Issued September 18, 1912.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—CIRCULAR 203.

A. D. MELVIN, CHIEF OF BUREAU.

A METHOD FOR THE DETERMINATION OF STARCH IN MEAT FOOD PRODUCTS.

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METHODS FOR DETERMINING STARCH.

A large amount of work has been done on methods for the determination of starch both in this country and abroad. Most of this work has been devoted to methods for estimating the starch in cattle foods and other vegetable substances. The first work undertaken in this country with a view to obtaining a reliable standard method for estimating starch was reported by Stone.[1] This report consisted in a comparison of methods for the determination of starch, the methods compared being:

1. Inversion of the starch with hydrochloric acid and titration with Fehling solution.
2. Inversion with nitric acid and polarization.
3. Modification of No. 2, the starch being first brought into solution by oxalic acid.
4. Inversion of starch by salicylic acid and polarization.
5. Precipitation of starch by barium hydroxid and determination of the excess of the latter by titration with standard acid.

The materials on which these methods were tried included potato starch, wheat bran, corn meal, and hay. The author concludes from his results that any one of the methods gives satisfactory results when dealing with starch alone, but it is recommended that in the presence

[1] Bulletin 43, Bureau of Chemistry, U. S. Department of Agriculture.—Proceedings Association of Official Agricultural Chemists, eleventh annual convention, 1894.

of such interfering substances as pentosans the starch be first brought into solution through the action of diastase or malt infusion.

Patterson [1] as referee made a report before the twelfth annual convention of the Association of Official Agricultural Chemists and gave the results obtained through the use of Sachsse's [2] and Maercker's [3] methods for starch determination. He recommended that Sachsse's method be adopted as a provisional method for the estimation of starch in by-product cattle foods.

At the twelfth annual convention of the same association certain methods of analysis were adopted and published,[4] and under "Methods of Analysis of Cattle Foods" a method is given for the determination of starch. This method consists in stirring 3 grams of the sample in a beaker with 50 c. c. of water for an hour and filtering. The insoluble residue is heated for 2½ hours, with 200 c. c. of water and 20 c. c. of hydrochloric acid, in a flask, provided with a reflux condenser, after which it is cooled and neutralized with sodium carbonate. The volume is completed to a quarter of a liter, filtered, and the dextrose is determined in an aliquot portion of the filtrate. The weight of dextrose obtained multiplied by 0.9 gives the weight of starch.

The next report on starch made before the association was presented by Lindsay [5] in 1897. Lindsay recommended that the diastase method be declared the provisional method for the determination of starch in all substances excepting commercial starch and potatoes.

During the next four years a few minor changes were made in the diastase and acid-inversion methods, but these were the only two methods for estimating starch that were recognized as provisional methods.

METHODS FOR DETERMINING STARCH IN CHOPPED MEATS.

At the meeting of the Association of Official Agricultural Chemists held in 1900 it was decided to make a systematic effort to outline methods for food examination, and with this in view the referee in charge of food adulteration was instructed to secure the cooperation of associate referees, each of whom should prepare methods for the examination of one or more classes of foods. The reports when completed were forwarded to the referee, printed, and distributed to about 250 chemists for suggestions and criticisms. The methods as amended were adopted provisionally at a meeting held in Novem-

[1] Bulletin 47, Bureau of Chemistry, U. S. Department of Agriculture.
[2] Chemisches Central-Blatt, 1877, p. 752.
[3] Chemiker Zeitung, vol. 9, p. 319.
[4] Bulletin 46, Bureau of Chemistry, U. S. Department of Agriculture.
[5] Bulletin 51, Bureau of Chemistry, U. S. Department of Agriculture.

ber, 1901.[1] The referee in submitting these methods stated that he considered that only a beginning had been made and that experience would indicate that numerous changes in the methods would be advantageous. Under the heading "Determination of Starch in Chopped Meats," the referee stated that the official methods of the association for the determination of starch will not answer for the examination of meat because of the presence in meats of bodies which hold a portion of the cuprous oxid in solution and thus give results that are too low, and in such cases recommends the following two methods:

Ambühl's method.[2] "From 2 to 10 grams of the meat under examination, according as it is finely or coarsely subdivided, are thoroughly macerated with 50 times their weight of water; boil for 30 minutes and dilute to 100 c. c. for each gram of meat employed. A portion of the clear liquid is cooled, treated with iodin, and the depth of color compared with solutions containing a known amount of the same kind of starch boiled for the same length of time."

This method has been adopted by the Swiss authorities. It is short and convenient, although the results obtained by it are only roughly approximate.

Bigelow's modification of Mayrhofer's method.[2] From 10 to 20 grams of the sample under examination (according as the iodin reaction shows a small or large amount of starch) are treated in a porcelain dish or casserole with 50 c. c. of an 8 per cent aqueous solution of potassium hydroxid, and the mixture heated in the water bath until the meat is entirely dissolved. The operation may be hastened by rubbing the larger pieces with a glass rod. An equal volume of 95 per cent (by volume) alcohol is now added and the mixture filtered (after the precipitate has subsided) through a starch-free filter paper and washed twice with a hot 4 per cent solution of potassium hydroxid in 50 per cent alcohol, and then with 50 per cent alcohol until a small portion of the filtrate does not become turbid on the addition of acetic acid. The precipitate and filter are returned to the original vessel and dissolved with 60 c. c. of a normal solution of potassium hydroxid with the aid of heat. A somewhat larger volume of alkali is required by sausage that has a high starch content. The filtrate is transferred to a 100 c. c. flask, acidified with acetic acid, diluted to a convenient volume, filtered through a ribbed filter, and the starch precipitated from an aliquot part of the filtrate by an equal volume of 95 per cent alcohol. The precipitate is then transferred to a weighed filter, thoroughly washed with 50 per cent alcohol, with absolute alcohol, and finally with ether, and dried to constant weight at 100°."

Since these provisional methods were adopted in 1902 there have been no changes made or suggested, and they are now given in Bureau of Chemistry Bulletin 107 (revised) as the provisional methods for determining starch in meat food products.

As Ambühl's method is only roughly approximate, it need not be considered here, especially in view of the fact that mixed starches

[1] Bulletin 65, Bureau of Chemistry, U. S. Department of Agriculture.
[2] Bulletin 13, Part 10, Bureau of Chemistry, U. S. Department of Agriculture.

are often used in the preparation of meat food products, and in such cases it is impossible to use standards that are recommended in carrying out this method.

Bigelow's modification of Mayrhofer's method, as given above, is generally considered the most practical method for the determination of starch in meat food products. However, the writer having had occasion to make determinations of starch in a number of sausages, made a thorough study of this method, from a standpoint of both accuracy and practicability, and found that it not only could not be depended upon to give accurate results, but it was not practicable, as it often took several days to make the necessary filtrations. The results of this study showed conclusively that some improvement in the method of determining starch in meat food products was necessary. Upon making an investigation of the methods used abroad, it was found that, besides those already referred to, a recent method had been proposed by Perrier[1] for determining starch in sausages. The method of Perrier is as follows:

Five grams (if the starch content is low—less than 2 per cent—which will be indicated by a preliminary test, use 10 grams) of finely divided sausage meat is placed in a 250 c. c. flask with 100 c. c. of water containing 3 c. c. of hydrochloric acid. The flask is then placed in the autoclave. When the temperature has reached 120° it is maintained there for 40 minutes; then the flask is removed. Almost all the material is found to be dissolved. After cooling, the liquid is decanted upon a funnel, the neck of which is closed by asbestos or glass wool lightly packed, which will retain the solidified fat and the undissolved particles. The filtrate is received in a 200 c. c. graduated flask. The residue remaining in the flask is washed twice with a little warm water, the wash water not being poured upon the funnel until it has been completely cooled.

The filtrate is then freed of the albuminoid substances which it contains by means of phosphotungstic acid in the presence of hydrochloric acid. To do this, add 2 c. c. of concentrated hydrochloric acid and a slight excess of a 20 per cent solution of phosphotungstic acid (10 c. c. is usually sufficient). Under these conditions the precipitate which is formed settles rapidly, and the supernatant liquid is clear and colorless. The volume is brought to 200 c. c. with distilled water, and after agitation the liquid is filtered. In the filtered liquid the glucose is determined by means of a copper potassium solution by using either the ordinary method or, by preference, by using the method of Bertrand. The latter method is always employed by us in the laboratory. The glucose present in 200 c. c. multiplied by $\frac{0.9 \times 100}{9}$ gives the percentage of starch in the sample analyzed.

In applying this method to meats containing known amounts of starch, excellent results were obtained. Unfortunately, however, in determining the amount of starch present, this method depends upon

[1] Bulletin des Sciences Pharmacologiques, 1908, p. 305.

the amount of reducing material in the meat food products, and as sugar is sometimes added to sausage, and at times certain sausages are made from meats that have been cured in pickling fluids containing sugar, together with the fact that reducing compounds other than starch naturally occur in the spices used in the preparation of sausages, the results obtained by this method for estimating starch in meat food products can not be relied upon as representing the actual amount of starch present.

As Bigelow's modification of Mayrhofer's method has some good features, and the method proposed by Perrier also some, it was thought advisable to try to combine the good features of the two methods with such modifications and additions as were thought necessary, thereby obtaining a method in which the objectionable features were eliminated. It is believed that such a method is given below.

THE WRITER'S METHOD.

In a 200 c. c. beaker treat 10 grams of finely divided meat with 75 c. c. of an 8 per cent solution of potassium hydrate in 95 per cent alcohol, and heat on the steam bath until all the meat is dissolved. This will require from 30 to 45 minutes. Add an equal volume of 95 per cent alcohol, cool, and allow to stand at least one hour. Filter by suction through a thin layer of asbestos in a Gooch crucible. Wash twice with warm 4 per cent potassium hydrate in 50 per cent alcohol and then twice with warm 50 per cent alcohol. Discard the wash water. Endeavor to retain as much of the precipitate in the beaker as possible until the last washing. Place the crucible with contents in the original beaker, add 40 c. c. of water and then 25 c. c. of concentrated sulphuric acid. Stir during the addition of the acid and see that the acid comes in contact with all the precipitate. Allow to stand about 5 minutes, add 40 c. c. of water, and heat just to boiling, stirring constantly. Transfer the solution to a 500 c. c. graduated flask, add 2 c. c. of a 20 per cent aqueous solution of phosphotungstic acid, allow to cool to room temperature, and make up to mark with distilled water. Filter through a starch-free filter paper, and determine the dextrose present in a 50 c. c. portion of the filtrate with Fehling's solution after neutralizing the acid, using Low's method, as given in Bureau of Chemistry Bulletin 107 (revised), page 241, for the determination of the copper in the cuprous oxid precipitate. The amount of dextrose multiplied by 0.9 gives the equivalent in starch.

The accuracy of the above-described method was demonstrated through its application to a number of samples of sausages to which

known amounts of pure cornstarch had been added. Some of the results obtained are given in the following table:

Starch added.	Starch found.
Per cent.	*Per cent.*
2.20	2.30
3.04	3.12
4.36	4.26
1.77	1.72
4.39	4.24
2.22	2.31
6.26	6.26
3.64	3.70
2.77	2.76
3.73	3.72
0.71	0.78

This method of determining starch in meat food products is not only much shorter than any of the other methods recommended, but it does not require the use of an autoclave. For these reasons it is well adapted to routine analytical work.

Approved:

JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, *D. C., June 11, 1912.*

[Cir. 203.]

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