DETERMINATION OF STARCH IN PLANT TISSUES

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Introduction

During a study of the carbohydrate distribution in grape canes, it became necessary to have a method for the estimation of small amounts of starch. A fairly rapid, accurate method yielding easily reproducible results was desired in order that the analysis of a great number of samples could be completed in a short time.

An excellent survey of the literature dealing with analytical methods for starch is given by Antolton (11). Of all the methods listed, one described by Thomas (9) for estimating starch in apple spurs seemed most suitable. The fundamental idea of Thomas’s method is the direct determination of the combined reducing power of glucose and maltose produced by the action of diastase on starch under prescribed conditions. This method avoids the destruction of sugars which always takes place if complete hydrolysis with acids is attempted or if acid hydrolysis after diastatic conversion of the starch is used according to the Official Methods (6). This fact was shown by Thomas and confirmed in the present work. Thomas determined the reducing sugars by means of the colorimetric method of Benedict and Osterberg (1), which involved the reduction of a picrate-picric acid solution to picramic acid. When this method was tested out on pure starch it always yielded high results (table I). The use of mercuric nitrate as a clarifying agent was also unsatisfactory since it yielded a precipitate which was slow in settling and difficult to filter. Moreover, mercuric nitrate exerts an oxidizing influence on sugar solutions (5). It is evident that the clarifying agent must be chosen to suit the particular type of substrate obtained from the different plants.

The procedure which was found most satisfactory is as follows:

Procedure

Three gm. of finely ground material (50 mesh), which had previously been extracted with 80 per cent. alcohol in order to remove soluble sugars, are heated to boiling on a steam bath for 30 minutes with 200 cc. water, and then cooled to 37° C. An aliquot portion of a solution of taka-diastase is added so that 0.1 gm. of the enzyme preparation is present. Two cc. of toluene are added and the mixture incubated for 48 hours at 37° C. The mixture should be shaken as often as possible during this digestion. The mixture is heated to boiling, filtered, and the insoluble material washed with hot water. One to 3 cc. of a saturated lead acetate solution are added and
the solution diluted to 250 cc. after cooling to 20° C. After shaking thoroughly and allowing the lead precipitate to settle as much as possible the solution is filtered. The excess lead is removed by adding finely powdered disodium phosphate and filtering again. A copper reduction is run on a 50-cc. portion according to the standard Munson-Walker procedure, using the Shaffer-Hartmann (8) iodometric method for determining the reduced copper. A blank must be run using 200 cc. water and 0.1 gm. taka-diastase treated exactly like the sample. The difference between the blank and the sample gives the copper reduction value of the digest. This is calculated in terms of glucose from the standard tables. The amount of starch is then obtained as follows:

Calculations

It has been shown (8) that the ratio of glucose to maltose in the diastase substrate is 2:1; hence if $g =$ glucose and $m =$ maltose, then $g = 2m$ and $m = \frac{g}{2}$ (1).

The average copper reducing value of maltose is 0.61 that of glucose, so if $R$ represents the copper reducing of the diastase substrate expressed in terms of glucose, then $g + 0.61m = R$ (2).

By combining equations (1) and (2):

$$2m + 0.61m = 2.61m = R; \text{ also } g + \frac{0.61g}{2} = R$$

or: $m = \frac{R}{2.61}$ and $g = \frac{R}{1.305}$

Starch value of maltose = 0.947
Starch value of glucose = 0.90

so $Sm =$ starch equivalent from maltose = $\frac{R}{2.61} \times 0.947 = 0.362 \times R$

and $Sg =$ starch equivalent from glucose = $\frac{R}{1.305} \times 0.90 = 0.689 \times R$

Total starch = $Sm + Sg = 0.362 R + 0.689 R = 1.051 R$

that is: to determine the amount of starch simply multiply the copper reducing value (R) expressed in terms of glucose by the factor 1.051.

Discussion

The preceding method and calculations are based on the following experimental facts:

1. The presence of glucose and maltose in the diastase substrate is in the ratio of 2:1. When the diastatic conversion is carried out as shown, the variation from the 2:1 ratio is very slight, and Thomas has shown that
these variations (1.8–2.3) do not materially affect the accuracy of the final percentage of starch.

2. The average copper reducing value of maltose is 0.61 that of glucose (6). This value varies somewhat, of course, as is evident from Munson and Walker’s tables and the choice of 0.61 is made purely arbitrarily since it has been found that this value gives good results.

3. The starch equivalents for glucose and maltose are the ones usually given (9).

It will be noted that the factor calculated from the experimental data is quite different from the stoichiometric factor calculated from the following equation:

\[
4C_{6}H_{10}O_{5} + 3H_{2}O \rightarrow 2C_{6}H_{12}O_{6} + C_{12}H_{22}O_{11} \quad (3)
\]

Since the average copper reducing value of maltose is 0.61 that of glucose,

\[
\begin{align*}
\text{then } 342 \times 0.61 &= 208 \\
\text{for glucose } 2 \times 180 &= 360 \\
\text{sum of glucose and maltose} &= 568 \\
\text{hence ratio should be } &\frac{648}{568} = 1.141 \text{ theoretically.}
\end{align*}
\]

However, there is no evidence for equation (3). The diastase substrate contains glucose and maltose in the ratio of 2 : 1 but this does not mean that four \(C_{6}H_{10}O_{5}\) units of starch produce two moles of glucose and one of maltose. In fact, the number of moles of glucose and maltose is less than these values and recent work indicates that maltose is not a primary cleavage product of starch but is produced by the synthetic action of the taka-diastase on the glucose. It is also evident from table I that the use of this factor would lead to high results for pure starch. The use of lead acetate as a clarifying agent and disodium phosphate as a de-leading agent is much more satisfactory and more rapid than the use of mercuric nitrate, and it avoids the reduction of any mercury with simultaneous oxidation of the sugars. It is essential that disodium phosphate be used for de-leading, as shown by Engris and Tsang (4), and especially so in the present method since the Shaffer-Hartmann iodometric titration method for estimating the reduced copper is used. The use of sodium oxalate or sodium carbonate as a de-leading agent is prohibited since both of these interfere with the determination of the reduced copper by this method.

The substitution of the Shaffer-Hartmann volumetric method for the colorimetric method has several advantages. In the first place it eliminates
the personal error involved in methods which depend on the matching of colors. The starch-iodine endpoint is easily observed. The titrimetric method is also much more rapid, requiring only one-half to one-third the time required for the colorimetric comparison. Although the objections to and disadvantages of the Munson-Walker reduction method are recognized, it has been found that with a little experience this method yields good results. Since the copper reducing ratio of maltose to glucose is only 0.61 while the picric acid reducing ratio is 0.91, it might be expected that this would result in a reduction in accuracy of the method. The data in table I, in which the present method is compared with other methods on a

**TABLE I**

**DATA ON PURE STARCH**

<table>
<thead>
<tr>
<th>Weight of pure starch taken (gm.)</th>
<th>Amount of starch found by</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diastatic conversion followed by hydrolysis with 0.5 per cent. HCl</td>
<td>Colorimetric according to Thomas</td>
</tr>
<tr>
<td>0.2000</td>
<td>0.1599</td>
<td>0.2162</td>
</tr>
<tr>
<td>0.2000</td>
<td>0.1591</td>
<td>0.2162</td>
</tr>
<tr>
<td>0.3000</td>
<td>0.2531</td>
<td>0.3563</td>
</tr>
<tr>
<td>0.3000</td>
<td>0.2476</td>
<td>0.3523</td>
</tr>
</tbody>
</table>

**TABLE II**

**SUMMARY OF STARCH DETERMINATIONS IN GRAPE CANE**

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Method</th>
<th>By hydrolysis with HCl after diastase copper reduction</th>
<th>Colorimetric according to Thomas</th>
<th>Writer’s method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>per cent.</td>
<td>per cent.</td>
<td>per cent.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1.92</td>
<td>2.79</td>
<td>2.66</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1.98</td>
<td>3.44</td>
<td>2.61</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3.84</td>
<td>5.44</td>
<td>4.98</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2.60</td>
<td>3.86</td>
<td>3.43</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3.25</td>
<td>4.87</td>
<td>3.93</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3.35</td>
<td>5.29</td>
<td>4.49</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>5.45</td>
<td>5.93</td>
<td>5.30</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>4.27</td>
<td>4.79</td>
<td>4.72</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>1.61</td>
<td>2.63</td>
<td>2.39</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>4.03</td>
<td>5.26</td>
<td>4.50</td>
</tr>
</tbody>
</table>
sample of purified arrowroot starch, indicate that this factor has had no appreciable effect on the accuracy of the results.

It will be noted (table I) that the colorimetric method yielded high results; the acid hydrolysis (0.5 per cent. HCl) after diastatic conversion yielded low results (indicating destruction of the glucose); while the direct copper reduction yielded results closely approximating the amount of starch taken. Since it has been shown that 2.5 per cent. hydrochloric acid possesses a destructive action on glucose (3, 7, 9, 10), 0.5 per cent. acid was used (9). This concentration is sufficient to hydrolyze the maltose and any remaining unhydrolyzed dextrins. It was also found that the use of 2.5 per cent. hydrochloric acid gave still lower results than those in table I.

In table II the same relationship between the methods is found to hold when applied to the determination of starch in ten different varieties of grape canes.

Summary

A method for the determination of starch is described which involves: (1) conversion of the starch into glucose and maltose by the action of taka-diastase; (2) determination of the combined reducing value of the glucose plus maltose by the SHAFFER-HARTMANN modification of the MUNSON-WALKER method. A simplified method of calculating the amount of starch from these data is given.

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LITERATURE CITED


