Saccharase activity is usually determined by finding polarimetrically the "time value," or the time required for a saccharase preparation to bring the rotation of a sucrose solution to 0. One may instead determine the invert sugar by one of the reduction methods and then obtain the time of 0 rotation by referring to a table of sugar values, prepared for this purpose. Since one cannot accurately calculate the time needed to reach 0 rotation from a single polarimetric reading, it is necessary to make several readings and the analysis takes from 1 to 2 hours to complete. According to the terminology of Willstätter (1, 2) the enzyme preparation contains 1 saccharase unit when 50 mg. have a time value of 1 when one employs 25 cc. of 16 per cent cane sugar at 15.5°. Willstätter expresses the purity of saccharase preparations by the enzyme value, or number of units in 50 mg. of enzyme preparation. Von Euler (3, 4) uses the Inversionsfähigkeit, where \( I_f = \frac{k \times \text{gm. substrate}}{\text{gm. enzyme}} \). However, this value is not serviceable when one desires a figure for enzyme units per cc. of solution.

It is our opinion that the polarimetric determination of saccharase is unduly time-consuming and that the Willstätter system of expressing units is needlessly complicated. We believed that determination of saccharase activity could be improved greatly by use of the dinitrosalicylic acid method for reducing sugar. Nelson and Vosburgh (5) have shown that in the presence of 5 to 10 per cent sucrose saccharase attains its maximum velocity. We have employed saccharase in such concentration that no more than 10 mg. of invert sugar are produced by the digestion of 6 cc. of 5.4 per cent sucrose in 5 minutes at 20°. Under these conditions
the velocity is only 1 per cent less than it is at 0 time, as we have calculated from the curve shown in Fig. 1. This difference of 1 per cent is less than the experimental error of the colorimetric analysis for invert sugar and the values obtained by our method can, therefore, be taken to represent the velocity at 0 time.

The reaction is stopped at the end of 5 minutes by the addition of 5 cc. of approximately 0.1 N sodium hydroxide and the invert sugar is determined by the dinitrosalicylic acid method (6). The curve in Fig. 1 gives some idea as to the accuracy of the dinitrosalicylic acid method for reducing sugar.

Our saccharase units are expressed directly in terms of the mg. of invert sugar formed in 5 minutes at 20°, at pH 4.5. To express the purity of the enzyme it is more logical to state the units per gm. of dry enzyme than it is to employ an enzyme value and compel the reader to hunt through the literature for a definition of this value.
The new method requires only from 20 to 30 minutes and is accurate to within about 2 per cent as compared with an accuracy of 1 to 2 per cent for the polarimetric method in our hands. The method can be employed directly with compressed yeast since so little yeast is required that the turbidity caused by the presence of yeast cells is negligible. Yeast preparations containing sugar must naturally be washed before being tested. We have found commercial taka-diastase to contain so little saccharase that it was necessary to continue the digestion for more than the usual 5 minutes. Here it was necessary also to subtract a blank for the reducing material in the diastase solution. A few of our results are given in the accompanying tabulation.

<table>
<thead>
<tr>
<th>Dry saccharase preparation</th>
<th>Our saccharase units per gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fleischmann's yeast</td>
<td>994</td>
</tr>
<tr>
<td>&quot;</td>
<td>987</td>
</tr>
<tr>
<td>Nectar Brewing Company yeast</td>
<td>830</td>
</tr>
<tr>
<td>Difco invertase</td>
<td>18,300</td>
</tr>
<tr>
<td>Taka-diastase</td>
<td>48</td>
</tr>
</tbody>
</table>

Method

Pipette 5 cc. of 6.5 per cent sucrose in acetate buffer into a test-tube and allow to come to 20° in a thermostat bath. Add 1 cc. of saccharase solution, or yeast suspension, at 20°, from a Folin-Ostwald pipette that delivers rapidly. Mix and allow to remain in the bath for 5 minutes, after which add 5 cc. of approximately 0.1 N sodium hydroxide. Mix and determine the invert sugar in a 1 cc. aliquot by the dinitrosalicylic acid method (6), with a 1 mg. glucose standard. The mg. of invert sugar multiplied by 11 will give the saccharase units per cc. of enzyme solution, or yeast suspension. If considerably more than 1 or less than 0.5 mg. of invert sugar is found per cc., the analysis will have to be repeated, with more dilute or more concentrated saccharase, or else with a longer or a shorter time for the digestion.

Sucrose-Buffer Solution

Place 43 cc. of N sodium acetate and 57 cc. of N acetic acid in a liter volumetric flask; dilute to the mark with water redistilled
from glass and mix. Dissolve 6.5 gm. of c.p. sucrose in 96 cc. of the buffer and add a few drops of toluene. This will give a 6.5 per cent solution of pH 4.5. The solution will remain serviceable at room temperature for 3 days.

Sugar Standard

We have not found any detectable difference between the reducing values for invert sugar and for d-glucose, by the dinitrosalicylic acid method. We therefore employ the usual standard containing 0.1 per cent glucose in saturated benzoic acid.

We wish to acknowledge our appreciation to the Sage Foundation for financial assistance in this research.

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A METHOD FOR DETERMINATION OF SACCHARASE ACTIVITY
James B. Sumner and Stacey F. Howell


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