

PART VI. USE OF TINTOMETER IN THE STUDY OF THE DEGRADATION PRODUCTS OF STARCH.

By H. P. Das Gupta.

There is an extensive literature on the degradation products of starch. In the study of its constitution, starch has been subjected to hydrolysis by enzymes, bacteria or chemical agents. In the preparation of products required in the industry, acid hydrolysis has been generally employed. Phillipp (*Zeit. für Chemie*, 1867, 10, 400), Allihn (*Jour. für Prac. Chemie*, 1880, N.F., 22, 46), Edmund (*Jour. Soc. Chem. Ind.*, 1905, 24, 450), and numerous other workers used dilute sulphuric acid for the conversion of starch into dextrin and glucose. Delarue (*Chemisches Centralblatt, Leipzig*, 1881, 404) used tartaric and oxalic acids while Lintner and Düll (*Ber.*, 1893, 26, 2533) and Friedrichs (*Jour. Chem. Soc.*, 1914, 106, 145) used oxalic acid only for the conversion of starch into various dextrans and other products. Dilute hydrochloric acid mixed with some oxidising agent, such as potassium permanganate or dichromate, has also been employed by Jules (*Jour. Soc. Chem. Ind.*, 1906, 25, 34) and others for the modification of starch. Even concentrated solution of hydrogen peroxide was used by Gerber (*Compt. rend.*, 1912, 154, 1543) and others to modify starch. In connection with the study of starch constitution, enzyme hydrolysis has been employed by O'Sullivan (*Chem. Soc. Trans.*, 1872, 25, 579), Sherman (*Jour. Amer. Chem. Soc., Easton*, 1919, 41, 1123), Pringsheim (*Ber.*, 1913, 46, 2959), Schmalz (*Biochem. Zeit.*, 1923, 142, 108), and numerous other workers. Pringsheim and Eissler (*loc. cit.*) utilized *B. macerans* for the same purpose. Small (*Jour. Amer. Chem. Soc., Easton*, 1919, 41, 113) used a mixture of alcohol and hydrochloric acid for the preparation of soluble starch.

There are at present, in the market, a few dozen varieties of thin boiling starches and many more of dextrans that find applications in the textile industry. Dextrin gives violet, red or yellow colour with iodine but neither violet nor the red can give us any concrete idea as we can have several shades in each colour. Moreover, it becomes almost impossible to differentiate between these two tints, especially when they tend to approach each other. Measurement of viscosity is no doubt a useful method, but it may be useful to combine it with some colour standards. Thus, if one definitely states whether violet or red is of so many units, then it provides a more quantitative estimate than a mere expression such as deep or pale violet.

Johnson (*Jour. Amer. Chem. Soc.*, 1908, 30, 798) proposed a method for the routine valuation of various diastatic preparations. A starch gel of certain concentration was subjected to different degrees of

enzymic hydrolysis and the hydrolysates thus obtained were tested with some iodine solution in test tubes. The colour in the test tubes were matched against a white paper. The rate of disappearance of blue colour was taken as a criterion for the comparative diastatic activity. Blake (*Jour. Amer. Chem. Soc.*, 1918, 40, 623) studied the digestibility of bread (*in vitro*) by enzymes and the rate of digestion was followed by the appearance of various shades of colour when iodine solution was added to the hydrolysates. The colours thus developed were matched in Dubosq colorimeter and, as standard colours, the Lovibond tint glasses were used. Lovibond tintometer itself was used for the present study, thus avoiding the necessity of any additional colorimeter for comparison.

Since the degradation of starch can be controlled to the finest degree by alcoholic acid treatment, mixtures of methyl alcohol (99 per cent.) and sulphuric acid were selected chiefly because of the fact that the other mineral acids such as hydrochloric acid may be more drastic in their effects, while, on the other hand, organic acids may be too slow in their action.

EXPERIMENTAL.

Preliminary studies with viscometer.—Jowar starch (95.5 per cent.) which had been originally prepared by the author, was subjected to alcohol-sulphuric acid hydrolysis at a temperature of 80°C. Methyl alcohol containing 0.5 per cent. sulphuric acid, was tried in the beginning. The acidified alcohol (200 c.c.) was mixed with starch (100 grams) and the suspension refluxed on a water-bath in a round bottomed flask, which had been provided with an arrangement at the side to tap out samples. At intervals of two hours, samples were taken out, freed from acid and dried.

Gels (2 per cent.) of the different samples were prepared and their rates of flow compared with those of some well-known commercial brands of thin boiling starches now on the market. The results have been presented in Table I.

TABLE I.

| Hours of treatment | | | | Flow in seconds at 25°C. (Ostwald's viscometer) |
|----------------------------------|----|----|----|--|
| Control (distilled water) | .. | .. | .. | 90 |
| 2 | .. | .. | .. | 195 |
| 4 | .. | .. | .. | 164 |
| 6 | .. | .. | .. | 150 |
| 8 | .. | .. | .. | 140 |
| Commercial preparation (Brand A) | .. | .. | .. | 198 |
| Commercial preparation (Brand B) | .. | .. | .. | 166 |

Thus, it was found that the preparations obtained after the second and fourth hour, corresponded to brands A and B respectively.

In the next set of experiments, the concentration of acid was raised to 2 per cent. as the rate of hydrolysis in the previous one was very slow. Samples were tapped out every hour for the first six hours and later on every six hours. Two per cent. gels of the samples were prepared and their flows determined as before (Table II).

TABLE II.

| Hours of treatment | Flow in seconds at 20°C. (Ostwald's viscometer) |
|--------------------|--|
| 0 | 1214.0 |
| 1 | 151.0 |
| 2 | 138.0 |
| 3 | 125.0 |
| 4 | 123.0 |
| 5 | 120.0 |
| 6 | 107.5 |
| 12 | 107.6 |
| 18 | 105.0 |
| 24 | 102.0 |
| 30 | 101.2 |
| 36 | 100.0 |
| 42 | 99.2 |
| 48 | 97.0 |
| 54 | 96.0 |
| Dextrin (Brand C) | 96.8 |

It may be seen from the above that the sample of commercial dextrin (Brand C) was degraded to about the same extent as the sample obtained after 54 hours of hydrolysis.

Use of Lovibond Tintometer.—Before taking the readings in the Lovibond tintometer, the conditions for the percentage of starch in the

suspension and the quantity of iodine solution to be used were first of all standardised by using pure soluble starch (E. de Haën's) and N/50 iodine solution. A 1 cm. prism was used for introducing the suspensions. It was found that if 1 c.c. of 2 per cent. gel of soluble starch is diluted to 100 c.c. and if 10 c.c. of this diluted solution be treated with 0.2 c.c. of N/50 iodine, the proportionality of colour with the concentration of starch remains approximately constant, when blue tint is taken into consideration. If more of iodine be used, the yellow

TABLE III.

| Hours of treatment | Lovibond readings | | |
|---------------------------------|-------------------|-----|--------|
| | Blue | Red | Yellow |
| Control (iodine solution alone) | .. | 0.2 | 0.5 |
| 0 | 13.0 | .. | 0.3 |
| 1 | 6.5 | .. | 0.3 |
| 2 | 7.3 | 1.0 | 0.3 |
| 3 | 7.0 | 2.2 | 0.4 |
| 4 | 6.8 | 2.5 | 0.3 |
| 5 | 6.5 | 2.9 | 0.3 |
| 6 | 7.0 | 4.0 | 0.3 |
| 12 | 5.2 | 4.5 | 0.3 |
| 18 | 3.2 | 4.6 | 0.3 |
| 24 | 2.7 | 4.2 | 0.3 |
| 30 | 1.5 | 3.5 | 0.4 |
| 36 | 1.0 | 3.9 | 0.4 |
| 42 | 1.1 | 3.9 | 0.3 |
| 48 | 1.1 | 3.4 | 0.3 |
| 54 | 0.5 | 2.4 | 0.3 |
| Commercial dextrin (Brand C) | 0.2 | 1.4 | 0.3 |

and red tints become prominent and mask the blue. On the other hand, too small a quantity of iodine does not develop the proper starch iodide tint. Every sample was, therefore, made of the same concentration, *i.e.*, 10 c.c. contained 0.002 g. of starch and 0.2 c.c. of N/50 iodine solution being added in each case. The tints that developed were matched against the coloured glasses in the tintometer. Readings of the degradation products of jowar starch were taken. The starch was degraded by treating with methyl alcohol mixed with 2 g. sulphuric acid per 100 c.c. of alcohol (Table III).

The above procedure was next extended to another kind of starch. Ragi (*Fleucine coracana*) (starch 96 per cent.), which was prepared by the author, was subjected to the same alcoholic acid treatment and samples were taken out as before. Their comparative flows (2 per cent. gel) and Lovibond readings were then taken (Table IV).

TABLE IV.

| Hours of Treatment | Flow in seconds at 25°C. | Lovibond readings | | |
|--------------------|--------------------------|-------------------|-----|--------|
| | | Blue | Red | Yellow |
| 0 | 240.0 | 8.2 | .. | 0.4 |
| 1 | 173.0 | 7.7 | 0.2 | 0.3 |
| 2 | 152.0 | 8.3 | 0.9 | 0.4 |
| 3 | 129.0 | 7.3 | 1.7 | 0.3 |
| 4 | 124.0 | 6.0 | 1.9 | 0.3 |
| 5 | 120.0 | 5.4 | 2.4 | 0.3 |
| 6 | 118.0 | 4.1 | 2.8 | 0.2 |
| 12 | 115.0 | 2.3 | 3.2 | 0.2 |
| 18 | 105.0 | 1.2 | 3.2 | 0.4 |
| 24 | 103.5 | 0.6 | 2.0 | 0.2 |
| 30 | 103.0 | 0.3 | 2.3 | 0.3 |
| 36 | 102.0 | 0.2 | 2.2 | 0.3 |
| 42 | 102.0 | 0.2 | 2.3 | 0.3 |
| 48 | 102.0 | 0.2 | 2.2 | 0.4 |

It may be seen from the above that, after the 30th hour, there was practically no change in either the time of flow or the tintometer readings. The observations would suggest that a certain stage has been reached after which, the alcoholic acid treatment would have no effect. The significance of this observation with special reference to the digestibility of starch would be of considerable interest. In later studies, hydrochloric acid was tried in place of sulphuric acid. Jowar

TABLE V.

| Hours of Treatment | Flow in seconds | Lovibond readings | | |
|--------------------|-----------------|-------------------|-----|--------|
| | | Blue | Red | Yellow |
| 6 | 116.0 | 3.9 | 4.8 | 0.2 |
| 12 | 111.5 | 1.5 | 4.2 | 0.1 |
| 18 | 109.0 | 0.5 | 3.2 | 0.2 |
| 24 | 108.0 | .. | 2.8 | 0.3 |
| 30 | 107.2 | .. | 2.6 | 0.5 |
| 36 | 106.0 | .. | 2.4 | 0.5 |

TABLE VI.
(Rice Starch)

| Hours of Treatment | Flow in seconds | Lovibond readings | | |
|--------------------|-----------------|-------------------|-----|--------|
| | | Blue | Red | Yellow |
| 2 | 150.0 | 5.4 | 3.1 | 0.2 |
| 4 | 145.5 | 2.1 | 2.8 | 0.4 |
| 6 | 140.8 | 1.6 | 1.9 | 0.3 |
| 8 | 136.0 | 0.2 | 2.4 | 0.3 |
| 10 | 127.0 | .. | 2.8 | 0.5 |
| 12 | 113.0 | .. | 2.5 | 0.4 |

and rice starches were taken. The hydrolysing agent contained 5 c.c. of 10 N hydrochloric acid for every 100 c.c. of 99 per cent. methyl alcohol. The details relating to sampling and examination were the same as those already outlined (Table V).

As may be naturally expected, the degradation of starch proceeds very much more quickly in presence of hydrochloric acid than in that of sulphuric acid. The results of the present study show that tintometer readings can be used as a measure of the extent of degradation of starch. The readings do in fact take one further than mere viscosity measurement, which represents a combined effect. Each of the different component colours represent, at any rate, a group of polysaccharides sharing a common characteristic, so that the changes in the degree of each colour would provide an approximate measure of the transformations of the associated constituents. It would be of much interest, therefore, to extend the technique to the study of other types of starch hydrolysis such as those brought about by enzymes. Further work in this direction is in progress and will form the subjects of later communication.

SUMMARY.

1. A few degradation products of starch have been prepared by alcoholic acid treatment.

2. A comparative flow of the gels prepared from samples thus obtained, as also the tint developed by adding iodine solution, have been studied by Ostwald's viscometer and Lovibond tintometer respectively.

3. A parallelism between the rate of flow and the intensity of the tint has been observed.

The author wishes to thank Prof. V. Subrahmanyan for his kind interest in the progress of the work and helpful criticism.

*Department of Biochemistry,
Indian Institute of Science,
Bangalore.*

[Received, 8-1-1936.]