

THE JOURNAL

OF THE

AMERICAN CHEMICAL SOCIETY.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF PURDUE
UNIVERSITY.]

THE QUANTITATIVE DETERMINATION OF CARBOHY- DRATES IN FOOD STUFFS.

BY W. E. STONE.

Received February 2, 1897.

IN various articles published by the author¹ during the past ten years, attention has been called to the character of the composition of the so-called "nitrogen-free extract" of food materials. Other writers² have also discussed the same subject and numerous specific researches have contributed a constantly increasing amount of definite knowledge bearing upon the matter. As a result, it is now generally recognized that that portion of food material broadly included under the term "nitrogen-free extract," consists of a considerable number of definite chemical compounds, mostly of the nature of carbohydrates, but of such evident variation as regards their food value or digestibility, as well as their chemical and physical properties, that it is highly inconsistent, if not absurd, to continue to regard them as of homogenous character or worth in food valuation.

When one refers to the very great number of food analyses now on record and notes how large a proportion of all vegetable foods consists of nitrogen-free extract, he cannot escape the thought that a more definite knowledge of this group of bodies

¹ *Am. Chem. J.*, 13, 73; 14, 9; 15, 653; 15, 660; *J. Anal. Appl. Chem.*, 5, 421; *Agri. Sci.*, 2, 315; 7, 6; U. S. Dept. Ag. Div. Chem. Bull., 28, 125; *Ber. d. chem. Ges.*, 23, 3791; 24, 3019; 25, 563; *Proc. Am. Asso. Advancement of Science*, 31, 159; *Proc. Soc. Promotion of Agr. Science*, 1890.

² See articles by Tollens and his colleagues in *J. f. Landwirtschaft. Versuch-Stationen*, etc.; E. Schulze, *Ber. d. chem. Ges.*; and Cross and Bevan, *J. Chem. Soc.*

is necessary, if any value is to be attached to work of this kind. Occasionally a statement of analysis may be found which refers to carbohydrates instead of nitrogen-free extract, but the same thing is meant in either case, and the results have been obtained by the same methods. In comparatively few cases is there any record of any analyst having attempted to estimate directly in a food analysis any of the various substances included under nitrogen-free extract; and anything like a complete determination of all these has rarely if ever been attempted. The present method, as almost universally followed for estimating the constituents of foods, consists in directly determining the amounts of moisture, ash, fat, fiber, and proteids in the material and the difference between the combined percentages of these and 100 is called nitrogen-free extract. This amounts, in many of the most important food materials, to more than fifty per cent. of the whole. It includes, of course, all of the known carbohydrates and doubtless other materials of unknown character, but gives no clue to the relative proportion of any of them.

In brief, the present stage of food analysis leaves us largely ignorant as to the character and value of the principal constituents of many of the most important foods. Little wonder that many have come to regard the ordinary food analysis as contributing little information regarding the actual food value of any material. It certainly affords no means of ascertaining whether the sixty to eighty per cent. of nitrogen-free extract in grains is to be valued as an easily digested carbohydrate or a practically valueless gum or hemicellulose; it tells us nothing as to the presence or absence of sugar or soluble forms of starch or their relative proportions; it furnishes, in short, no accurate information as to the quantity or quality of the carbohydrates which constitute one of the three essential classes of nutrients and are among the chief constituents of vegetable foods.

Recent contributions to our knowledge of the carbohydrates have revealed to a surprising degree not only their specific chemical and physical properties, but indicate even more subtle and delicate distinctions as regards their physiological values; their susceptibility to organized and unorganized ferments; their origin and functions in the growing plant, etc. The time is therefore fully ripe when analytical methods ought to show equal progress

and differentiate sharply between the various kinds of carbohydrates. That something of the kind is highly important will be acknowledged, I think, by every chemist who has to deal with this subject. That such a practice is not already established is due, without doubt, to the lack of general information with regard to the properties of these different substances and the absence of an accurate and easily performed method of separately estimating their amounts.

Any method of analysis intended to apply to this case and which shall be expected to become generally adopted, must have the qualities of simplicity and accuracy. It must be borne in mind, however, that the class of materials included under the general head of nitrogen-free extract, are of such a nature as to render them in many cases difficult of accurate determination.

It is believed that the method here presented satisfies these requirements better than any yet proposed. Although as here presented, some details have been modified, the general method has been employed in an investigation upon the carbohydrates of wheat and maize, the results of which have been published by the U. S. Department of Agriculture as Bulletin 34 of the Office of Experiment Stations. The analytical data presented here are selected in part from this report by permission.

The carbohydrates to be found in food materials of a vegetable origin, include sugars of different kinds, starch in various modifications and a certain kind of material, intimately associated with the true cellulose, variously designated as gums, pentosans, hemicelluloses, oxycellulose, and finally the cellulose itself.

For the determination of many of these substances, methods already exist which permit of very fairly accurate determinations, but these methods, as a rule, assume that all other carbohydrates shall be absent so that their application to a material containing several of these substances would lead to serious errors and false conclusions. The most notable example of this is the determination of starch, the ordinary method for which consists in heating the starch containing materials with dilute acids and determining the sugar thus produced by titration with Fehling's solution.

This, as well as other methods for the determination of starch in the absence of other carbohydrates, is of practically sufficient

accuracy, but as has been shown, none of these methods are applicable to the determination of starch in the presence of such other carbohydrates as the pentosans or the hemicelluloses.¹

Again, the methods proposed for the determination of the pentosans are all based upon a destructive distillation of the original material with strong acids and the subsequent determination of the furfurool thus produced. However accurate this final determination may be according to one or the other of several methods, it is obvious that only an approximate knowledge is obtained as to the actual amount of pentosans present in the original material.

It seems also desirable that any plan for the differentiation of carbohydrates in any given material, should, so far as possible, approximate the selective action of the digestive organs in dealing with these same materials. This principle has been recognized in the familiar Weender method for the estimation of crude fiber, which has so long been made use of by food chemists. For example, those substances which are readily soluble in mild reagents will evidently be more easily digested and assimilated than those which are practically insoluble, so that although we may not possess complete knowledge of these same insoluble substances or of their value as food materials, we may still separate them in our analytical tables from those of which we do know more and which evidently have a greater food value.

The following method for the detailed determination of the carbohydrates in vegetable materials is based upon the successive treatment of the same sample: 1, with boiling alcohol to remove sugars; 2, with cold water to remove dextrin and soluble forms of starch; 3, with diastase or malt infusion to remove starch; 4, with dilute boiling hydrochloric acid to convert the gums, pentosans, hemicelluloses, etc., into soluble, reducing sugars; 5, with boiling 1.25 per cent. sodium hydroxide, as is customary in the determination of the crude fiber by the Weender method, leaving behind crude fiber.

In all of these successive operations, linen filters are to be used in order to facilitate the subsequent removal of the sample. With very fine starchy materials it may be advisable to fold a paper filter within the linen one.

¹ This Journal, 16, 726.

In each of the above successive extracts the respective carbohydrates may be estimated by optical or chemical methods, although they are all susceptible of titration by Fehling's solution in the ordinary way.

This method presents certain marked advantages :

1. It permits of the estimation of these constituents in one and the same sample.
2. The process consumes comparatively little time.
3. The final determinations are made by methods already in common use, well understood, and capable of being accurately performed by persons of no great analytical skill.
4. If, by any error of manipulation, any portion of the carbohydrates sought for at any stage escapes solution, it can scarcely fail to be obtained at the succeeding stage, and so there can be no failure to sum up in the end all of the carbohydrates actually present. For instance, any sugar escaping solution in alcohol would be removed by the subsequent treatment with water. Any starch not dissolved by the malt extract would certainly be removed in the subsequent treatment with dilute acid.
5. No violence is done to our present classification of food constituents, only those portions not heretofore classified being separately estimated and recorded. Even the crude fiber remaining after this treatment would be fairly comparable with that determined by the Weender method.

The successive operations may be outlined as follows :

1. The material being finely powdered or grated, from 50 to 100 grams, according to its nature, are accurately weighed and boiled with 500 cc. of strong alcohol, under a reflux condenser, during two hours, or the material may be extracted with boiling alcohol in a Soxhlet extractor. The sugars are thus dissolved and removed by filtering off the alcoholic extract. If this contain only sucrose it may be brought to a given volume and estimated in the polarimeter. If more than one kind of sugar, as for instance sucrose and invert sugar, are present, the alcohol may be carefully evaporated, the sugars taken up in water to a given volume and estimated by titration with Fehling's solution before and after inversion, according to the ordinary treatment in such cases.

2. *The residue from the alcoholic extraction* may contain carbo-

hydrates soluble in water, as for instance soluble starch and dextrin, which are specially to be considered in materials which have been subjected to the action of heat. To this residue, therefore, is added 500 cc. of water and the whole allowed to stand eighteen to twenty-four hours with frequent agitations. The watery solution is then filtered, using a linen filter. With ordinary materials where only dextrin-like substances are to be expected, this filtrate may be evaporated to a small volume, inverted with dilute acids, and titrated with Fehling's solution in the ordinary way. If, however, the solution contain soluble starch in any of its forms, which may be recognized by the iodine test, a further distinction between this starch and dextrin becomes necessary.¹ The watery solution is brought to a volume of 200 cc., and of this an aliquot part is inverted with dilute acid and titrated with Fehling's solution in order to determine the total carbohydrates in the solution. Another aliquot part may be treated with an excess of a solution of barium hydroxide, which precipitates the soluble starch. In the filtrate from this precipitate, the dextrin may be determined by inversion and titration as before. The difference between the dextrin and the total carbohydrates determined in the first aliquot portion may be considered as soluble starch. The use of barium hydroxide for the estimation of the soluble starch directly, according to Von Asboth's method, is not permissible in this place because there are other substances in solution besides starch, which seem to combine with the alkali.²

3. *The residue from the aqueous extract* of the material is now brought to an air-dried condition and its weight determined in order to establish its quantitative relations to the substance as originally weighed out. This step is advisable because smaller portions are more conveniently employed for the subsequent operations.

This material now contains the starch and other insoluble carbohydrates. Two grams are weighed, 100 cc. of water added

¹ By soluble starch is meant starch soluble in water but still giving the characteristic blue color with iodine, in distinction from some of the more completely broken down forms of starch, like dextrin, which no longer give blue or purple colors with iodine. In only rare cases would it be necessary to make this distinction.

² It is thought that barium hydroxide combines with the albuminous substance in solution. At least a much larger amount is precipitated than can be accounted for by the total amount of carbohydrates present.

and boiled thoroughly for thirty minutes in order to convert the starch into a soluble form, then cooled to 60° C. In the meantime an infusion of malt has been prepared as follows: Ten grams of finely ground fresh malt are covered with fifty cc. of water and kept at an ordinary temperature, with frequent agitation, from two to three hours, filtered, and ten cc. of the infusion added to the starch paste, cooled as above.

The starch is subjected to the action of malt extract at a temperature not exceeding 65° C. from two to three hours, or until the blue starch reaction with iodine disappears. The solution is filtered on a linen filter and washed thoroughly with hot water. In many cases this filtration will be facilitated by previously heating the solution to boiling and filtering hot. The residue on the filter should give no starch reaction with iodine. The filtrate is evaporated to 100 cc., transferred to a 200 cc. flask, receives ten cc. of concentrated hydrochloric acid, and the flasks with contents kept at the temperature of a boiling water bath for one hour in order to convert all of the malt sugar into dextrose. The solution is then neutralized, made up to a volume of 200 cc., and titrated with Fehling's solution. A correction must of course be employed for the amount of sugar introduced in the malt extract.

4. *The residue from the malt extraction* still contains the gums, pentosans, hemicelluloses, and true cellulose. It is carefully removed from the filter, receives 100 cc. of water and two cc. strong hydrochloric acid, and is heated to boiling under a reflux condenser or in a water bath for one hour.

The action of the acid converts the gum and pentosans into reducing sugars, which may be regarded as xylose.¹ This is then filtered, neutralized, made up to 200 cc. and titrated with Fehling's solution.

5. We have now reached a stage corresponding to that attained in the determination of crude fiber by the Weender

¹ Repeated examinations of the character of this material shows that in most of the fibrous or woody vegetable materials it is xylan and yields on hydrolysis mainly xylose. This is the case especially in all materials containing any considerable amount of hydrolyzable carbohydrates of this kind, although in some special cases mannose, galactose, or arabinose have been noted as products of this treatment. It is thought sufficient, however, for all general purposes to regard this product as xylose, since the error introduced by the varying reducing coefficients of small amounts of the other sugar would be nominal.

method after the material has been boiled with one and one-fourth per cent. sulphuric acid. It remains, therefore, to treat this residue with 1.25 per cent. sodium hydroxide, to filter, dry, weigh, ignite and weigh again, in order to obtain what is commonly known as "crude fiber."

It is necessary now to consider the accuracy of each of these steps in detail, and to present examples of their application. For the purpose of illustration the following materials have been examined according to the plan here presented: Wheat, two samples; wheat flour, two samples; maize; bread made from each of the samples of whole wheat, flour, and maize; sugar beets, and a sample of timothy hay.

1. THE ESTIMATION OF SUGARS BY EXTRACTION WITH BOILING ALCOHOL.

It is hardly necessary to discuss this method since it is the generally accepted way of separating sugars from other materials either for qualitative or quantitative analysis. All of the ordinary sugars are soluble in boiling alcohol of ninety-five per cent. strength, especially when sufficient volume is employed as is the case in the specifications of the method here presented. It is even applicable to fresh materials, such as the sugar beet, potato, etc., in the presence of a large amount of moisture.

In the treatment of many materials, the hot alcohol also dissolves waxes and oils, which are readily removed by evaporating the solution nearly to dryness and then taking up in water. The polarimeter may be employed in many cases for determining the sugar thus extracted, in preference to the method of inversion and titration.

The following examples will serve to show how sugars, in small or large quantities, may be determined in a variety of materials and with accuracy, by means of this method:

SUGAR OBTAINED BY EXTRACTION WITH BOILING ALCOHOL.

	Sucrose. Per cent.	Invert sugar. Per cent.
Wheat I	0.51	0.08
Wheat II	0.72	0.00
Wheat flour I	0.18	0.00
Wheat flour II.....	0.20	0.00
Maize	0.27	0.00
Sugar beet (Fresh)	8.38	0.07
Hay (Timothy).....	2.53	0.00

	Sucrose. Per cent.	Invert sugar. Per cent.
Bread (Whole wheat I).....	0.05	0.32
Bread (Whole wheat II)	0.06	0.37
Bread (Wheat flour I)	0.01	0.10
Bread (Wheat flour II)	0.15	0.38
Corn cake (Maize).....	0.16	0.19

2. THE ESTIMATION OF DEXTRIN AND SOLUBLE STARCH.

These materials, while insoluble in alcohol, are readily dissolved in water at ordinary temperature.

Usually grains and seeds in their natural condition contain very small quantities of materials of a carbohydrate nature, insoluble in alcohol and soluble in cold water which it is sufficient to designate here as dextrin. Some attempts to identify these bodies in a more specific manner, have been made. In this place, however, it is only necessary to say that they are readily changed into sugars by inversion with acids and behave, to all intent and purpose, like dextrin. If, however, the material has been subjected to heat or friction, as in the grinding of corn or flour, or to the action of acids, then a portion of the starch may have been changed into soluble forms and will accompany the dextrin in the watery solution. The presence of such starch will be readily detected by means of the blue color produced with iodine.

In the absence of such starch, it is sufficient to estimate the dextrin by inversion with acids. When, however, soluble starch is present, the separate estimation of the two becomes a more difficult matter. Ordinarily the small amount of dextrin would render a separate estimation of the two hardly necessary, in which case the watery extract might be at once inverted with acids and the total soluble carbohydrates estimated in this way.

Evidently there will be little occasion to assign different values to these two forms of starch products even after they are separately estimated. If desirable, however, the starch may be completely precipitated with barium hydroxide, according to the plan proposed by Von Asboth, and a separate estimation made as indicated above.

These separations have been made in some of the analyses presented below, and there is no reason to doubt that they are sufficiently accurate.

DEXTRINS AND SOLUBLE STARCH EXTRACTED BY COLD WATER.

	Dextrin. Per cent.	Soluble starch. Per cent.
Wheat I.....	0.27	0.00
Wheat II	0.41	0.00
Wheat flour I.....	0.90	0.00
Wheat flour II.....	1.06	0.00
Maize	0.32	0.00
Sugar beet (Fresh)	0.35	0.00
Hay	1.18	0.00
Bread (Whole wheat I).....	0.68	1.37
Bread (Whole wheat II)	0.23	2.36
Bread (Flour I).....	0.27	1.99
Bread (Flour II).....	0.91	1.74
Corn cake (Maize).....	0.00	2.80

3. THE ESTIMATION OF STARCH BY TREATMENT WITH MALT EXTRACT.

The residue from the preceding treatment consisting of many grams of material, is too bulky for subsequent operations. In order, therefore, to secure smaller portions of definite quantitative relations to the original fifty or one hundred grams taken, these residues are desiccated, weighed, and the amount of dry matter contained therein is determined. The proportion between this dry matter and that in the original material may then be established. From this, then, two grams are taken for the determination of starch and other remaining carbohydrates.

Many methods have been proposed for the determination of starch, any of which are fairly satisfactory when applied to starch in the absence of the other carbohydrates. A review of these methods and comparison of their values will be found in a former paper by the author,¹ and also in papers by H. C. Sherman² and by P. Hibbard,³ but all of these methods give inaccurate results when applied to materials which contain any of the pentosans or gums or easily hydrolyzable forms of cellulose, since these latter materials are affected in the same way as starch and give similar products with the same reagents.

They are not, however, appreciably affected by the action of diastase,⁴ and thus far it appears that this is the only agent which

¹ This Journal, 16, 726.

² *School of Mines Quarterly*, Columbia College, 17, 356.

³ This Journal, 17, 64.

⁴ The somewhat obscure action of malt extract on cellulose mentioned by Brown and Morris, as well as a similar action upon hemicelluloses, is of very uncertain extent under any conditions and is practically checked at 60°. J. Grüss: *Wochenschr. Brauerei*, 1895, 1257.

may be employed for the accurate separation of starch from these other substances.

As to the efficiency of this method for the complete removal of starch, there seems to have been some question. It also appears from recent investigation by the author that not all starches are affected in the same manner or degree by diastase. It may be found necessary in different cases, to subject the material to the action of diastase for a longer or shorter time. The operation, however, is wholly within the control of the analyst, since the final extraction of starch from the material can be detected by the iodine reaction. Being once in solution, it is then readily separated from the insoluble residue and converted into sugar by the action of dilute acids as above described.

The following table shows the results obtained upon the material previously extracted and examined for other carbohydrates as described above.

These data were obtained by the use of more dilute acids for the hydrolysis of the malt extract, than is recommended above. The percentages are therefore somewhat lower than those usually ascribed to these materials. With the strength of acid prescribed there is no difficulty in obtaining complete conversion of the starch products into sugar.

NORMAL STARCH DISSOLVED BY MALT EXTRACT.

	Per cent.
Wheat I.....	30.94
Wheat II.....	30.36
Wheat flour I.....	46.19
Wheat flour II.....	34.04
Maize	42.50
Sugar beet(Fresh).....	0.00
Hay.....	7.49
Bread (Whole wheat I).....	27.93
Bread (Whole wheat II).....	27.08
Bread (Wheat flour I)	39.70
Bread (Wheat flour II).....	31.99
Corn cake (Maize).....	40.37

4. ESTIMATION OF THE PENTOSANS AND HEMICELLULOSES BY DILUTE ACIDS.

After removing the more easily soluble constituents of the material as already described, there remains the somewhat insoluble substance incorporated in the cell walls and intimately associated with the true cellulose.

This woody or fibrous material is very far however from possessing a homogenous character, but consists of some of the most complex and least understood of the carbohydrate group, as well as some substances which cannot be regarded as carbohydrates at all.

Tollens regards the lignified cells as composed of three general classes of substances: cellulose, wood gum or pentosans, and bodies belonging undoubtedly to the aromatic group. This last is of non-carbohydrate nature. Of the first two, cellulose may be regarded as resistant to any of the reagents here proposed, while the wood gum or pentosan is easily hydrolyzed by dilute acids. In many materials other substances similar to the pentosans will occur, as for instance, galactan, inulin, dextran, even of substances of the nature of pectin, but all these behave like the pentosans to hydrolytic agents and yield specific sugars. Recent researches by Cross and Bevan throw much light upon the constitution of these non-cellulose materials. The hydrolyzable portions seem to be largely of the nature of pentosans or their derivatives.¹ The most rational treatment of the material at this stage would therefore seem to be such as would dissolve this group of similar substances and convert them into a form capable of ready estimation. For this purpose, treatment with dilute hydrochloric acid has been found preferable to any other.

The reagent was selected because, as shown by Couclicr,² it possesses superior hydrolytic power to sulphuric acid towards these very substances. Repeated use of hydrochloric acid in this laboratory for the purposes of hydrolyzing large quantities of gums and similar materials has shown that its action is quicker and that fewer by-products result than when sulphuric acid is used.

A recent paper by Cross, Bevan and Smith,³ suggests the use of one per cent. sulphuric acid for this purpose, and heating in an autoclave during thirty minutes at a pressure of three atmospheres. Their results do not indicate a greater degree of efficiency than the method here presented, while the operation would prove less convenient for many analysts.

With regard to the duration of treatment and the strength of

¹ *Chem. News*, 74, 175; *Ber. d. chem. Ges.*, 29, 1457.

² *Chem. Ztg.*, 16, 1719.

³ *Ber. d. chem. Ges.*, 29, 1457.

acid to be employed, a systematic study has indicated that one hour's boiling under a reflux condenser with 100 cc. of one per cent. hydrochloric acid, gave the most satisfactory results. The accuracy of this conclusion was determined by ascertaining the conditions which yielded the largest amount of reducing sugar without appreciably diminishing the amount of crude fiber obtained at the close of the analysis, it being thought undesirable to increase the intensity of this reaction at the expense of the fiber. After one hour's boiling with the acid of the concentration mentioned, there remains in the material only very slight traces of soluble matters which could be converted into reducing sugars.¹

In the absence of other bodies similar to the pentosans this method will undoubtedly furnish a quicker and more accurate method of determining the latter than the well known destructive distillation with strong hydrochloric acid and subsequent estimation of the furfural thus found.

As a factor for the reducing coefficient of the sugars thus formed, that of xylose has been chosen as probably representing the most common and most abundant sugar present under these conditions (one cc. Fehling's solution requires for complete reduction 0.004617 gram of xylose.)²

The equivalent of xylose in xylan as originally present in the material may be estimated after the manner of finding the equivalent of dextrose in starch, according to the proportion



PENTOSANS DISSOLVED BY BOILING WITH ONE PER CENT. HYDROCHLORIC

	ACID.	Per cent.
Wheat I.....		4.54
Wheat II.....		4.37
Wheat flour I.....		0.00
Wheat flour II.....		0.00
Maize.....		5.14
Sugar beet (Fresh).....		4.89
Hay.....		7.14
Bread (Whole wheat I).....		4.16
Bread (Whole wheat II).....		4.34
Bread (Wheat flour I).....		0.00
Bread (Wheat flour II).....		0.00
Corn cake (Maize).....		3.54

¹ Cross, Bevan and Beadle, *This Journal*, 18, 8, state that the residue from a successive treatment by boiling alcohol, dilute sodium hydroxide, and dilute hydrochloric acid is free from pentosans and all the more readily hydrolyzable constituents.

² *Am. Chem. J.*, 13, 73.

5. ESTIMATION OF CRUDE FIBER.

The residue from the preceding treatment still contains in addition to the actual fiber or cellulose a considerable amount of a substance not hydrolyzable to reducing sugars, by means of dilute acids, but which is readily soluble in dilute alkalis. This frequently forms a considerable proportion of the nitrogen-free extract. Repeated treatment of such alkaline solutions with dilute acids (after neutralizing the alkali) have failed to produce any considerable amount of reducing sugars. An investigation of the nature of this substance is now in progress, but there seems little ground at this time for regarding it as of the nature of a carbohydrate. It probably deserves separate classification as non-nitrogenous and non-carbohydrate in its nature.

The residue from treatment with dilute acid is therefore boiled under a reflux condenser for thirty minutes with 200 cc. of 1.25 per cent. sodium hydroxide, filtered, washed, dried, weighed, and ignited in the manner prescribed for the ordinary estimation of crude fiber.

Following are the results obtained in this way :

CRUDE FIBER RESULTING AFTER TREATMENT WITH BOILING ALCOHOL,
COLD WATER, MALT EXTRACT, BOILING ONE PER CENT. HYDRO-
CHLORIC ACID AND BOILING ONE AND ONE-FOURTH
PER CENT. SODIUM HYDROXIDE.

	Per cent.
Wheat I.....	2.68
Wheat II.....	2.51
Wheat flour I.....	0.25
Wheat flour II.....	0.25
Maize	1.99
Sugar beet (Fresh).....	1.00
Hay.....	34.34
Bread (Whole wheat I).....	2.70
Bread (Whole wheat II).....	2.02
Bread (Wheat flour I).....	0.34
Bread (Wheat flour II).....	0.17
Corn cake (Maize).....	2.22

The percentage of crude fiber obtained in this way will not be essentially different from the results obtained by the Weender method.

A better comprehension of the significance of these results

will be had from their collective presentation in the following table. From this it will be seen that in each material six, or in some cases, seven forms of carbohydrates have been quantitatively determined, the results showing at a glance the comparative value of the material for use either as a food or for technical purposes, as no other existent method of analysis could do. The sum of these various items constitute of course the total carbohydrates in the respective materials.

TABLE SHOWING THE RESPECTIVE PERCENTAGES OF DIFFERENT CARBOHYDRATES IN CERTAIN FOOD-STUFFS.

Material analyzed.	Sucrose.		Invert sugar.		Dextrin.	Soluble starch.	Normal starch.	Pentosans.	Crude fiber.
	Per cent.	Per cent.	Per cent.	Per cent.					
Wheat I.....	0.52	0.08	0.27	0.00	30.94	4.54	2.68		
Wheat II.....	0.72	0.00	0.41	0.00	30.36	4.37	2.51		
Wheat flour I.....	0.18	0.00	0.90	0.00	46.19	0.00	0.25		
Wheat flour II.....	0.20	0.00	1.06	0.00	34.04	0.00	0.25		
Maize	9.27	0.00	0.32	0.00	42.50	5.14	1.99		
Sugar beet (Fresh)....	8.38	0.07	0.35	0.00	0.00	4.89	1.00		
Hay	2.53	0.00	1.18	0.00	7.49	7.18	34.34		
Bread (Whole wheat I)	0.05	0.32	0.68	1.37	27.93	4.16	2.70		
Bread (Whole wheat II)	0.06	0.37	0.23	2.36	27.08	4.34	2.02		
Bread (Wheat flour I)	0.01	0.10	0.27	1.99	34.70	0.00	0.34		
Bread (Wheat flour II)	0.15	0.38	0.91	1.74	31.99	0.00	0.17		
Corn cake (Maize)....	0.16	0.19	0.00	2.80	40.37	3.54	2.22		

In many cases it is believed that the total amount of carbohydrates thus definitely determined in food materials will be found to be appreciably less than the nitrogen-free extract estimated by difference. If subsequent research should justify this belief, the character of this non-carbohydrate and non-nitrogenous substance would remain to be determined. This question is now being investigated and for the present the writer desires to reserve the subject of inquiry to this laboratory.

This outline is thought to present an analytical method superior to anything at present available for the estimation of carbohydrates in foods, although it cannot be regarded as in any sense perfected. It will have served its purpose if it shall suggest to others the necessity of something better and assist them in the search for it.