

the large scale by the municipal authorities, but I can not think that the Pasteur filter should be swept aside like the worthless contrivance the paper calls it. My experience is that, with proper care, it is efficient. Extended tests were made with it for the Connecticut Board of Health in 1892, which show that it may be depended upon, if the porcelain cylinder be cleaned and sterilized once a week.

Freundenreich has obtained similar results, and has also shown that the length of time during which the filter is efficient depends upon the temperature.

RENSSELAER POLYTECHNIC INSTITUTE,
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THE PROTEIDS OF COTTONSEED.¹

BY THOMAS B. OSBORNE AND CLARK G. VOORHEES.

THE only reference to the proteids of cottonseed which we can find was made by Ritthausen in 1881 (*J. prakt. Chem.*, **23**, 485), who stated that he had been unable to obtain crystals of proteid matter from this seed and also that he would soon publish his complete investigations of the proteid bodies of this as well as of several other seeds which he named. Papers on the proteids of the other seeds mentioned by him were subsequently published, but we have not found anything relating to those of cottonseed. Since so long a time has elapsed, we feel warranted in assuming that Ritthausen has abandoned his intention of reporting the results of his investigation. The importance which cottonseed-meal has assumed as a cattle-food of late years, makes it desirable to understand its chemical composition, especially as regards the nitrogen compounds which it contains so abundantly. Our results are not as satisfactory as we hoped for when we undertook this work but we have decided to publish them as they stand and shall endeavor to make them more complete in the future. The difficulties encountered are due to the presence of substances which render filtration of the extracts extremely slow and to the large amount of coloring matters taken up from the seed together with the proteids, which could be separated only with difficulty and large loss of substance. The material used in our investigation consisted partly of seed

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from which the cotton had been removed, but which otherwise was in the condition in which it was harvested. We separated the kernels from these seeds by chopping them in a wooden bowl and sifting out the larger fragments of husk. We thus succeeded in obtaining meal nearly free from hulls. This meal was bruised in a mortar and freed from most of the oil by extraction with benzine and from benzine by drying in the air at the temperature of the room. Samples of commercial cottonseed-meal from which the oil had been expressed by the usual process were also used and gave the same result when extracted, and therefore in the following account of our experiments, no special mention is made of the origin of the meal employed.

a. Extraction with Water.—100 grams of cottonseed-meal were extracted with three liters of distilled water and the filtered extract saturated with ammonium sulphate. The small precipitate so produced was filtered off, dissolved in water, the solution filtered clear and placed in a dialyzer. After remaining a week in running water, no precipitation occurred, showing the absence of any notable quantity of globulin soluble in the dilute saline solution produced by the salts of the seed with the water used in the extraction. The dialyzed solution gave no immediate coagulum on boiling, proving the absence of albumens. The solution was then evaporated over a lamp and, after boiling some time, a light, bulky coagulum gradually separated. When the solution became quite concentrated, the coagulum was filtered off, washed with water, alcohol, and ether, dried over sulphuric acid and found to weigh 0.25 gram. The filtrate from this coagulum was evaporated to small volume on a water-bath and precipitated by pouring into absolute alcohol. The precipitated proteid was then washed with absolute alcohol and ether and weighed, when dry, 0.40 gram. Accordingly, 0.65 per cent. of the oil-free meal, consisted of water-soluble proteose-like matter. The amount of this substance was so small, and the difficulty of preparing it in a state of purity so considerable, that it was not further examined. Other experiments, both with water and saline solution, fully confirmed the results here described and left no doubt that the amount of water-soluble proteid is very small.

b. Extraction with Sodium Chloride Solution.—When treated with ten to twenty per cent. sodium chloride solution, the cottonseed-meal yields a slightly acid extract of a yellowish-pink color which is viscid, and difficult to filter. When heated slowly, this extract becomes turbid at 44° , flocks, in small amount, separating at 64° . Filtered after heating to 70° , the solution becomes turbid when heated again to 70° and flocks in larger amount separate at 93° .

Saturation with sodium chloride gives a slight precipitate. Dilution of the extract with water yields an abundant precipitate which is redissolved on warming and again separates in the form of spheroids when cooled.

Fifty grams of oil-free meal were extracted with ten per cent. sodium chloride solution, and the meal washed with the same solution as long as any proteid could be extracted. The extract and washings were saturated with ammonium sulphate, the precipitate produced filtered off, dissolved in dilute sodium chloride brine, filtered, and dialyzed four days. The solution was then removed from the dialyzer and the precipitated globulin filtered off, washed with water, alcohol, and ether, and dried over sulphuric acid. There was thus obtained seven grams or fourteen per cent. of light yellowish proteid which, when dried at 110° , had the composition given below, preparation 1.

A second preparation was made by treating 100 grams of meal with three liters of twenty per cent. sodium chloride solution for forty-eight hours and, after filtering, saturating the extract with ammonium sulphate. The meal-residue was again treated with twenty per cent. sodium chloride brine and after filtering the extract, it was saturated with ammonium sulphate and added to the first.

The ammonium sulphate precipitate was filtered off and dissolved in ten per cent. sodium chloride solution, yielding a deep brown liquid. This was filtered clear and dialyzed till free from chlorides when the precipitate was filtered off and treated in the usual way. There resulted 15.83 grams of globulin of a slightly yellowish color which had the composition stated below, preparation 2.

A third preparation was obtained by extracting 100 grams of

meal with water and then treating the residue with twenty per cent. sodium chloride solution.

The salt-extract was filtered, saturated with ammonium sulphate and otherwise treated in the manner already described in making preparations 1 and 2. Only 8.39 grams of globulin were recovered, indicating a conversion into the insoluble modification through contact with water.

The composition of this preparation 3, is shown by the following figures.

COTTONSEED GLOBULIN.										
	1				2				3	
	I.	II.	Av.	Ash-free.	I.	II.	Av.	Ash-free.	Ash-free.	
Carbon.....	51.85	51.85	51.91	51.75	51.75	51.86	51.66	51.77
Hydrogen..	6.78	6.78	6.79	6.87	6.87	6.88	6.73	6.74
Nitrogen...	18.02	18.12	18.07	18.09	17.90	18.15	18.03	18.07	17.93	17.97
Sulphur ...	0.68	0.68	0.68	0.67	0.67	0.67	0.71	0.71
Oxygen	22.53	22.52	22.81
Ash	0.11	0.11	0.21	0.21	0.22
	100.00				100.00				100.00	

The properties and composition of this substance are so similar to those of the vegetable vitellin found in the seeds of flax, hemp, wheat, and other seeds, it seemed probable that the three preparations just described were not entirely pure and that if freed from all foreign matters they would agree more closely with the globulin of the seeds just named. Three preparations were accordingly made, substantially in the manner already described and, after drying over sulphuric acid, they were redissolved in ten per cent. sodium chloride brine and the resulting clear solutions were dialyzed. The globulin thus reprecipitated, was filtered off, thoroughly washed with water, alcohol, and ether and when dried at 110° analyzed with the following results:

COTTONSEED GLOBULIN. EDESTIN.										
	4		5		6					
	Ash-free.		Ash-free.		I.	II.	Av.	Ash-free.		
Carbon	51.59	51.75	51.56	51.93	51.68	51.58	51.63	52.05		
Hydrogen	6.68	6.70	6.92	6.97	7.11	6.92	6.97	7.02		
Nitrogen	18.72	18.78	18.40	18.52	18.66	18.40	18.53	18.68		
Sulphur	0.75	0.75	0.50	0.51	0.66	0.66	0.66		
Oxygen	22.02	22.07	21.59		
Ash	0.33	0.74	0.82	0.82		
	100.00		100.00		100.00					

We next attempted to detect the presence of other globulins in the sodium chloride extract. One kilogram of cottonseed-meal was extracted with ten per cent. sodium chloride solution and as the extract after straining through cloth was quite concentrated and could not be filtered, it was shaken with ether so as to remove oil and other substances soluble in that liquid. On standing, a part of the aqueous solution separated, leaving a supernatant layer consisting of an emulsion which long standing failed to resolve. Addition of alcohol, instead of breaking up the emulsion, transformed it into a jelly-like mass of considerable solidity. The unemulsified part of the extract, after standing some time, was decanted and dialyzed free from chlorides. The dialyzed solution was allowed to stand until the globulin had deposited when the supernatant liquid was decanted. The separated globulin was treated with ten per cent. sodium chloride brine and the solution filtered very nearly clear. This solution was then dialyzed for eighteen hours, during which time a large precipitate formed which was filtered off, washed with water, alcohol, and ether, and, when dried over sulphuric acid, weighed 23.63 grams, preparation 7. The filtrate was found to be practically free from proteids. The solution decanted from the first precipitation of the globulin contained a large amount of very finely divided substance that would not settle. A little sodium chloride was therefore added which dissolved the suspended globulin. The solution was next saturated with ammonium sulphate and the large precipitate filtered off, dissolved in water, the solution filtered perfectly clear, and dialyzed for several days. It was then removed from the dialyzer and allowed to stand until the suspended globulin had mostly settled out. The milky solution was then decanted from the sediment and the latter washed with water, alcohol, and ether. After drying over sulphuric acid it weighed 8.58 grams. Preparation 8. The solution decanted from 8, after repeated filtration was obtained clear and was again dialyzed. After several days a very small precipitate formed which, when subjected to the usual treatment, weighed but 0.82 gram. This was much colored and evidently impure. The solution filtered from this precipitate was saturated with ammonium sulphate and yielded

only a very small precipitate which appeared to consist mostly of proteose. The emulsion obtained by shaking the original extract with ether, after standing some days, gave no evidence of re-resolution. The jelly-like mass was therefore broken up and thrown on a filter. A clear quick-running filtrate was obtained which was dialyzed for five days and deposited a globulin, that, after the usual treatment, weighed 8.43 grams. Preparation 9. All these preparations were analyzed with the following results:

	7				8		9	
	I.	II.	Av.	Ash-free.	Ash-free.		Ash-free.	
Carbon	51.59	51.45	51.52	51.75	51.38	51.44	51.10	51.33
Hydrogen	7.04	6.70	6.87	6.90	6.70	6.70	6.88	6.91
Nitrogen	18.76	18.71	18.74	18.82	18.49	18.51	18.47	18.55
Sulphur	0.61	0.61	0.61	} 23.05	0.60	0.60
Oxygen	21.92	22.61
Ash	0.46	0.46	0.12	0.46
	100.00				100.00		100.00	

The above analyses show that the first three preparations were not quite pure and the extract, last made, affords satisfactory evidence that no other salt-soluble globulin exists in the cottonseed in noteworthy amount. In the following table, the analyses of the purer preparations may be compared together.

SUMMARY OF ANALYSES OF COTTONSEED GLOBULIN. EDESTIN.							
	4	5	6	7	8	9	Average.
Carbon	51.75	51.93	52.05	51.75	51.44	51.33	51.71
Hydrogen ..	6.70	6.97	7.02	6.90	6.70	6.91	6.86
Nitrogen...	18.78	18.52	18.68	18.82	18.51	18.55	18.64
Sulphur ...	0.75	0.50	0.66	0.61	} 23.05	0.60	0.62
Oxygen	22.02	22.08	21.59	21.92		22.61	22.17
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The table subjoined shows that this substance agrees in composition with the vitellin which exists in the seeds of wheat, maize, hemp, castor-bean, squash, and flax. As the properties of the preparations obtained from all these sources are substantially alike, there can be little doubt that one and the same proteid exists in them all. For this body we adopt the name *Edestin*, from the Greek *εδεστος*, signifying edible, in view of its occurrence in so many important food-stuffs.

COMPOSITION OF EDESTIN FROM VARIOUS SEEDS.							
	Wheat kernel.	Maize kernel.	Hemp- seed.	Castor- bean.	Squash seed.	Flax- seed.	Cotton- seed
Carbon.....	51.03	51.71	51.28	51.31	51.66	51.48	51.71
Hydrogen..	6.85	6.85	6.84	6.97	6.89	6.94	6.86
Nitrogen...	18.39	18.12	18.84	18.75	18.51	18.60	18.64
Sulphur ...	0.69	0.86	0.87	0.76	0.88	0.81	0.62
Oxygen	23.04	22.46	22.17	22.21	22.06	22.17	22.17
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The considerable differences in carbon and nitrogen in the above analyses amounting, in the extreme cases, to 0.7 per cent. are, in general, not greater than the discrepancies between analyses of preparations from the same seed. In the seeds of wheat and maize, other water and salt-soluble proteids occur in considerable quantities and it is probable that the crystallized preparations obtained from hemp, castor-bean, squash, and flax more truly represent the composition of edestin than the amorphous or spheroidal substance yielded by the cereal seeds.

The slight differences in the deportment of solutions of these preparations of edestin may be reasonably attributed to admixtures of traces of other proteids.¹

c. Extraction with Potash Water.—After extraction with water and sodium chloride solution a considerable amount of proteid was always found in the residual-meal which could be partly removed by two-tenths per cent. potash solution. All attempts to obtain the proteid thus extracted in a pure state, have hitherto entirely failed. Much coloring matter passes into the alkaline solutions, and when the proteid is precipitated, goes down with it and can not be removed by any process we have hitherto applied.

When freshly prepared, the alkaline extracts, as well as the meal moistened with the alkali, are of a bright reddish-yellow color, but on exposure to the air they rapidly darken and finally become greenish-black. So much gummy matter is also extracted by alkaline solutions that it is almost impossible to filter them clear. As a result no preparations were obtained, the analysis of which could shed any light on the composition of the proteid which they represented. The residue of meal after treatment with potash still contains a notable quantity of nitrogen.

¹ See paper on Crystallized Vegetable Proteids, by T. B. Osborne, *Am. Chem. J.*, 168.

d. Amounts of the Different Forms of Proteids in Cottonseed.—As already shown, the proteid matter of cottonseed soluble in water, consists almost wholly of proteose. Making full allowance for incomplete extraction and loss, this does not exceed 0.75 per cent. of the oil-free meal. The highest yield of globulin recovered in the preceding extractions was 15.83 per cent. of the oil-free meal, and contained 42.3 per cent. of the total nitrogen. After repeated extraction with potash water, the residue contained, in the case where extraction was most complete, 11.4 per cent. of the total nitrogen, showing that 88.6 per cent. of the total nitrogen had been dissolved. The difference between the percentage of nitrogen removed by sodium chloride solutions and that extracted by weak potash, represents the proteid dissolved by potash water which is not soluble in saline solutions, and which corresponds to 46.3 per cent. of the total nitrogen, assuming all this nitrogen to be present in proteid form.

These data may be tabulated as follows:

	Air-dry and oil- free meal.	Per cent. of Total nitrogen.
Proteose.....	0.75	2.0
Salt-soluble proteid. Edestin.....	15.83	42.3
Alkali-soluble and salt-insoluble proteid.	44.3
Proteid insoluble both in salt and alkali.	11.4
		100.0

ON THE ANALYSIS OF AMERICAN REFINED COPPER.¹

BY HARRY F. KELLER.

IT is generally conceded that the presence even of minute quantities of other elements in metallic copper has a marked influence upon its physical properties, and especially upon its electric conductivity.

The copper produced in the Lake Superior region is relatively free from injurious admixtures, and is, therefore, generally preferred when a high conductivity is desired. "Lake" copper is always quoted a trifle higher than the metal from other localities.

Of late years the production of copper from impure sulphide ores has grown enormously, and, at the same time, the quality of the product has steadily improved. It has, indeed, become

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