$C_{21}H_{18}O_6$. An estimation of the molecular weight by the freezing-point method with benzene as solvent, gave as an average 326. The formula $C_{14}H_{12}O_4$ gives 244.12; the formula $C_{21}H_{18}O_6$ gives 366 for the molecular weight. A study of other derivatives will be required for the establishing of the exact formula of xanthoxylin S. This work is to be continued.

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[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

# INVESTIGATIONS ON THE PROPERTIES OF WHEAT PROTEINS.

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## I. THE SEPARATION OF THE PROTEINS.

THE proteins of wheat according to the work of Osborne and Voorhees,[1] are five in number, *viz.*, gliadin, glutenin, an albumin, a globulin and proteose. The first of these, gliadin, is soluble in 70 per cent. alcohol. Glutenin is insoluble in alcohol, and together with gliadin constitutes about 80 to 85 per cent. of the total proteins of the wheat. The other three proteins, *viz.*, the albumin, the globulin and the proteose are soluble in dilute salt solutions and together constitute about 15 to 20 per cent. of the total proteins present.

This separation of the proteid substance of wheat into these five individual proteins is not accepted by all who have worked upon the problem, *e. g.*, F. Kutscher[2] claims, from a study of the cleavage products, that there are but three proteins in wheat, *viz.*, gluten casein, corresponding to Osborne and Voorhees' glutenin, gliadin, readily soluble in 60 per cent. alcohol, and gluten fibrin, slightly soluble in 60 per cent. alcohol. Ritthausen[3] had previously distinguished four proteins, one insoluble in alcohol, gluten casein, and three soluble in alcohol, *viz.*, gliadin, gluten fibrin and mucedin. Kutscher[4] claims, from a study of the products of hydrolysis, that mucedin and gliadin are the same.

[1] Am. Ch. J. 15, 392 (1893).

[2] Z. physiol. Chem. 38, 111 (1903).

[3] "Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Oelsamen" (Bonn, 1872, Dissert.).

[4] Loc. cit.

Fleurent[1] claims three different proteins present in wheat gluten, and König and Rintelen[2] agree with Ritthausen that there are four. One explanation of this difference of opinion is, that some of the above investigators worked with wheat gluten alone, while others worked with the wheat itself. As will be shown later on, wheat gluten is not of uniform composition, unless prepared by exactly the same methods and under the same conditions, nor does it contain all of the proteins of the wheat.

Without going more into detail in regard to the views of these and other writers on this subject the author wishes to speak of some results that have been obtained in an effort to make a quantitative separation of the proteins of wheat. Osborne and Voorhees[3] state, as the general quantitative result, that the amounts of the different proteins in wheat are:

TABLE I.

| | Protein. Per cent. of wheat. | Protein. Per cent. of total proteins. | |
|---|---|---|---|
| Glutenin | 4.428 | 40.25 | |
| Gliadin | 3.936 | 35.78 | |
| Globulin | 0.625 | 5.68 | 14.25 |
| Albumin | 0.621 | 5.64 | |
| Proteose | 0.322 | 2.93 | |
| $H_2O$ washings | 1.076 | 9.78 | |
| Total | 11.009 | 100.06 | |

ACTION OF HOT ALCOHOL.

It is practically impossible to extract a flour with a salt solution and then, after filtering it, to extract the residue with alcohol, because of the difficulty of filtering off the salt solution completely and the effect of the presence of a small amount of salt on the solubility of the alcohol-soluble proteins. The following table gives results obtained when the flour was extracted directly with 70 per cent. alcohol, and examination was made to see if hot alcohol would dissolve out more protein than cold. In this and the following tables the results are the averages of a series of determinations in each case.

[1] Ann. Agron. 1898, 371.

[2] Z. Nahr. Genussm. 8, 401, 721 (1904).

[3] Loc. cit.

TABLE II.

| | Protein. Per cent. of flour. | Protein. Per cent. of total proteins. |
|---|---|---|
| Cold alcohol on air-dry flour..... | 7.47 | 56.80 |
| Hot alcohol on air-dry flour..... | 7.32 | 54.43 |
| Cold alcohol on dry flour..... | 4.58 | 34.82 |

These results show that the use of hot alcohol has a diminishing effect due, no doubt, to the coagulating action of the hot solution and its effect on the solubility of the proteins. If, also, the flour is dried before the extraction is made, the effect on the solubility is even greater and the difference between alcohol-soluble proteins extracted by cold alcohol, on air-dry flour and on dried flour, is about 20 per cent. of the total proteins present. Some investigators have used, for extracting gliadin, flour which had been extracted with ether. If this could be accomplished without first drying the flour by means of heat it might do, but otherwise it would be liable to the same errors as the hot extraction.

## EFFECT OF DIFFERENT AMOUNTS OF FLOUR, IN PROPORTION TO THE EXTRACTING LIQUID, ON THE AMOUNT OF PROTEIN EXTRACTED.

Osborne and Voorhees in their work used large quantities of wheat or flour and determined the amount of protein by weight, after purifying it by fractional precipitation and drying. Taking their analysis of the pure proteins as correct and using, therefore, the factor 5.68 or 5.7, the amounts of protein extracted in these investigations were determined by nitrogen determinations, kindly made by Mr. T. C. Trescott, of the Bureau of Chemistry. Repeating as nearly as was possible the operations of Osborne and Voorhees, and determining the nitrogen in the numerous filtrates and residues, calculating all to the basis of the original dry flour, the results in Table III were obtained. In the first column are the results obtained when relatively large amounts of flour were used. In these cases 1,000 grams of flour were extracted with 4,000 cc. of 70 per cent. alcohol for twenty-four hours, and subsequent extractions were made, using 2,000 cc. alcohol each time until the total amount of alcohol used equaled 10,000 cc. In the second column are the results obtained when relatively small amounts of flour were used. In these cases 2 to 4 grams of flour were extracted once with 100 cc. of 70 per cent. alcohol for twenty-four hours.

TABLE III.—PROTEIN IN PER CENT. OF TOTAL PROTEINS.

| | Using large amounts of flour. | | Using small amounts of flour. | |
|---|---|---|---|---|
| Direct extraction with alcohol | 43.56 | 49.54 | 47.15 | 52.66 |
| Extraction with salt solution after preceding extraction with alcohol | 5.98 | | 5.51 | |
| Direct extraction with salt solution | 13.54 | 46.25 | 16.26 | 45.52 |
| Extraction with alcohol after preceding extraction with salt solution | 32.71 | | 29.26 | |

Thus we see that the amount of protein extracted depends upon the relative amounts of solvent and solute. Several extractions, aggregating seventy-two hours, in addition to the twenty-four hour extraction, failed to yield more than a mere trace of protein when the small amounts of substance were used, whereas when large amounts were used, four separate extractions were made and the last extraction yielded about 7.0 per cent. of the total proteins extracted, and in another case about 1.5 per cent.

TABLE IV.

| | Protein. Per cent. of flour. | | Protein. Per cent. of total proteins. | |
|---|---|---|---|---|
| Direct extraction with alcohol | 7.47 | 8.04 | 56.80 | 61.13 |
| Extraction with salt solution after preceding extraction with alcohol | 0.57 | | 4.33 | |
| Direct extraction with salt solution | 2.18 | 7.68 | 16.57 | 55.83 |
| Extraction with alcohol after preceding extraction with salt solution | 5.50 | | 39.26 | |

As is shown by both Tables III and IV, the amounts of both the alcohol-soluble protein and the salt solution-soluble proteins are different when the wheat is extracted directly and when it has been previously extracted with the other solvent. In the case of the alcohol extraction preceded by the extraction with salt solution, this difference is undoubtedly due to the fact that the salt retained in the extracted flour or wheat affects the solubility of the alcohol-soluble proteins. When the salt solution extraction was preceded by the alcohol extraction, it would seem, that, by air-drying the sample after the alcohol extraction, if the alcohol had no solvent effect upon the salt solution-soluble proteins, its effect would disappear with its evaporation, and we should obtain by the following salt solution extraction the same amount of protein

as we do from a direct extraction with salt solution. But this is not the case, as is seen from Table IV, for we obtain, by direct extraction with salt solution, 16.57 per cent. of the total proteins, whereas after extracting with alcohol we obtain only 4.33 per cent. This can mean only one thing, *viz.*, that alcohol dissolves, with the gliadin, a large part of the albumin, globulin and proteose, which are soluble in salt solutions. That it does not extract all is shown by the fact that extraction with salt solution, after alcohol extraction, always yields protein material, usually about 4 or 5 per cent. of the total. Osborne and Voorhees found globulin to be about 5.6 per cent. of the total proteins, and it seems probable that dilute alcohol (70 per cent.) dissolves out, with the gliadin, the albumin and proteose and leaves the globulin. This is shown also from the following data in Table V. In this table are given the results obtained from a series of determinations in which both the alcohol extraction and the salt solution extraction were made directly on the flour, and, also, each extraction was made on flour previously extracted with the other solvent. In addition the salt solution extract itself was extracted with alcohol in the following manner: Alcohol of 95 per cent. was added to the salt solution extract in such quantity as to make a resulting alcohol equal to 70 per cent. The precipitate formed was then filtered off and the protein remaining in solution in the alcohol was determined.

TABLE V.—PROTEIN IN PER CENT. OF TOTAL PROTEINS.

| | |
|---|---|
| Direct extraction with alcohol | 56.73 |
| Direct extraction with salt solution | 20.80 |
| Extraction with alcohol after preceding extraction with salt solution | 41.20 |
| Extraction with salt solution after preceding extraction with alcohol | 4.92 |
| Extraction of the salt solution extract with alcohol | 12.70 |

In this table, as in Table IV, the results show that direct extraction with 70 per cent. alcohol dissolves out, not only the protein gliadin, but also a portion of the proteins characterized as salt solution-soluble, and that this portion may be extracted by alcohol, either directly from the flour or from the salt solution extract itself.

From this portion of the work we can conclude, therefore:

(1) For the proper extraction of the proteins of wheat by means of alcohol, cold 70 per cent. alcohol should be used directly upon the air-dry wheat or flour. Relatively large amounts of solvent, in proportion to the flour, should be taken, *viz.*, 2 to 4 grams flour

per 100 cc. alcohol, and the extraction continued for twenty-four hours. Either hot alcohol or dry flour gives abnormal results.

(2) The same conditions of extraction should be observed in using the salt solution. The author has found that 5 per cent. potassium sulphate solution extracts practically the same as 10 per cent. sodium chloride and is better in practice, because it avoids the evolution of hydrochloric acid gas when digested in the Kjeldahl operation. Four to 6 grams of flour are taken per 100 cc. of solvent and the extraction continued for twenty-four hours with frequent or continued shaking.

(3) In the extraction with alcohol there is extracted, with the alcohol-soluble protein, gliadin, a large part of the salt solution-soluble proteins, probably the albumin and proteose. Corrections for such overlapping of the extractions must be made whenever a quantitative separation of the proteins is attempted.

(4) As recommended by the author, in the Association of Official Agricultural Chemists,[1] the separation of the proteins of wheat into more than two groups, *viz.*, (1) alcohol-soluble, and (2) alcohol-insoluble, seems unwarranted, both because of the difficulty of making a further quantitative separation and because of the indefinite value of such separation.

## II. WHEAT GLUTEN.

The proteid bodies of the wheat grain are especially interesting and important because it is to them that wheat flour owes its exceptional adaptability to the making of bread.

Before the proteins of wheat were known or studied as individuals it was known that practically all of them were present in what is termed wheat gluten. This gluten is obtained by the simple mechanical process of washing away starch and soluble constituents by kneading a ball of dough in the hand in a small stream of water. The residue is a tough elastic mass rich in nitrogen and containing the larger part of the proteid constituents.

The determination of the amount of this gluten, either wet or dry, or both, has long been considered as of value in judging the character of wheat and flour for bread-making purposes. The determination is, however, one in which so many errors are involved that it seems hardly to be of value as a chemical factor

[1] A. O. A. C. Proceedings, 1903, 1904. Bull. 81, 118, and Bull. 90, 121, 127, Bur. Chem., U. S. Dept. Agr.

when the corresponding factor of total proteins, by nitrogen determination, is so reliable and easy to carry out.

It is for the purpose of showing some of the errors in the ordinary gluten determination that the following facts have been brought together from the results obtained by the author during the last year or so.

It has been admitted by those who have worked on this method that differences in temperature of wash-water, its salt content and the time and manner of manipulation all have a strong influence in varying the final result of the amount of gluten. Fleurent,[1] the most ardent advocate of the value of the gluten determination, claims that by using water of a certain arbitrary salt content and exact methods as to time and manner of washing, uniform results may be obtained.

It is true that concordant duplicates can be obtained by any one after becoming thoroughly acquainted with the operation so as to be able to duplicate conditions exactly. But even though the results are concordant, do they mean anything, or if so, do they mean more than other more reliable determinations?

F. A. Norton[2] has studied the composition of crude gluten and the relation between gluten and total proteins, by nitrogen determination. His results are based on the analysis of the gluten itself and the determination of non-proteid material in it.

The following results are based on an examination of the proteid material lost in the process of washing out the gluten and contained in the wash-water therefrom. It will be seen that the data and conclusions obtained in this investigation are in agreement with those obtained by Norton.

## THE EFFECT OF PHYSICAL CONDITION OF THE SAMPLE ON THE DETERMINATION OF GLUTEN.

The average of the determinations made on some fifty samples of wheat, when simply ground as whole wheat, give an amount of dry gluten about 2.31 per cent. lower than the total proteins calculated from total nitrogen multiplied by 5.7. If we allow for the amount of amino bodies present, this excess of total proteins over dry gluten will be reduced by about 1.0 per cent. or

[1] Ann. chim. anal. 10, 129, 195, 238, 276, 309 (1905); Compt. rend. 140, 99 (1905).

[2] This Journal, 28, 8 (1906).

less. If the samples worked with are the finest patent flours the results for dry gluten will average nearly as much in excess of total proteins as the others did below:

TABLE VI.

| | Total nitrogenous bodies. | Amino bodies. | Total proteins. | Dry gluten. |
|---|---|---|---|---|
| Flour[1] | 13.07 | ..... | 13.07 | 14.30 |
| Difference | ..... | ..... | ..... | + 1.23 |
| Whole wheat | 10.26 | 1.26 | 9.00 | 7.95 |
| Difference | ..... | ..... | ..... | — 1.05 |

The amount of true proteid material in the two classes of glutens is, however, nearly the same.

TABLE VII.

| | Nitrogen. Per cent. | Proteins. Per cent. (N × 5.7). | Non-proteins. Per cent. |
|---|---|---|---|
| Whole wheat gluten | 13.11 | 74.72 | 25.28 |
| Patent flour gluten | 13.54 | 77.17 | 22.83 |
| Theoretical | 17.60 | ..... | ..... |
| Average | ..... | 75.94 | 24.06 |

These facts can readily be understood if we consider the physical condition of the material, which makes it easy for a considerable loss of proteins, mechanically, in washing out the whole wheat so as to wash away all particles of bran, and the corresponding smaller loss when patent flour is used, the amount of non-proteins retained by the gluten being in one case approximately the same as in the other.

An examination of the wash-water from whole wheat, after the gluten has been obtained, shows us where the loss of proteins occurs. Collecting all wash-water, after allowing it to pass through a bolting-cloth sieve to retain bran, and later allowing the filtrate to settle for twenty-four hours until the greater part of the starch had settled and the supernatant liquid could be siphoned off, and analyzing all these portions for nitrogen, gave the following results:

TABLE VIII.

| | | Nitrogen compounds calculated as protein. Per cent. | Per cent. of the total nitrogen compounds. |
|---|---|---|---|
| Filtrate | | 2.16 | 21.23 |
| Residue | Starch | 0.51 | 5.03 |
| | Bran | 1.71 | 16.76 |
| Total | | 4.38 | 43.02 |

[1] Bull. 70, Bur. Plant Ind., U. S. Dept. Agr.

The loss of nitrogenous compounds, calculated as protein, in the washing out of gluten is, therefore, equal to 4.38 per cent., or 43.02 per cent. of the total nitrogenous compounds present. If we allow for the amino bodies found to be present, by the ordinary Stutzer method we have, considering that all amino compounds are in the filtrate from the gluten washing, the following results:

TABLE IX.

| | | Per cent. of wheat. | Per cent. of total proteins. | |
|---|---|---|---|---|
| Proteins in gluten (13.11 per cent. N). | | 5.81 | 64.56 | |
| Proteins in washings | Filtrate | 0.91 | 10.11 | 34.77 |
| | Starch residue | 0.51 | 5.66 | |
| | Bran residue | 1.71 | 19.00 | |
| | | 8.94 | | |
| Total proteins (Alb. N × 5.7) | | 9.00 | 99.33 | |
| Amino compounds (amino N × 4.25) | | 0.93 | | |
| Total N. comp. | | 9.93 | | |

We thus see that the gluten is only about 76 per cent. pure protein, and that of the total proteins present in wheat about 65 per cent. is found in the gluten and 35 per cent. in the washings.

From the average composition of wheat bran and shorts, (2.65 per cent. nitrogen), and the fact that they constitute about 30 per cent. of the wheat, there would be in the bran of the whole wheat, not present in flour, about 34 per cent. of the total nitrogenous compounds present. The loss in nitrogenous compounds during the washing is, as we have seen from Table VIII, 43 per cent., so that, in the washing out of the gluten, there is lost, over and above the nitrogenous compounds present in the bran, about 9.0 per cent. of the total nitrogenous compounds.

Also, we see that in the gluten there is, calculated as per cent. of the total proteins, about 25 per cent. (Table VII) of non-proteins. The difference between the proteins lost in washing, 35 per cent., and the non-proteins present in the gluten, 25 per cent., is, therefore, the absolute difference between the total proteins and the amount of dry gluten. This is equal to about 10 per cent. in favor of the total proteins, making the dry gluten less than the total proteins. This agrees practically with the statements in the preceding paragraph. When gluten determinations are made with flour, however, the loss in bran is nothing and the only loss is in soluble amino compounds, which is so small

in amount as to be practically negligible, and in dissolved and mechanically lost particles of protein, so that the non-proteins in the gluten more than balance the loss of proteins during the washing, and the dry gluten will equal or usually exceed the total proteins calculated from total nitrogen. Another table showing results of a later series of determinations gives practically the same conclusions, though the figures are not exactly the same.

TABLE X.

| | Proteins in per cent. of wheat. | Proteins in per cent. of total proteins. | |
|---|---|---|---|
| Gluten | 5.41 | 59.32 | |
| Filtrate | 2.05 | 22.47 | |
| Starch residue | 0.63 | 6.91 | 39.38 |
| Bran residue | 0.91 | 10.00 | |
| | 9.00 | 98.70 | |
| Total protein ($N \times 5.7$) | 9.12 | | |

The determination, by the extraction method, described in the preceding part of this paper, of the amounts of the different proteins present in whole wheat, gave as the average of all the samples represented in Tables IX and X the following results:

TABLE XI.

| | Proteins in per cent. of wheat. | Proteins in per cent. of total proteins. |
|---|---|---|
| Salt solution-soluble proteins | 2.16 | 23.68 |
| Gliadin and glutenin | 6.96 | 76.31 |

Comparing Tables IX, X and XI, it will be seen that, calculated as per cent. of total proteins, about 15 per cent. of the proteins gliadin and glutenin, which are the true agglutinating proteins of wheat, are not accounted for in the gluten; and, that, on the other hand, there is present in the washings about 15 per cent. more protein than is represented by the salt solution-soluble proteins of the wheat.

This means that there is lost in the operation of washing out gluten about 15 per cent. of those proteins of which gluten is composed.

The following conclusions seem justified from the preceding facts:

(1) Dry gluten is about 75 per cent. proteins and 25 per cent. non-proteins.

(2) Of the total proteins present in wheat about 60 to 65 per cent. are present in the gluten, and about 35 to 40 per cent. are lost in the washings.

(3) The balance between the non-proteins present in the gluten and the loss of proteins in washing, makes gluten determinations agree roughly with total proteins calculated from total nitrogen, but they will usually fall below with whole wheat and above with flours.

(4) The amount of total proteins present in gluten is about 15 per cent. less than the sum of the gliadin and glutenin determined by extraction of the wheat, and the loss of proteins in washing out gluten is more than equal to the salt solution-soluble proteins. Therefore, the loss of proteins, in the determination of gluten, is at the expense of gliadin or glutenin, the true gluten proteins of wheat.

(5) On account of these losses and errors it would seem that the determination of gluten is not able to yield any information that cannot be gained either from the determination of total proteins or that of the alcohol-soluble and insoluble proteins.

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## NOTE.

*Two New Weighing-bottles.*—The weighing-bottles described were devised by the writer some time ago and have been found useful by some of his colleagues and himself. The advantages

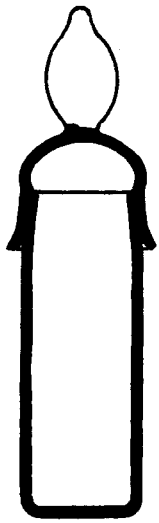

Fig. 1.

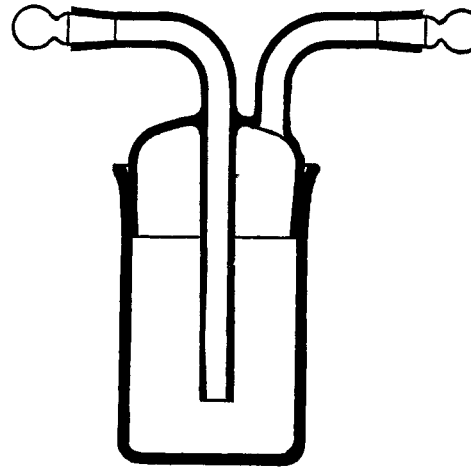

Fig. 2.