[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF PURDUE UNIVERSITY.]

A COMPARISON OF METHODS FOR THE DETERMINATION OF STARCH.

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THERE is no longer much doubt among chemists that, in food analysis, the present practice of classifying a large number of widely varying substances under the head of non-nitrogenous extract matter, as an homogeneous material, is wholly erroneous and misleading. The variety of substances covered by this term is well known, and, while it is granted that they are mainly of carbohydrate nature, it is equally clear that, so far as food valuation is concerned, there should be some degree of differentiation between sugars, starches, gums, and the more or less soluble forms of cellulose.

The position of the pentosans in this class of bodies has been pointed out in its different aspects by myself and others at intervals during the past four or five years. It has been shown that these bodies form a distinct constituent of the non-nitrogenous extract matter to a greater or less degree in all fodder articles; that under present analytical methods their individuality as food constituents is wholly merged into that of the more valued carbohydrates; some light has also been thrown upon their digestibility, and analytical methods of a fair degree of accuracy have been devised for their estimation in the presence of other similar bodies.

Mention is made of this instance here to show that really some progress has been made, and that too within a short time, in our knowledge of what, until recently, has been an obscure constituent of the non-nitrogenous extract matter.

The object of this present paper is to call attention along the same line to certain proposed methods for the quantitative estimation of probably the most important of these bodies; *viz.*, starch. At first thought it may appear that the existing methods for the determination of starch are sufficiently satisfactory and accurate; and this is, doubtless, true so far as starch alone is concerned. But it must be remembered that starch almost always occurs in connection with other soluble non-nitrogenous matters, under which conditions its estimation becomes quite another matter and constitutes exactly the question under discussion. The pentosans or gums are, doubtless, the most common of these accompanying substances and, unfortunately for the analyst, behave toward many analytical methods precisely like starch. Hence, a result which is interpreted to mean starch by a strict adherence to the method, may be due, to a very large degree, to other substances. For example, brewers' grains, which from the very process through which they have passed contain little or no starch, will by the ordinary method of inversion and titration be credited with a large amount of starch. Again, hay or straw yield considerable amounts of reducing sugars on inversion, although they contain a minimum of starch, or even none at all. In both these cases the results are due to the inversion of insoluble gums which are present in large quantities.

With these facts in mind it is apparent that a food analysis should give some information with regard to the relative amounts of these different substances present, and should include determinations of the pentosans, sugars, and starches separately.

In this connection the inquiry arises as to how accurately it is possible to determine these bodies as they occur in connection with each other in the ordinary feeding stuffs, by current and accepted methods. As regards the pentosans and sugars, the state of our knowledge seems far more satisfactory than in the case of the starches, in proof of which proposition the following data are offered.

Five methods of starch determination have been compared upon a variety of materials. Four of the methods involve the inversion of the starch and the estimation of the sugar thus formed by volumetric or optical methods; the fourth method is based upon a combination of the starch with one of the alkaline earths. Following are the details of the different methods:

1. Inversion of the starch with hydrochloric acid and titration with Fehling's solution, known as Sachsse's method: Three to five grams of the starch bearing material are heated in a water-bath with 200 cc. of water and twenty cc. of concentrated acid,

during three hours; the acid is neutralized with soda, the solution filled to 500 cc., and titrated with Fehling's solution in the usual manner.

2. Inversion with nitric acid and polarization, proposed by Guichard, *Bull. Soc. Chim.*, [3,] **7**, 554: Three grams of the material are heated in a water-bath during one hour with 100 cc. of ten per cent. nitric acid, filled to 200 cc. and polarized. The readings are calculated to dextrose.

3. A modification of the preceding: Three grams of material are heated in the water-bath with 100 cc. of a saturated solution of oxalic acid during one hour, the object being to bring the starch into solution. The liquid is cooled and filled to 200 cc. with ten per cent. nitric acid, filtered, and the filtrate heated in the water-bath one hour, attached to an inverted condenser. The solution is then polarized. This method is preferable to the other in that the cellulose and other insoluble substances are removed from the influence of the nitric acid.

4. Inversion of the starch by salicylic acid and polarization, proposed by A. Baudry, *Zeit. f. Spiritus Industrie*, **15**, 41, 42: A normal weight, 5.376 grams is adopted for the Laurent polariscope. This weight, or some multiple of it, is placed in a 200 cc. flask with 100 cc. of water and four-tenths to one-half gram of salicylic acid and heated in a water-bath for thirty minutes; fill to within twenty cc. of the mark with hot water, cool rapidly, clarify by adding a few drops of ammonia, filter and polarize in a 400 mm. tube. The readings on the percentage scale indicate percentages. In our practice, sodium hydroxide was used instead of ammonia for clarifying.

5. Precipitation of the starch from the condition of paste by barium hydroxide, and determination of the excess of the latter by titration with a standard acid, proposed by A. von Asboth, in 1887: One gram of the material, from which the fat has been extracted, is boiled with water to convert the starch into a paste; twenty-five cc. of a solution of barium hydroxide of known strength is run in and the whole allowed to cool; then add 100 cc. of eighty-five per cent. alcohol and fill to 200 cc. A compound of starch is formed containing 19.1 per cent. of barium oxide, which is precipitated by the dilute alcohol. The solution is fil-

tered and the excess of barium hydroxide is determined by titrating aliquot portions with standardized hydrochloric acid, using phenolphthalein as an indicator.

The materials to which these methods were applied included nine different samples, as follows: Pure potato starch, wheat bran, wheat middlings, wheat flour, dried potatoes, corn-meal, hay, cottonseed-meal, and a mixture of starch, sugar, and dextrine. Four of these were samples sent out by the reporter on feeding stuffs of the Association of Official Agricultural Chemists. Before examination, the samples were extracted with water and ether to remove soluble carbohydrates and fats.

The results obtained are best shown by tabulation, as follows:

Materials.	Inversion by HCl.	Inversion by HNO_3.	Inversion by oxalic acid and HNO_3.	Solution by salicylic acid.	Precipitation by $Ba(OH)_2$.
1. Pure potato starch·······	85.75	85.50	85.75	85.47	85.58
2. Dried potato·············	70.92	69.79	68.53	....	64.25
3. Wheat flour ·············	77.69	70.65	65.29	69.38	59.76
4. Corn-meal ···············	73.24	66.81	70.55	....	62.11
5. Wheat bran··············	65.86	40.25	38.68	....	70.77
6. Hay ····················	3.48	19.10	19.10	....	66.47
7. Wheat middlings········	30.00	63.09	60.24	....	60.44
8. Cottonseed-meal ········	4.15	....*	....*	....	54.65
9. Mixture of starch, sugar, and dextrine··········	9.58	21.00	24.08	18.8	33.99

* These solutions were laevorotatory.

From a survey of these data it appears that any of the methods employed, give satisfactory results when dealing with starch alone. It is important to recognize this in order that the responsibility for the discrepancies in connection with the other materials may be properly placed where it belongs; *viz.*, that the variations are due to the complex nature of the materials and not to the method. Allowing for moisture and cellulose, the starch was pure and each method gave credit for the entire amount present. Turning to the other materials the following is to be noted.

1. Inversion by hydrochloric acid. The starch is converted into dextrose and under carefully controlled conditions the reaction is quantitative. Wohl has shown that a very small quantity of acid is efficient in producing complete inversion.

(*Ber. d. chem. Ges.*, **23**, 2084.) According to Märcker, the method of Sachsse here employed gives slightly low results owing to a slight destruction of dextrose by long heating with the acid. This objection does not appear here in comparison with the other methods. There is undoubtedly danger in the other extreme in not heating long enough. The samples numbered 6, 7, 8 and 9 were treated according to the directions sent out by the A. O. A. C. which call for the removal of heat as soon as the starch-iodine reaction disappears. It is possible that under these conditions which are accomplished in a very short time, an appreciable amount of starch does not pass beyond the dextrine stage and escapes detection by Fehling's solution. In this connection it is noticeable that the samples 6, 7, 8 and 9 treated in this way, gave lower results by this than by the other methods.

2. The methods calling for the use of nitric acid seem unusually destructive, yet in the case of pure starch they have given results fairly comparable with the others. As originally proposed by the author, it was feared that the cellulose would be attacked, and the use of oxalic acid as in the third method was proposed. This provides for the solution and removal of the starch by a comparatively mild reagent and this solution is then inverted by the nitric acid apart from the accompanying constituents of the material. The method has a certain advantage of ease and rapidity; the solutions obtained for polarization are also beautifully clear and admit of accurate readings. The effect of the direct action of the acid in the second method is not so marked as might be expected.

3. Both salicylic and benzoic acids completely dissolve starch; the solutions have a right-handed polarization and the degree of rotation is directly proportional to the amount of starch present. The method was originally proposed for the valuation of commercial starches and with such materials is fairly accurate. With feeding stuffs, however, the resulting solutions are frequently too highly colored and opaque to admit of examination in the polarimeter. The method is therefore of very limited application.

5. The use of barium hydroxide as a means of combining and

precipitating starch from solution has attractive features and has been both highly praised and severely criticised. It necessitates the previous removal of all oils from the material. In our hands the results obtained have been somewhat erratic.

One fact is apparent in connection with the above data, if we leave out of consideration the pure starch sample. The results are more or less discordant and in some cases quite unexplainable. For instance, the hay and cottonseed-meal when boiled with water, do not give the iodine reaction for starch, yet each of these special methods for the determination of starch credit them with from four to sixty per cent. of the same. Even fifteen minutes warming with very dilute hydrochloric acid gives an appreciable amount of what we are bound to interpret as starch. This brings us to the inevitable conclusion, that other bodies than starch are present and respond toward each of these reactions in the same way as starch does. We know moreover that the pentosans are such bodies and that they are invariably present to a greater or less degree in materials of the kind under examination.

In order to obtain some experimental data bearing upon the subject, portions of an isolated sample of pentosans were subjected to each of the methods under discussion. The material was, properly speaking, *xylan*, since on inversion it yields only xylose. It was obtained from wheat straw by boiling the same with lime-water and precipitating the xylan with alcohol. It possessed as high a grade of purity as has thus far been attainable in the preparation of these materials. It was free from any other form of carbohydrate and contained about six per cent. of ash materials.

One gram of xylan heated for thirty minutes with fifty cc. of water and four cc. of strong hydrochloric acid was entirely dissolved. The solution was neutralized and made up to 100 cc. Of this 6.7 cc. were required to reduce the copper from ten cc. of Fehling's solution, a result equivalent to 67.16 per cent. of starch.

One gram of xylan was heated with fifty cc. of ten per cent. nitric acid for one hour and became completely dissolved. The solution was made up to 100 cc. and polarized. The reading was equivalent to that produced by 44.73 per cent. of starch.

One gram of xylan was treated with fifty cc. of a concentrated solution of oxalic acid for one hour by which it became completely dissolved, made up to 100 cc. with ten per cent. nitric acid and heated another hour. The polariscope readings were the same as in the previous case, equivalent to 44.73 per cent. starch.

The salicylic acid method was not applicable.

One gram of xylan was boiled with thirty cc. of water, twenty-five cc. of barium hydroxide solution was added and afterwards thirty-five cc. of eighty-five per cent. alcohol and the whole made up to 100 cc. with water. Upon titration of the excess of barium hydroxide, an equivalent of 47.48 per cent. of starch was indicated.

These results show conclusively that the pentosan which is most characteristic of feeding stuffs and which has been shown to occur in all such materials, behaves, towards the reagents named, in precisely the same manner as starch and in a no less-marked degree. The conclusion is unavoidable that none of the methods for determining starch, based upon the above principles, can be employed with any degree of accuracy upon grains or forage plants or any materials where the presence of these pentosans is probable.

The most hopeful way of avoiding these difficulties would seem to be some method of bringing the starch into solution and removing it from its accompanying carbohydrates without any corresponding influence upon them. One method seems to offer this advantage; *viz.*, the application of diastase or malt infusion to the starch containing material. This method is recommended for use among the European Experiment Stations; it has been shown to yield accurate results with pure starch and can be objected to, if at all, only on account of length of time required and the difficulty of always having a proper preparation of diastase or malt infusion at hand. These are, however, minor objections in the light of greater accuracy secured. The general features of the method are as follows: The weighed material is boiled with water (most effectively under pressure) to convert the starch into a paste; after cooling to 65° C. a small quantity of malt infusion is added and the temperature maintained at 60° to 65° for a half hour. The starch reaction having disappeared the solution may be filtered off washing the residue thoroughly, the filtrate warmed with a few cc. of acid to complete the conversion into dextrose and finally neutralized, made up to definite volume, and titrated. It remains to be seen what the effect of this process may be upon the pentosans or other similar bodies, concerning which there are few data at hand. I have made some preliminary tests which indicate that these bodies are not affected by diastase. One gram of the xylan, already mentioned, after boiling with water received ten cc. of a fairly strong infusion of malt and was kept at 65° C. for a half hour. At the same time a sample of starch was boiled to a paste, and treated with the malt infusion in the same way. The starch reaction disappeared and the solution reduced Fehling's solution strongly but the xylan did not change the Fehling's solution in the least nor did it appear to have been altered.

It seems, therefore, that in this last method the difficulties presented by the more common methods are to be avoided. The ordinary inversion methods on the other hand furnish no accurate conclusion when applied to the determination of starch contained in vegetable tissues.

For assistance in much of the analytical work cited I am indebted to Mr. D. B. Hoffmann.

NOTES ON THE HARDENING OF MORTAR.[1]

BY WM. P. MASON.

THE following is extracted from the graduating thesis of Mr. J. A. McPherson, of the class of 1894, Rensselaer Polytechnic Institute, the work having been done in my department:

It is common belief among builders, that it is better practice to mix lime mortar and let it lie in a heap some days previous to use, rather than to employ it directly after preparation. In order to test this point, samples of mortar were taken, on successive days, from two separate heaps, of large size; briquettes were made therefrom, and, after an interval of some weeks, were broken for estimation of tensile strength with the following results:

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[1] Read at the Brooklyn Meeting, August 16, 1894.