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ESTIMATION OF STARCH IN SORGHUM AND OTHER GRAINS

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SUMMARY

A polarimetric method suitable for the routine estimation of starch in sorghum, wheat and maize grains and arrowroot is proposed. Commercial starches of the four types were chemically analysed and used as standards and to determine the specific rotation of each starch. A recovery of $99.8\% \pm 0.2\%$ was obtained in recovery experiments with added commercial sorghum starch.

Introduction

A rapid method for the analysis of starch which would fulfil two main conditions was required by the Agricultural Chemical Laboratory. Firstly, the method must be applicable to the analysis of sorghum grain, and secondly, it must involve a minimum number of time-consuming manipulations. The methods tested with these requirements in mind were those of the Association of Official Agricultural Chemists (1965), Steiner and Guthrie (1944), Earle and Milner (1944), Clendenning (1942, 1945a, 1945b, 1948), Pucher and Vickery (1936) and Clendenning and Wright (1945).

Each of the methods, except that of Clendenning (1945b), required the use of centrifuging to separate starch from either alcohol and sodium hydroxide solutions or a starch-iodide complex from excess reagent. Tests with sorghum showed that, even with prolonged centrifuging as specified in these methods, some of the sample always floated on the liquid surface. Unless great care was taken during decantation, loss of starch resulted. After evaporation of the decanted liquid, the residue gave a positive starch-iodide test. In some cases this loss could not be prevented. Because of the inconsistency in starch results and the laborious time delays associated with centrifuging, further work with these methods was abandoned.

The A.O.A.C. method does not precipitate proteins from the starch-calcium chloride solution. It was found that filtration under these conditions was impossibly slow and the turbidity of the filtrate rendered polarimetry difficult. Clendenning (1942, 1945a) advocated the use of stannic chloride as a protein precipitant but gave a warning on its use. Hoffpauir and Guthrie (1945) made a similar report. Steiner and Guthrie (1945) and later Clendenning (1945b) recommended the use of uranyl acetate with most types of grain. Tests with sorghum, maize, wheat and arrowroot showed that uranyl acetate-protein precipitates were easy to filter. Zinc uranyl acetate was tested in place of uranyl acetate because of its higher solubility. It was just as efficient as the uranyl acetate and so it was adopted as a modification in the proposed method.

Filtration media was tested for speed and efficiency. Hyflo supercel efficiently removed the protein precipitate but sometimes difficulties were experienced with filtration. Macerated paper was not quite as efficient but it was more convenient to use, because filtration was quite rapid. The macerated paper was supported in a gooch filter crucible and filtration was carried out using gentle suction. The filtrates generally were clear but a few did show a very slight turbidity.

Materials and Methods

Apparatus.—Hot plate; polarimeter fitted with sodium lamp; polarimeter tube (20 cm or 40 cm) with water-jacket; constant temperature (20°C) water supply; hydrometer.

Reagents.—

(1) Ether—lab. reagent.
(2) Ethanol (70% w/v).
(3) Calcium chloride solution (sp. gr. 1·30 at 20°C). Dissolve 500 g AR "dried" CaCl₂ in 600 ml distilled water. Cool to 20°C. Adjust the specific gravity of the solution to 1·30 using an hydrometer. Add a few drops of phenolphthalein to the solution. Adjust the solution to a faint pink colour by using either approximately 0·1N sodium hydroxide or acetic acid. Filter the solution until it is clear and then store it in a plastic container.

(4) Acetic acid (0·15N).

(5) n-octanol.

(6) Zinc uranyl acetate solution. Prepare just before use. Dissolve 5 g zinc uranyl acetate BDH lab. reagent in 100 ml calcium chloride solution.

(7) Macerated filter paper—filter aid.

Procedure.—Fold a 9-cm Whatman No. 4 filter paper to fit a glass filter funnel 5 cm diameter. Wet the paper with 70% ethanol. Weigh accurately about 2 g of finely ground sample (40 mesh). Transfer it to the filter paper. Wash the sample with 30 ml ether followed by 50-100 ml 70% ethanol, using a strong jet of liquid to break up any lumps. Allow the paper to drain well.

Transfer the paper and sample to one 150-ml beaker. Add 60 ml calcium chloride solution a few millilitres at a time. Use a glass rod to break up the paper and any lumps. Add 3 ml of 0·15N acetic acid solution and stir well. Add 5 drops of n-octanol to control foaming during refluxing.

Leave the rod in the beaker. Cover the beaker with a glass and bring the contents to the boil in 5 min. Transfer the beaker to a hot plate maintained at about 140°C after marking the level of the suspension on the outside of the beaker. With occasional strong stirring, allow the suspension to reflux gently for 15 min.

Notes.—(a) Do not remove glass cover during refluxing because spitting may cause a loss of starch. (b) If necessary, add distilled water to maintain the volume in the beaker at the level of the mark on the outside of the beaker. (c) More n-octanol may be added as required to control foaming.

Remove the beaker from the hot plate and after moving the glass cover a little to one side add 10 ml zinc uranyl acetate solution from a dip pipette. Stir the solution vigorously during the addition of the protein precipitant. Replace the cover glass and quickly cool the beaker and contents to room temperature. Quantitatively transfer the contents of the beaker to a 100-ml volumetric flask, using the calcium chloride reagent as wash solution. Make almost to the mark. Cool the flask and contents to 20°C and adjust to volume, ignoring the volume occupied by the filter paper and sample. (A correction is made for the paper when the results are calculated. The volume of the sample can be ignored.)

Using gentle suction, filter 15-20 ml of the starch solution through a gooch filter crucible containing about 1 g macerated paper. Transfer the filtered solution to a 20 cm or 40 cm water-jacketed polarimeter tube maintained at $20 \pm 0\cdot1^\circ\text{C}$ and allow to equilibrate in the instrument before reading the optical rotation. The mean of at least 10 readings is taken as the observed rotation of the sample ($A^\circ_{\text{observed}}$). A correction factor of 0·995 is used to compensate for the volume occupied by the filter paper in the volumetric so that

$$A^\circ_{\text{actual}} = A^\circ_{\text{observed}} \times 0.995$$

Calculate the starch percentage in the sample from the formula

$$\text{Starch percentage} = \frac{A^\circ_{\text{actual}} \times 10^4}{(a^\circ_D)^{20^\circ\text{C}} \times C_x \int}$$

where

- | | |
|----------------------------------|--|
| A°_{actual} | = true rotation of the sample; |
| $(a^\circ_D)^{20^\circ\text{C}}$ | = 200 = specific rotation of starch; |
| C_x | = number of g sample per 100 ml of solution; |
| \int | = path length of polarimeter tube in decm. |

Discussion

Removal of sugars.—Experiments showed that about 9 mg glucose or 20 mg sucrose would cause a change in rotation of $\pm 0.01^\circ$. This indicated that under the conditions of refluxing in calcium chloride, sucrose is hydrolysed to invert sugar while glucose mutarotates. No other sugars were available for testing but they would be expected to behave in a similar way to refluxing, especially in the case of the monosaccharides. Amounts of 100 mg each of glucose and sucrose were separately treated according to the procedure of the proposed method. No rotation was observed for either sugar. These weights of sugars were then added to separate 2-g samples of ground sorghum grain and the samples analysed as before. Again, no difference in the rotations of the two samples was observed. In view of the weights of glucose and sucrose removed by the wash procedure (5% of the sample weight) it should also be possible to reduce the concentrations of any other sugars that may be present in the sample so that they will make no contribution to the observed rotation.

Volume correction for filter paper (9 cm Whatman No. 4).—This was determined by rotation and specific gravity tests. One paper from each of six packets with different batch numbers, together with a known weight of commercial sorghum starch, was subjected to analysis. From these observed rotations and those for sorghum starch without paper in the 100-ml volumetric flask, the correction factor was calculated as 0.995 (Table 1). In the specific gravity test, the weight of the filter paper was subtracted from the weight of paper plus calcium chloride solution together occupying a volume of 100 ml. The ratio of this difference in weight to that of 100 ml calcium chloride solution alone gave a correction factor of 0.997. Since rotations are used to calculate starch the correction factor 0.995 was adopted for use in the method.

TABLE 1
CORRECTION FOR 9 CM WHATMAN NO. 4 PAPER

Sample	Weight of Starch (g)	A° observed†	A° observed/g Starch†
Starch + paper	2.0167*	7.69*	3.81 \pm 0.01*
Starch only (From Table 3)	—	—	3.79 \pm 0.01
Correction factor = 3.79/3.81 = 0.995			

* Mean of 6 replications.

† 22 cm tube.

The difference between the two means in Table 1 would not seem to cause much error in the starch content of the sample, but because the rotation is multiplied by 12.5 to calculate percentage starch in a 2-g sample (20 cm path), a difference of 0.01° is enlarged sufficiently to affect the first decimal place in the calculated starch. This error can be reduced by increasing the sample weight and the length of the polarimeter tube, but there is a practical limit to this

Table 2 shows the effect both the correction factor (0.995) and a change of 0.01° rotation have on the calculated starch percentage. The rotations selected are those approximating the analysis of 2 g sorghum grain at about 10% moisture, using a 20-cm tube. If the factor is not used, an error of 0.4% starch could be introduced in addition to 0.1% starch for every 0.01° error in rotation. However, if sufficient observations are made (usually 10) on each sample, the error in the mean rotation can be expected to be a little greater than 0.01° .

TABLE 2
EFFECT OF CORRECTION FACTOR (0.995) ON THE CALCULATED STARCH

Rotation (A°)*		Calculated Starch %†	
Observed	A° obs. $\times 0.995$	From A° observed	From A° actual
5.30	5.27	66.3	65.9
5.31	5.28	66.4	66.0
5.32	5.29	66.5	66.1
5.33	5.30	66.6	66.3
5.34	5.31	66.8	66.4

* Practical values for 2 g sorghum in 20 cm tube.

† Starch percentage = (rotation $\times 12.5$) assuming $[\alpha]_D^{20} = 200$.

Calculation of specific rotations.—Commercial starches of four types were analysed for moisture, ash, fat and protein. The starch percentage was calculated by difference (Table 3). Seven to 10 samples of each commercial starch were analysed by the procedure and a total of 100 observations were made. A° actual was the mean of the 100 observations per gram commercial starch. Using this mean and the corresponding starch percentage by difference, the specific rotation of the starch was calculated (Table 4). In the last column of the table the starch percentage has been calculated using A° actual rounded off to the second decimal place and a specific rotation of 200. In this method, 200 was adopted as the specific rotation because the calculation of starch percentage by difference obviously contains all the errors incurred in estimating each of the analysed fractions and so can be regarded only as a very close approximation of the true starch content.

TABLE 3
CHEMICAL ANALYSIS OF COMMERCIAL STARCHES

Starch Type	Water (%)	Ash (%)	Fat (%)	Protein (%)	Starch Percentage (by difference)
Sorghum	13.82	0.12	0.12	0.34	85.60
Wheat	13.00	0.18	0.10	0.43	86.29
Maize	11.84	0.09	0.04	0.36	87.67
Arrowroot	15.47	0.12	0.02	0.03	84.36

TABLE 4
SPECIFIC ROTATION OF STARCHES

Starch Type	Starch Percentage (by difference)	A° actual*	$[\alpha^\circ]_D^{20^\circ}$ calculated	Starch Percentage for $[\alpha^\circ]_D^{20^\circ} = 200$
Sorghum ...	85.60	3.788 \pm 0.014	201.1	86.14
Wheat ...	86.29	3.777 \pm 0.013	199.2	85.90
Maize ...	87.67	3.862 \pm 0.014	200.2	87.73
Arrowroot ...	84.36	3.698 \pm 0.011	199.3	84.10

* Per g starch for 22 cm tube (mean of 100 observations).

Recovery tests on sorghum starch.—Six recovery experiments were made. Each was with 2 g of the same sorghum grain sample, which contained varying weights of added commercial sorghum starch. A specific rotation of 200 was used and the commercial starch was taken as being 86.14% starch (from Table 4). The results of the recovery tests are shown in Table 5.

TABLE 5
RECOVERY OF SORGHUM STARCH

mg Starch Added		A° actual*	Starch		
Commercial	Pure†		mg Found	mg Recovered	Percentage Recovery
0	0	5.70	1,295.45	—	—
110.8	95.44	6.12	1,390.91	95.46	100.0
227.4	195.88	6.56	1,490.91	195.46	99.8
318.6	274.44	6.90	1,568.18	272.73	99.4
423.4	364.72	7.30	1,659.09	363.64	99.8
523.4	450.87	7.68	1,745.45	450.00	99.8
					Mean 99.8 \pm 0.2

* 22 cm tube.

† Calculated using 86.14% starch in commercial starch.

Conclusions.—The method is rapid and capable of giving at least a 99.6% recovery of the starch present in the sample. Where possible, a 40-cm tube is recommended for use in observing the rotation, since the increased rotation and consequently the calculated starch are less affected by the accuracy ($\pm 0.01^\circ$) with which the instrument can be read.

As in all polarimetric methods, the greatest single error is that in the determination of the specific rotation. Until a more accurate value is obtained, the specific rotation has been adopted as 200.

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