H_{12}O_6=2C_2H_5OH+2CO_2.$$

In case a measurable quantity of hexose-sugars were present it was determined by fermentation previous to the hydrolysis of the starch.

Duplicate determinations on a sample of corn containing 14.96 per cent. of water gave the following results:

Corn taken	3.35	2.98	gms.
Carbon dioxid found	1.02	0.92	"
Starch equivalent	1.877	1.693	"
Starch in dry matter	65.88	66.80	per cent.

Aside from the determination of fiber as commonly made and reported in proximate analyses and Atwater's estimation of sugar (see page 134), nothing further of importance concerning the chemical composition of the carbohydrates of corn is found until 1887, when Archbold[2] gives the following percentages of different carbohydrates in corn, as representing "the average of many samples analyzed in the course of one year's working" in a large starch factory:

Water	11.20	Dry
Starch	54.80	61.71
Cellulose	16.40	18.47
Gum and sugar	2.90	3.27

[1] Annalen der Chemie und Pharmacie (1846) **58**, 212.

[2] Journal Society Chemical Industry (1887) **6**, 84

Archbold's report shows[1] that 55.6 per cent. of starch are actually obtained from corn (dry basis) in the commercial process of starch manufacture, and that several different by-products still contain traces of starch.

In 1889 Washburn[2] reported an investigation of the cane sugar content of corn. By extracting 1400 gms. of ordinary field corn, to which 3 gms. of magnesia had been added to prevent possible inversion of sugar, with 72 per cent. alcohol, shaking the solution with ether to separate fat, and purifying the sucrose in the filtered aqueous layer by repeated precipitation as strontium sucrate and decomposition of the precipitate by carbon dioxid (method of Schultze[3]), 1.105 gms. of pure cane sugar were obtained by crystallization. American sweet corn yielded larger amounts, 10.5 gms. of sugar being obtained from 2000 gms. of corn. Washburn states that all of the sugar in the corn is not obtained by this process.

Marcacci[4] has found over 1 per cent. of sugar in corn.

Pentosans ($C_5H_8O_4$), which are also termed *wood gum* and *hemicellulose*, were found in corn by Stone[5]. These carbohydrate bodies[6] yield pentoses ($C_5H_{10}O_5$), also called *penta-glucoses*, by hydrolysis with dilute acids ($C_5H_8O_4+H_2O=C_5H_{10}O_5$), and furfurol ($C_5H_4O_2$) by distillation with moderately concentrated acids ($C_5H_{10}O_5-3H_2O=C_5H_4O_2$), reactions which serve as a basis for their quantitative determination. Either the pentose is determined by Fehling's method[7] for reducing

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[1] Based upon six years' experience as chemist to a starch factory.

[2] Über den Rohrzucker des Maiskorns, etc, — Inaugural Dissertation zur Erlangung der Doctorwürde,—Göttingen (1889); Journal für Landwirtschaft (1889) **37**, 503.

[3] Landwirtschaftliche Versuchs-Stationen (1887) **34**, 403.

[4] Le Stazioni Speriment, Agrar. Ital. (1889) **17**, 266; Central-Blatt für Agricultur-Chemie (1890) **19**, 352.

[5] American Chemical Journal (1891) **13**, 73.

[6] Two pentosans are well known: Xylan, found quite commonly in grains and grasses; and araban, occurring especially in gums such as arabic, tragacanth, cherry, etc. Xylan and araban have the same empirical molecular formula, but they may be distinguished by the difference in the specific rotation and melting points of the respective pentoses, xylose and arabinose, into which they are converted by hydrolysis. For xylose $[\alpha]_D=18°$ to 19° and M. P.=144° to 145°; while for arabinose $[\alpha]_D=103°$ to 105° and M. P.=154° to 157°. Cf. Koch, Pharmaceutische Zeitschrift für Russland (1886) **25**, 619 and other pages; Berichte der deutschen chemischen Gesellschaft (1887) **20**, III, 145; Bauer, Landwirtschaftliche Versuchs-Stationen (1889) **36**, 304; Stone and Tollens, Annalen der Chemie (1888) **249**, 227; Wheeler and Tollens, ibid. (1889) **254**, 304; Schulze, Zeitschrift für physiologische Chemie (1890) **14**, 227; (1892) **16**, 387; (1894) **19**, 38.

[7] Bauer, Landwirtschaftliche Versuchs-Stationen (1889) **36**, 304; Stone, American Chemical Journal (1891) **13**, 78.

sugars; or the furfurol is determined, preferably by precipitation with phenyl hydrazine as a hydrazone ($C_5H_4ON_2HC_6H_5$)[1].

Stone found corn bran to contain 1.25 to 2.67 per cent. of pentosans.[2] Schulze,[3] after separating considerable other matter from corn bran, obtained a residue which yielded 43.37 per cent. of a pentosan which he showed to be xylan.[4]

In 1896 Stone[5] reported a somewhat extended study of the carbohydrates of corn, in which sucrose, dextrine, starch, pentosans, and fiber were determined quantitatively. The general method employed may be briefly described as follows:

Sucrose.—Finely ground corn meal was extracted with 95 per cent. alcohol which was then evaporated nearly to dryness, the residue taken up with water, treated with hydrochloric acid, the inverted sugar estimated by Fehling's solution and calculated to sucrose.

Dextrine.—The residue of meal was extracted with cold water which was then evaporated to a small volume, the dextrine being precipitated by alcohol, inverted by hydrochloric acid, and estimated by Fehling's solution.

Starch.—A known proportion of the residue of meal was treated with malt extract, the solution hydrolysed and the sugar obtained estimated by Fehling's solution, and calculated to starch.

Pentosans.—The residue from the starch determination was boiled with 1 per cent. hydrochloric acid, the pentose formed estimated by Fehling's solution and calculated to xylan.

Fiber.—The residue still remaining was boiled with 1.25 per cent. sodium hydroxid, and the insoluble matter (less ash) given as fiber.

A sample of corn which contained 80.69 per cent. of total carbohydrates, when estimated "by difference," gave by the above method the following results:

Sucrose	0.27 per cent.
Dextrine	0.32 "
Starch	42.50 "
Pentosans	5.14 "
Fiber	1.99 "
Total carbohydrates	50.22 "

[1] Flint and Tollens, Landwirtschaftliche Versuchs-Stationen (1893) **42**, 381. Cf. Berichte der deutschen chemischen Gesellschaft (1891) **24**, II, 3575; (1892) **25**, II, 2912.

[2] The results were published (American Chemical Journal (1891) **13**, 73) in terms of furfuramid, but are here calculated to pentosan.

[3] Zeitschrift für physiologische Chemie (1894) **19**, 41.

[4] The statement by Stone (U. S. Dept. of Agr., Exp. Station Bul. (1896) **34**, 16) that Tollens and Flint (Berichte der deutschen chemischen Gesellschaft (1892) **25**, II, 2916) had estimated the amount of pentosans in corn bran to be 38.17 per cent. appears to be erroneous, as the work referred to was with corn cobs *(Maiskolben)*.

[5] U. S. Dept. of Agr., Exp. Station Bul. (1896) **34**.

In discussing his results, Dr. Stone says:

"This method not only permits the separation of the more delicate and easily decomposed carbohydrates from those which offer greater resistance to reagents, but from the very beginning of the process any carbohydrate not wholly removed at any particular step would hardly fail of being detected at the next succeeding and more searching reaction. It is considered pertinent to the subject under discussion to call attention to the apparent discrepancy between less than 50 per cent. of carbohydrates found in our most prominent cereal grains by direct and fairly accurate methods of determination and the 70 to 80 per cent. commonly ascribed to them by the indirect method of estimating 'by difference.' From 20 to 30 per cent. of the grain or flour is not accounted for. Under the conditions this matter cannot be conceived of as possessing a similar nature to the sugars, starches, or even the more easily soluble forms of gum or celluloses."

When we remember that Krocker had shown (see page 165) by a direct and positive method that corn contains over 65 per cent. of fermentable[1] carbohydrates (at least almost entirely starch), and that Archbold, from long experience in the manufacture of corn-starch, reports over 60 per cent. of starch present in corn and at least 55 per cent. actually recovered in the commercial process (see page 166), the previously existing evidence of an error in Stone's results is apparent. Dr. Stone has subsequently discovered and reported[2] a large error in the starch determination, due to the use of too dilute hydrochloric acid and consequent imperfect hydrolysis. The percentage of starch is now given as 65.45 instead of 42.50 as first reported. The total carbohydrates thus found by determination become 73.17 per cent. as compared with 80.69 per cent. estimated by difference. Dr. Stone concludes that:

"This discrepancy may arise from one of two sources, viz.: 1. Error in the determination of the carbohydrates. 2. The existence of a substance which is free of nitrogen and is of a character not usually ascribed to carbohydrates and resistant to the ordinary reactions for such. While the first alternative is not excluded, the writer is inclined to the latter conclusion and expects to continue the investigation along this line."

In a recent report of extended investigations of methods for the estimation of starch, Wiley and Krug[3] refer to their experiments with the conversion of starch into maltose and dextrine by the use of malt extract, as follows:

"The residues from the diastase digestion were all thoroughly washed with hot water and then examined with iodine under the microscope. In every case a large number of cells was found which contained undigested starch, showing that the sample[4] had not been ground to a sufficient degree of fineness. This is, therefore,

[1] The pentosans are classed as strictly non-fermentable carbohydrates. Cf. Koch, Pharmaceutische Zeitschrift für Russland (1886) **25**; Stone and Tollens, Annalen der Chemie (1888) **249**, 257; Stone, American Chemical Journal (1891) **13**, 82.

[2] Journal American Chemical Society (1897) **19**, 183, 347.

[3] Ibid. (1898) **20**, 255.

[4] A sample of wheat previously analyzed by Stone.

another source of error in Professor Stone's work. The sample was then reground and the starch determined. . . . . The residues were again examined and in every case found free from starch, showing that the conversion had been complete. . . . . The number for starch thus obtained, added to our per cents. of the other constituents gives us a total of 99.28."

In summarizing their results Wiley and Krug express the following opinion:

"The small quantity of matter unaccounted for in the cereal grains is doubtless of a carbohydrate nature, belonging to that complex class, pentosan-ligno-celluloses, whose chemical and physical properties are so nearly alike as to make their exact separation and determination extremely difficult. The quantity of these undetermined bodies in cereal grains is very minute."

THE OIL OF CORN.—The presence of oil in the corn kernel was discovered by Bizio[1] in 1823. A partial analysis by Hoppe-Seyler[2] gave the following as the percentage composition[3] of the oil:

Cholesterol	2.65
Protogon	3.95
Saponifiable fats etc	93.40

The statement is made that the oil contains stearin, palmitin, and much olein, and the melting point of the fatty acids is given as 51° to 54° F. [11°] to 12° C.].

Some of the so called physical and chemical "constants," which have been determined by several investigators are given below:

	Specific gravity of oil. (at 15°C.)	Unsaponifiable substance. (per cent.)	Iodin absorption. (per cent.)
Spüller[4]		1.35	119.7
Smith[5]	0.9244	....	122.9
Hart[6]	0.9239	1.55	117.0
Rokitianski[7]	0.8360	....	75.8

The oil used by Spüller was the ordinary ether extract. Rokitianski used a petroleum ether extract. Hart worked with a "dark brown" sample presumably found on the market. Smith's material was obtained on the market, but was of a "bright golden color" and was probably a fair sample of corn oil.

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[1] Journal für Chemie und Physik (1823) **37**, 377.

[2] Medicinische-Chemische Untersuchungen, **1**, 162; Bulletin Société Chimique de Paris (1866) [2] **6**, 342; Jahresbericht über die Fortschritte der Chemie (1866) 698.

[3] I have not been able to see Hoppe-Seyler's original paper. Presumably the protogon is the substance now termed lecithin, and the methods employed in estimating it and cholesterol were similar to those which are discussed herein.

[4] Polytechnisches Journal (Dingler) (1887) **264**, 626.

[5] Journal Society Chemical Industry (1892) **11**, 504.

[6] Ibid. (1894) **13**, 257, from Chem. Zeit. **17**, 1522.

[7] Inaugural Dissertation, St. Petersburg (1894); Pharmaceutische Zeitschrift für Russland (1894) **33**, 712; Chemisches Central-Blatt (1895) [4] **7**, I, 22.

Spüller observed that the oil absorbed no oxygen from the air even after fourteen days' exposure. Smith states that the freezing point of the oil is below —20°. Hart gives the melting point of the fatty acids as 25°. Rokitianski reports further qualitative chemical work which showed the oil to contain oleic and linolic acids. It is evident from the specific gravity and the iodin absorption that the material with which he worked was not ordinary corn oil.

Willey and Bigelow[1] have recently found the heat of combustion of oil of corn to be 9280 calories per gramme.

EXPERIMENTAL.

In a preliminary study a small amount of oil was obtained by collecting the ether extract from a large number of proximate analyses of corn. In this, advantage was taken of the fact that the oil is moderately soluble in alcohol when hot and but slightly so at ordinary temperatures.[2]

The oil was transferred from the small flasks, used in its extraction, by means of hot alcohol to a single vessel. On cooling the oil precipitated and settled to the bottom, the alcohol being each time decanted from the collected oil and used in transferring the next lot. Finally the alcohol was evaporated and the oil dried to constant weight in a water oven. When freshly obtained from white dent corn the oil is nearly colorless, but on standing a pale yellow and finally a deep golden color develops, plainly indicating a gradual change in its condition, presumably due to absorption of oxygen. This was confirmed by determining the iodin absorption which was found to be 115.5 per cent.

A large quantity of corn oil, including samples from four different sources[3], was then secured in order to make a more thorough investigation. The oil is obtained as a by-product in the manufacture of corn-starch and glucose-sugar, and all of the samples secured were of a pale straw color and evidently fresh and pure.

Specific Gravity.—Three of these samples of corn oil were sufficient in quantity to enable me to make determinations of their specific gravity by means of a delicate Westphal balance which by trial gave the specific gravity of pure water at 15° as 1.0000. The samples of oil gave the following results:

	1.	2.	3.
Specific gravity.... (15°)	0.9245	0.9262	0.9258

[1] Journal American Chemical Society (1898) **20**, 309

[2] Smith has found the solubility of corn oil in alcohol by volume to be 2 per cent. at 16° and 13 per cent. at 63°.

[3] Samples of corn oil were very kindly furnished me by President Wm. F. Piel, Jr., of The National Starch Manufacturing Company, New York City; by The Chas. Pope Glucose Company, Geneva, Ill.; by The Glucose Sugar Refining Company, Chicago; and by Messrs. Elbert and Gardner, New York City.

Melting Point.—Preliminary experiments confirmed the observation of Smith that the oil is still fluid at —20°, a temperature of —23° (obtained with snow and concentrated sulfuric acid) failing to solidify the oil. It was found, however, that the oil became hard and solid at about —36°.

The melting point was determined by a modification of the method of the Association of Official Agricultural Chemists[1].

In a tall beaker of about 2.5 liters capacity was placed a small quantity of concentrated sulfuric acid (to absorb water vapor so that the apparatus would remain transparent at low temperatures). A second beaker of about 2 liters capacity was placed in the first, being supported by the rim without touching the bottom. A 1-liter beaker taller than the second was placed in the latter and filled with alcohol, the space between the two being filled with solid carbon dioxid. A glass tube 30 mm. in diameter and closed at the bottom was fitted into the inner beaker with a large cork, the tube being about one-third filled with a mixture of 1 volume of concentrated sulfuric acid and 3 volumes of absolute alcohol, and then nearly completely filled with absolute alcohol. The temperature of the alcohol in the beaker was kept uniform throughout by constant stirring with a wire which passed through the cork and terminated in a ring surrounding the glass tube. A heavy glass spoon and a glass spatula were placed in the alcohol.

When the temperature reached —50°, the spoon was removed and a drop of the oil at once let fall upon it. A thin, solid, white, opaque disc formed and was quickly made to drop into the inner tube by using the glass spatula. The disc of solidified oil settled through the absolute alcohol to the denser liquid below and there remained in suspension.

The beaker which had contained carbon dioxid was replaced by another and the temperature allowed to slowly rise. An alcohol thermometer was used for reading the temperatures below the freezing point of mercury. Above —38° a delicate mercury thermometer was employed.

As the temperature rose the disc remained unchanged until at —19° it began to lose its opacity. At —14° it had become perfectly transparent, but no change in shape could be detected below —7°. The disc was much contracted and thickened at —5° and became entirely symmetrical in form at —2.3°. A second determination gave practically the same results, the final reading being —2.4°. The change in temperature (when near the melting point) required 5 to 6 minutes for one degree.

To determine the change in the consistency of the oil, a thin-wall tube of 8 mm. diameter, closed at the bottom, and containing 1 cm. of

[1] U. S. Dept. of Agr., Div. of Chem. Bul. (1895) **46**, 34.

the oil, was placed in alcohol at —45°. After the oil had become solid a glass rod 20 cm. long and 2 mm. thick (the lower end being widened to 5 mm. diameter) was placed in the tube so that its weight was entirely supported by the solidified oil. At —13° the oil had become transparent but still supported the rod. At —10° the rod began to settle appreciably and at —9° it had passed through the centimeter of oil to the bottom, although a disc of oil suspended beside the tube in the same liquid had not changed appreciably in shape. The change of temperature from —10° to —9° required 5 minutes.

Iodin Absorption.—The method of Hübl[1] was employed for this determination, except for certain details of the process.

Standard sodium thiosulfate solution was prepared by dissolving 47.2 gms. of the crystallized salt ($Na_2S_2O_3\,5H_2O$) in water and diluting to 2-liters. From theory 1 cc. of this solution should be equivalent to 12.06 mgs. of iodin if the salt were pure[2]. The solution was standardized with resublimed iodin with the following results:

Iodin taken	0.5160	0.5574	gms.
Thiosulfate solution required	42.9	46.4	cc.
Iodin equivalent to 1 cc	12.03	12.01	mgs.

The average of these results, 12.02, was used in the following work:

The iodin solution, containing 50 gms. iodin and 60 gms. mercuric chlorid in 2 liters of alcohol, was standardized whenever used.

Little pipettes of about 0.5 cc. capacity were placed in 5 cc. vials nearly filled with the corn oil, the bulb of the pipette being immersed, and the whole weighed. The measure of oil was then transferred to a 500 cc. glass stoppered bottle, the pipette returned to the vial, and the exact weight of oil taken determined by difference. The duplicate is taken immediately and necessitates only one more weighing. 10 cc. of chloroform and 40 cc. of iodin solution were added to the oil. After 2 hours 25 cc. of 10 per cent. potassium iodid solution and about 125 cc. of water were added and the excess of iodin determined by titrating with the sodium thiosulfate solution, starch indicator being added near the close of the reaction.

Duplicate determinations of four different samples of oil from as many different sources gave the following results:

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[1] Journal Society Chemical Industry (1884) **3**, 641.

[2] Sutton's Volumetric Analysis, (1890) 115, states that standard sodium thiosulfate solution may be made by simply dissolving an exact weight of the crystallized salt, $Na_2S_2O_3\,5H_2O$, in water and diluting to a definite volume.

	Oil taken. gms.	Iodin absorbed. gms.	Iodin absorbed. per cent.
1	0.3473	0.4255	122.5
	0.3844	0.4729	123.0
2	0.4251	0.5179	121.8
	0.4714	0.5729	121.5
3	0.4281	0.5212	121.7
	0.4742	0.5772	121.7
4	0.4326	0.5324	123.1
	0.5168	0.6351	122.9

Oxygen Absorption.—In order to afford a large surface for the absorption of oxygen, the oil was placed in a low crystallizing dish of 75 mm. diameter. This was allowed to stand at the room temperature, the weight of the oil being determined from time to time as follows:

Weight of oil taken	2.1732 gms.
Weight after 1 day	2.1722 "
" " 7 days	2.1718 "
" " 11 "	2.1718 "
" " 12 "	2.1718 "

These results confirm those of Spüller, showing that the oil does not take up oxygen under these conditions.

The dish was then placed in a water oven and the following data[1] obtained:

Weight after 1 hour	2.1726 gms.
" " 1 day	2.1996 "
" " 2 days	2.2488 "
" " 3 "	2.2590 "
" " 4 "	2.2588 "
" " 5 "	2.2558 "
" " 6 "	2.2513 "
" " 7 "	2.2448 "

The first action of air upon the hot oil is evidently the direct addition of oxygen; but after 2 or 3 days the oil began to turn noticeably darker in color and finally to lose weight, evidently due to a secondary reaction which effects some decomposition of the oil with formation of volatile products.

Lecithin[2].—A weighed quantity of oil was mixed with potassium nitrate and sodium carbonate in a platinum dish and ignited until the carbon was completely burned. The fused mass was dissolved in dilute

[1] These results emphasize the importance of avoiding the presence of oxygen in drying corn or corn oil in analytical work.

[2] Lecithin is commonly regarded as a compound of the base, neurine, with distearyl-glycero-phosphoric acid, although one or both of the stearic acid radicals may be replaced by radicals of palmitic or oleic acid, and the neurine (trimethylhydroxyethyl ammonium hydroxid) is sometimes replaced by another base; e. g., betaine.

hydrochloric acid, and the total phosphoric acid determined[1]. The amount of lecithin was calculated by multiplying the weight of magnesium pyrophosphate obtained by the factor 7.25[2]. Duplicate determinations gave the following results:

Oil taken	10.728	6.435	gms.
KNO_3 used[3]	10.0	35.0	"
$Mg_2P_2O_7$ obtained	0.0221	0.0132	"
Lecithin	0.1602	0.0957	"
Lecithin in oil[4]	1.49	1.49	per cent.

Cholesterol[5].—To determine cholesterol[6] about 50 gms. of the oil were saponified on the water bath with 20 gms. of potassium hydroxid and 100 cc. of 70 per cent. alcohol. The soap was transferred to a large separatory funnel with 200 cc. of water and shaken first with 500 cc. of ether and then 3 times with 250 cc. of ether. The four portions of separated ether were combined, and the ether distilled, the residue being resaponified with 2 gms. of potassium hydroxid and 10 cc. of 70 per cent. alcohol. The solution was then transferred to a small separatory funnel with 20 cc. of water and shaken with 100 cc. of ether. After separating the aqueous layer the ether solution was washed four times with 10 cc. of water, the ether solution being finally transferred to a weighed flask, the ether distilled and the weight of the dry residue (cholesterol) determined. Three determinations gave the following results:

Oil taken	50.16	53.50	54.24	gms.
Cholesterol obtained	0.7002	0.7114	0.7512	"
Cholesterol in oil	1.40	1.33	1.38	per cent[7]

The cholesterol was recrystallized from absolute alcohol in characteristic glistening plates, melting at 137° to 137.5°. It also gave the characteristic color reactions[8] for cholesterol: 1, when shaken with chloroform and sulfuric acid; 2, when evaporated to dryness with nitric acid; 3, when warmed with hydrochloric acid and ferric chlorid.

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[1] Cf. Hoppe-Seyler, Jahresbericht uber die Fortschritte der Chemie (1866) 744; Schulze and Frankfurt, Landwirtschaftliche Versuchs-Stationen (1893) **43**, 207.

[2] 7.25 parts of lecithin ($C_{44}H_{90}O_9PN$) yield 1 part of $Mg_2P_2O_7$.

[3] The proportions of KNO_8 used were purposely varied, but the results indicate that the smaller proportion was sufficient.

[4] By extracting corn with ether and alcohol, successively, Schulze and Frankfurt (reference above) have obtained amounts of phosphoric acid equivalent to 0.25 to 0.28 per cent. of lecithin in the corn.

[5] A monatomic alcohol, $C_{26}H_{43}OH$.

[6] Cf. Bömer, Zeitschrift für Untersuchung der Nahrungs- und Genussmittel (1898) 21, for recent work on the details of this method.

[7] Spüller had obtained 1.35 per cent. and Hart 1.55 per cent. of unsaponifiable matter.

[8] Watt's Dictionary (1889) **2**, 147.

Total Fatty Acids.—After removing the cholesterol from about 50 gms. of oil the remaining soap solution (about 500 cc.) was acidified with hydrochloric acid and shaken in a separatory funnel. An ethereal layer of about 150 cc. at once separated. After adding 100 cc. more ether and thoroughly shaking, the aqueous layer was drawn off, the ether solution of the fatty acids was washed with several portions of water and then transferred to a weighed flask, the ether distilled off, a few cubic centimeters of absolute alcohol dissolved in the residue and evaporated to remove traces of water, and the weight of the total fatty acids determined:

Oil taken	50.160	gms.
Fatty acids obtained	46.935	"
Fatty acids in oil	93.57	per cent.

The fatty acids form a solid mass at 15°, but melt nearly completely at one or two degrees above, the last particles of solid disappearing at 23°. Prepared as described the fatty acids absorbed only 126.4 per cent. of iodin instead of 130.7 per cent. as calculated from the iodin absorption of the oil. This indicates that oxygen had been absorbed by the acids during the process of separation. It was found that oxygen is slowly absorbed by the fatty acids while standing in a desiccator at the ordinary temperature. At 100° the absorption is much more rapid although, as with the oil, secondary reactions soon begin at the higher temperature. The change in weight was found to be as follows:

Time, in days.	Weight of fatty acids, gms., in desiccator.	in water oven.
0	1.9685	2.2740
1	1.9692	2.3106
2	1.9717	2.3366
3	1.9777	2.3366
4	1.9847	2.3282
8	2.0231	
12	2.0665	
16	2.0911	
22	2.1157	
28	2.1293	
34	2.1297	

All action apparently ceased after about one month's time. A considerable portion of the fatty acids had separated in the solid form and of a pure white color, while the other portion remained a colorless, oily liquid.

It is of interest to note the apparent relation between the iodin absorption and the oxygen absorption by the fatty acids. As already shown the fatty acids as prepared absorbed 126.4 per cent. of iodin. If an equivalent amount of the bivalent oxygen may be absorbed instead

of the univalent iodin, then 8.0 per cent. of oxygen should be taken up. The results show that 1.9685 gms. of the fatty acids absorbed 0.1612 gms. of oxygen, an amount equal to 8.2 per cent.

Time would not permit the preparation of the fatty acids in a manner which would prevent the absorption of oxygen during the process, and then a repetition of the quantitative determination of the absorption. This is especially desirable in order to confirm the results as given above, and the writer expects to investigate this point more fully in the near future.

Volatile Acids.—About 5 gms. of oil were saponified in a 500 cc. flask with 2 gms. of potassium hydroxid and 40 cc. of 80 per cent. alcohol. After evaporating the last of the alcohol, 100 cc. of recently boiled water were added, the soap solution acidified with 40 cc. of dilute sulfuric acid (1 to 10), a few pieces of freshly ignited pomace stone added, the flask connected with a condenser by means of a safety bulb tube, and 110 cc. of distillate collected. After mixing, 100 cc. were passed through a dry filter and titrated with one-twenty-fifth normal barium hydroxid solution.

Four determinations gave the following results:

Oil taken.....	4.506	5.894	5.671	5.718 gms.
N/25 $Ba(OH)_2$ required.	1.3	1.5	1.4	1.3 cc.

As two blank determinations required 1.3 and 1.5 cc., respectively, of the barium hydroxid solution it is evident that the oil contains no volatile acids.[1]

Separation and Determination of Fatty Acids.—It has been found especially by Hazura[2] and his associates that the oxidation of unsaturated fatty acids by alkaline potassium permanganate serves as a basis for the approximate separation of several fatty acids. Under proper conditions the oxidation is chiefly confined to the direct addition of the hydroxyl group (OH) wherever "free valences" exist. The following shows the relations among several acids in the series containing eighteen atoms of carbon in the molecule[3]:

Unsaturated Acids.	Saturated Acids.
	Stearic, $C_{18}H_{36}O_2$.
Oleic, $C_{18}H_{34}O_2$, oxidizes to....	dihydroxy stearic, $C_{18}H_{34}(OH)_2O_2$.
Linolic, $C_{18}H_{32}O_2$, oxidizes to..	tetrahydroxy stearic, $C_{18}H_{32}(OH)_4O_2$.
Linolenic, $C_{18}H_{30}O_2$, oxidizes to.	hexahydroxy stearic, $C_{18}H_{30}(OH)_6O_2$.

[1] Spüller gives Reichert's number for the volatile acids as 0.33; Smith states that the oil examined by him contained volatile acids equivalent to 0.56 per cent. of KOH; and Morse (New Hampshire Experiment Station Bulletin (1892) **16**, 19) gives volatile acids as 3.2 per cent. in a sample of corn oil which absorbed 112.8 per cent. of iodin.

[2] Monatshefte für Chemie (1886) to (1889), Vols. **7** to **10**.

[3] Cf. Hazura, ibid. (1887) **8**, 269.

After removing the cholesterol from 53.5 gms. of oil, the combined soap solution was heated till the dissolved ether was distilled, cooled, and diluted to 2 liters. Two liters of a 1.5 per cent. potassium permanganate solution were then gradually added with constant stirring. After 10 minutes the precipitated manganese hydroxid was filtered off, and the clear filtrate acidified with hydrochloric acid. The precipitate thus formed was filtered off, washed, air-dried, and then extracted with ether. The residue insoluble in ether weighed, after drying, 18 gms. It was extracted with boiling water until but 2 gms. remained, which when again extracted with ether, left a residue of only 0.6 gm. and soluble in boiling water.

The substance dissolved in hot water was practically completely precipitated as the solution cooled[1] and proved to be sativic acid (tetrahydroxy stearic acid), as is indicated by the method of formation and by its solubility in hot water. The melting point[2] of the dried substance was 157°–159°.

The quantitative synthesis of the potassium salt was effected by dissolving a weighed amount of the acid in warm alcohol and titrating with standard alcoholic potassium hydroxid solution:

Sativic acid taken.	Potassium hydroxid required.	Per cent. potassium in product.[3]	Per cent. potassium (theory) [4]
1.000	0.1604	10.08	10.14

The ether solutions obtained as described above were combined and the ether distilled. The residue was solid at the room temperature, melted gradually as the temperature rose from 40° to 60°, and was found to absorb 79.2 per cent. of iodin, thus showing very incomplete oxidation of the unsaturated acids.

A second lot of corn oil (54.24 gms.) was oxidized by alkaline permanganate, the cholesterol and then the dissolved ether having been previously removed. The soap was diluted to 2 liters and cooled to 0° by ice kept in the solution. A solution of potassium permanganate containing 80 gms in 2 liters of water was slowly added with constant stirring. After 30 minutes precipitated matter was filtered off and washed; the clear filtrate was acidified with 150 cc. of concentrated hydrochloric acid; the precipitated acids were filtered off, dried, and extracted with ether. The residue insoluble in ether (17.7 gms.) was

[1] 2000 cc of the filtrate from the precipitated sativic acid required only 0.5 cc. of N/5 KOH to show alkalinity with phenol phthalein.

[2] Bauer and Hazura, Monatshefte für Chemie (1886) **7**, 225, give 160° as the melting point of several samples of sativic acid, prepared in a manner similar to the above.

[3] Calculated weight $= 1.000 + 0.1604 \frac{39.14 - 1.008}{56.148}$

[4] For $C_{18}H_{31}(OH)_4\ O_2K$.

dissolved in boiling 95 per cent. alcohol. On cooling, the sativic acid separated in the crystalline form, melting at 161°–163°.

By distilling the ether from the solution obtained as above described, a brown residue (9.5 gms.) was obtained which melted at 55° to 60° and showed an iodin absorption of only 9.2 per cent.

The aqueous acid solution from which the insoluble organic acids had been precipitated by hydrochloric acid was evaporated nearly to dryness, a black tarry mass gradually separating, showing that, although a small amount of unsaturated acids had been unacted upon, the oxidation had gone far beyond the simple addition of hydroxyl groups to the unsaturated compounds.

To further investigate the fatty acids, a method essentially that of Muter[1] was tried for their separation and determination. It is based upon the fact that the lead salts of the unsaturated acids, oleic, linolic, etc., are soluble in ether; while the lead salts of the saturated acids, stearic, palmitic, etc., are not.

About 1.5 gms. of the oil were saponified with alcoholic potash and the soap dissolved in water, the unsaponifiable substance (cholesterol) being separated from the soap solution by shaking with ether. The solution was then neutralized with acetic acid, and the fatty acids precipitated with lead acetate, a slight excess being added. The lead salts were washed with water, and then transferred with 50 cc. of ether to a glass cylinder of about 60 cc. capacity, which was stoppered and then violently shaken for 5 to 10 minutes. The small quantity of matter insoluble in ether was then allowed to settle. A stopper carrying two glass tubes similar to those used in the ordinary washing bottle was placed in the cylinder, the long tube reaching nearly to the undissolved sediment. By blowing in the short tube the clear solution is transferred almost completely without disturbing the sediment. The undissolved substance was then shaken with more ether, allowed to settle, and the ether transferred as before as completely as possible. This treatment was twice more repeated. The undissolved lead salt was then warmed with about 25 cc. of dilute hydrochloric acid, till the fatty acids separated; and, after cooling sufficiently the whole was transferred to a 250 cc. graduated bulb tube, ether being used to complete the transfer. The portion of the tube below the bulb contained 50 cc. and was graduated to 0.2 cc. A small glass tube carrying a stopcock was sealed in just below the 50 cc. mark. The tube was filled to the 250 cc. mark (above the bulb) with ether, and thoroughly shaken. The aqueous layer, containing the excess of hydrochloric acid and the precipitated lead chlorid was allowed to separate.

The volume of ether solution was observed, and 200 cc. of it were

[1] Analyst (1877) **2**, 73.

drawn off into a weighed flask, evaporated to dryness, and the weight of the residue determined.

Duplicate determinations gave the following:

Oil taken	1.600	1.610	gms.
Volume of ether solution	222.4	221.0	cc.
Ether solution taken	200.0	200.0	cc.
Saturated acids obtained	0.0670	0.0648	gms.
Saturated acids in oil	4.66	4.44	per cent.

The residue of saturated acids formed a white solid mass. It was dissolved in hot alcohol and allowed to crystallize. The melting point was 57°. The quantity of the saturated acids thus obtained was considered too small for further satisfactory examination (see foot note below).

Before the lead salts of the saturated acids were completely washed by decantation[1] the clear ether solution of the lead salts of the unsaturated acids absorbed oxygen, and became cloudy, a white precipitate forming in considerable amount. Two samples of the atmosphere in the cylinders above the solutions were drawn off in gas burettes; and, after removing the ether vapor, the residual air was found to contain only 15.3 per cent. and 13.9 per cent., respectively, of oxygen instead of 20.8 per cent. as found in the air of the laboratory.

By subtracting the percentage (4.55) of saturated acids found in the oil from that of the total fatty acids (93.57) the amount of total unsaturated acids is found to be 89.02 per cent., consisting of oleic and linolic acids. (The melting point of the sativic acid obtained and the composition of its potassium salt prove the absence of linusic acid in the products of oxidation, and, hence, of linolenic acid in the total fatty acids.)

From the iodin absorption, the amounts of oleic and linolic acids can be accurately determined. Thus:

Oleic acid, $C_{18}H_{34}O_2 + I_2 = C_{18}H_{34}I_2O_2$, diiodo stearic acid.
Linolic acid, $C_{18}H_{32}O_2 + 2I_2 = C_{18}H_{32}I_4O_2$, tetraiodo stearic acid.

As 89.02 gms. of these unsaturated acids in the ratio in which they exist in corn oil absorb 122.3 gms. of iodin, the following equation can be stated, x being the number of gms. of oleic acid:

$$x\,\frac{254}{282} + (89.02 - x)\,\frac{508}{280} = 122.3$$

[1] At least two days' time is required for this process, and even this was found more satisfactory than filtration. I have no doubt that, if centrifugal force were substituted for gravity, the washing by decantation could be done much better and so quickly that the unsaturated acids could also be determined before the absorption of any appreciable amount of oxygen. Quantities of the separated materials sufficient for further examination could doubtless be obtained in a short time. No suitable centrifuge was at hand for this work.

The oleic acid is found to be 42.92 gms. and the linolic acid 46.10 gms.

By subtracting from the amount of saturated acids the equivalent of the stearic acid contained in the lecithin, and calculating to the respective glycerol esters the remaining saturated acids (as stearic acid), the oleic acid, and the linolic acid, the following summary is obtained as the composition of the oil of corn:

Cholesterol	1.37	per cent.
Lecithin	1.49	" "
Stearin (?)	3.66	" "
Olein	44.85	" "
Linolin	48.19	" "
Total	99.56	

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