THE PROTEINS OF THE WHEAT KERNEL

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INTRODUCTION.

Of the protein substances used as food none is of more importance than those contained in the seeds of wheat. Although these bodies attracted the attention of investigators more than one hundred years ago and have since then been many times the subject of study, the published statements respecting them are so conflicting and uncertain that it has heretofore been impossible to know what the truth regarding them actually is. With the purpose of clearing up the existing confusion and determining the real value of the evidence offered, as well as extending, as far as possible, our knowledge of these important substances, the writer some years ago undertook an investigation of this seed which has recently been concluded by work done under grants from the Carnegie Institution of Washington. As the results of these investigations have been published from time to time in a number of different papers, appearing in four different journals, it has been thought desirable to bring all this work together in one paper. In so doing the details have been reproduced in full, for the nature of the evidence is such that its value largely consists in concordant results of many experiments, repeated under different conditions, since it is not yet possible to establish the chemical individuality of different protein substances by demonstrating their possession of definite physical properties, as may be done with the simpler organic compounds.

The experience of the writer in his endeavors to understand and repeat the work of many of his predecessors has made him feel the importance of these details to future workers along the same lines and is his excuse for giving with so much minuteness the results of his own work, which to those not familiar with the difficulties of the subject must appear to a large extent unnecessary. In order to make this work available to those who wish simply to know the results, a comprehensive summary of this paper is given at the end of this publication, and the details of the many operations and experiments need be read only by those who wish to become familiar with these.

The account of this work is preceded by a review of the literature of the subject, from which an idea of the unsatisfactory state of our previous

knowledge can be obtained. This review is interesting, also, as it shows the slow development of the study of vegetable proteins and how the several investigators have been influenced by the knowledge of the animal proteins prevailing at the time the work was done. In carrying out his investigations of these proteins the writer has received the assistance of Messrs. Voorhees, Campbell, Harris, and Clapp, for which he wishes here to make acknowledgment; but especially is he indebted to Prof. S. W. Johnson, under whose direction and with whose advice and encouragement this work was first undertaken in the laboratory of the Connecticut Agricultural Experiment Station, where it has since been continued.

REVIEW OF THE LITERATURE.

The fact that gluten can be obtained from wheat flour by washing with water appears to have been first published by Beccari.[1]

That alcohol extracts a protein substance from wheat flour was first stated by Einhof,[2] who considered this to be the same as the gluten.

Taddei[3] found that gluten consists of two substances, one of which is soluble in alcohol, which he named "gliadin," the other insoluble in alcohol, which he named "zymom."

De Saussure[4] obtained from wheat gluten about 72 per cent of plant-albumin in the insoluble form, about 20 per cent of plant-gelatin, or, as he proposed to call it, "glutin," and about 1 per cent of mucin, which latter substance he considered to be similar to the mucin described by Berzelius.

Berzelius[5] thought that the alcoholic extract contained another protein substance, which he called "mucin," and that the part of the gluten which was insoluble in alcohol was so similar to albumin that he called this "plant-albumin."

Boussingault[6] agreed with Einhof that the part of the gluten that was soluble in alcohol was the same as the entire gluten protein.

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[1] Beccari. Reference to this publication has, for many years, appeared in the literature as Comon. Bonon. I. 1, p. 122. It should be De Bononiensi Scientiarum et Artium Instituto atque Academia Commentarii, 1745, II, part 1, p. 122. In this paper Beccari refers to the fact that in 1728 he had orally communicated to the Academy the previously unpublished fact that wheat flour can be separated into two parts, one of vegetable, the other of animal character. The substance of this communication was published in the above-cited paper in which the separation of gluten from wheat flour, by washing with water, is described.

[2] Einhof, Journal der Chemie von Gehlen, 1805, V, p. 131.

[3] Taddei, Annals of Philosophy, 1820, XV, p. 390.

[4] De Saussure, Schweiger's Journal, 1833, LXIX, p. 188.

[5] Berzelius, Lehrbuch der Chimie, Auflage 3, 1837, VI, p. 453.

[6] Boussingault, Annales de Chimie et de Physique, 1838, LXV, p. 30.

Liebig[1] named the part of the gluten that was insoluble in alcohol "plant-fibrin," on account of its supposed resemblance to blood-fibrin. The substance soluble in alcohol he called "plant-gelatin" and considered it to be a compound of a protein with an organic acid. In the aqueous extract of the flour he recognized the presence of albumin.

Scherer[2] prepared plant-fibrin by dissolving gluten in dilute alkali, filtering, neutralizing with acetic acid, and extracting the precipitate with hot alcohol and then with ether.

Bonchardat[3] considered that wheat gluten contained a protein soluble in extremely dilute acids, which he named "albuminose."

Dumas & Cahours[4] found four protein substances in wheat flour, namely, plant-fibrin, which remained after extracting gluten with alcohol; a substance which they considered similar to casein, which was deposited by cooling the alcoholic extract; glutin, which was obtained by concentrating and cooling the alcoholic extract, and albumin, which was present in the aqueous washings of the gluten and was coagulated by boiling. The plant-fibrin they considered to be identical with blood-fibrin, as both had the same ultimate composition, and the albumin to be identical with egg-albumin for the same reason.

Mulder[5] considered the plant-gelatin, obtained by extracting gluten with alcohol, to be a compound of sulphur with "protein," which contained the same proportion of sulphur as blood-albumin.

Von Bibra[6] recognized three proteins in gluten—plant-fibrin, which formed 70.8 per cent; plant-gelatin, 16.2 per cent; and plant-casein, 7.1 per cent. In the water used for washing out the gluten he found 1.34 per cent of albumin.

Günsberg[7] considered that Taddei's view that there were only two proteins in wheat gluten was correct. By boiling wheat gluten with water he obtained five preparations which separated on cooling and had the same ultimate composition as has been established for gliadin. By treating gliadin in the same way he obtained a body of the same composition. The substance which Günsberg thus obtained was unquestionably gliadin, which is sparingly soluble in hot water, and he appears to have been the first to obtain correct analyses of this protein.

[1] Liebig, Annalen der Chemie und Pharmacie, 1841, XXXIX, p. 129.
[2] Scherer, Annalen der Chemie und Pharmacie, 1841, XL, p. 7.
[3] Bonchardat, ibid., 1842, XLII, p. 124.
[4] Dumas & Cahours, Journal für praktische Chemie, 1843, XXVIII, p. 398.
[5] Mulder, Annalen der Chemie und Pharmacie, 1844, LII, p. 419.
[6] Von Bibra, Die Getreidearten und das Brod, Nüremberg, 1860.
[7] Günsberg, Journal für praktische Chemie, 1862, LXXXV, p. 213.

Commaille[1] recognized, as protein constituents of flour, sitosin, soluble in water and coagulable by heat; imesin, soluble, after drying, only in water containing 0.1 per cent hydrochloric acid; sitesin, soluble in 0.1 per cent hydrochloric acid; glutin, nearly insoluble in dilute acid, easily in strong acid, forming an emulsion with alcohol, which is separated by much water; and mucin, which dissolves easily, even after drying, in water and in cold 80 per cent alcohol.

Ritthausen[2] next published the results of his extensive investigations in a volume in which he discussed at length the proteins of the wheat kernel. The composition, properties, and the proportions in which they occur in the seed were given in detail, and also the evidence which he considered showed the individuality of each. He recognized five proteins, namely, gluten-casein, gluten-fibrin, plant-gelatin or gliadin, and mucedin, as constituents of the gluten, and also albumin, which he found in the aqueous extracts of the seed.

The gluten-casein was not soluble in water, very slightly soluble in dilute alcohol, and readily soluble in very dilute acids and alkalis. When decomposed by boiling with sulphuric acid it yielded tyrosine, leucine, 5.3 per cent of glutaminic acid, and 0.33 per cent of aspartic acid. In the dry gluten he found from 26 to 31.4 per cent of gluten-casein, which he considered to be minimal quantities.

Table giving results as ascertained by Ritthausen.

	Gluten-casein.	Gluten-fibrin.	Plant-gelatin.	Mucedin.	Albumin.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Carbon	52.94	54.31	52.76	54.11	53.12
Hydrogen	7.04	7.18	7.10	6.90	7.18
Nitrogen	17.14	16.89	18.01	16.63	17.60
Sulphur	0.96	1.01	0.85	0.88	1.55
Oxygen	21.92	20.61	21.37	21.48	20.55
	100.00	100.00	100.00	100.00	100.00

The gluten-fibrin formed that fraction of the alcohol-soluble proteins which was soluble in the strongest alcohol and separated from a hot concentrated solution in 50 to 60 per cent alcohol on cooling. Owing to the difficulty encountered in separating gluten-fibrin from the other alcohol-soluble proteins, it was impossible to determine its amount. Usually from

[1] Commaille, Journal de Pharmacie, 1866 (4), IV, p. 108.
[2] Ritthausen, Die Eiweisskörper, etc., Bonn, 1872.