METHODS OF ESTIMATING CARBOHYDRATES. II. THE ESTIMATION OF STARCH IN PLANT MATERIAL.

The use of Taka-Diastase.

By WILLIAM A. DAVIS and ARTHUR JOHN DAISH.
(*Rothamsted Experimental Station.*)

In 1884 C. O'Sullivan (*Trans. Chem. Soc.*, 1884, **45**, 1) described a method of estimating starch in cereals and other starch-containing substances which was based on the conversion of starch by means of diastase into a mixture of "dextrin" and maltose. This method has been improved at various times by H. T. Brown and his fellow workers, especially by Brown and Millar (*Trans. Guinness Research Lab.*, 1903, 79) in its application to malt and barley. Having encountered difficulties in applying this method to the estimation of starch in leaf material, we have been led to make a careful study of the various processes available for this purpose and to devise a method to obviate the errors to which the ordinary diastase method is subject in such cases.

For the estimation of starch in plant materials, such as foliage leaves, seeds, grain, etc., the modified Sachsse method which is official in the United States of America (*Bureau of Chemistry*, Bulletin 107; *Allen's Commercial Organic Analysis*, 4th Ed., Vol. I. p. 420) and is based on the hydrolysis of the starch present with boiling dilute hydrochloric acid, is quite valueless, not only because such tissues invariably contain pentosans and other substances which yield reducing sugars that count as dextrose, but because of the actual destruction of dextrose which occurs during the prolonged treatment with acid[1].

[1] We have found in a series of analyses made by this method of purified potato starch dried *in vacuo* at 120° C. (see p. 167), results varying from 93·8 to 94·3 % of starch, whereas by the ordinary diastase method an average result of 100·1 % (see p. 165), and with taka-diastase an average result of 99·6 % was obtained with the same sample. That actual destruction of dextrose occurs on prolonged heating with dilute acid we have recently shown (*Journ. of Agric. Sci.*, 1913, 5, p. 437). This destruction of dextrose is a source of error in all the methods which make use of hydrochloric acid to effect hydrolysis, such as that of Märcker and Morgen, even when the primary conversion of the starch has been carried out with diastase.

Although ordinary diastase gives with purified starch results by O'Sullivan's method which are approximately correct (see p. 165), values 15 to 20 °/₀ lower than the actual starch content may be obtained when it is applied to leaf material or plant tissues in general, owing to the loss of dextrin. In the majority of cases, plant material, which has been previously deprived of sugar by prolonged extraction with alcohol, still contains tannins, amino-acids, proteins, etc.; during the hydrolysis by diastase these pass into solution and exercise a very marked effect on the reducing power and optical activity of the solution. These substances have therefore to be removed by the addition of basic lead acetate, which we have found almost invariably produces a heavy precipitate in the filtered solution obtained from the diastase conversion. Although basic lead acetate *does not of itself precipitate dextrin*, when dextrin is present in solutions in which a precipitate is produced, as in the purification of the solutions obtained from the diastase conversions, it is *carried down with this precipitate and is thus lost to the analysis*[1].

Taka-Diastase as an Agent in Estimating Starch.

To estimate starch in foliage leaves and in similar cases in which it is necessary to purify the solution after hydrolysis has been effected, it appeared probable that the so-called "taka-diastase" would be more suitable than ordinary diastase, as it is said to give rise only to maltose and dextrose (compare Croft-Hill, *Proc. Chem. Soc.*, 1901, **240**, 184) free from dextrin[2]. If this were the case, it would be possible to add basic lead acetate or other clarifying agents without losing sugars. We therefore carried out a series of experiments with taka-diastase at two different temperatures, namely 38° and 55° during different lengths of time. In all cases, about 2·5 grms. of potato starch were

[1] When basic lead acetate is added to the solution obtained by the diastase conversion of *purified starch* not the slightest precipitate is produced with the dextrin existing in solution; but the results given on pp. 165–167 show that if sodium carbonate is subsequently added, or hydrogen sulphide is passed so as to precipitate the lead, a greater or smaller proportion of the dextrin is removed by co-precipitation.

[2] In 1898 Stone and Wright (*J. Amer. Chem. Soc.* **20**, 639–647) attempted to estimate starch by means of taka-diastase; but as they assumed maltose to be the only sugar formed and measured the products of the action solely by the reducing power without reference to their rotation, it is not surprising that they concluded that under their conditions "taka-diastase is not adapted for use in the quantitative estimation of starch." It will be seen that we have come to an exactly opposite opinion.

dried *in vacuo* over phosphorus pentoxide at 120° in a small wide-necked, round bottomed flask, fitted with a ground-in stopper carrying a tube connected with the vacuum through a small Wurtz flask containing the pentoxide; the heating was effected by means of a Meyer vapour bath containing a mixture of toluene and xylene which gave the required temperature. The starch was dried until the weight was constant; 2·5 grms. of the original starch gave approximately 2·0 grms. of the vacuum-dried product. This was gelatinised with 200 c.c. of water at 100°, the solution cooled to the proper temperature and mixed with 0·100 grm. taka-diastase[1]; the volume was kept at 200 c.c. by the addition of water as required and the temperature was maintained constant at 38 or 55° to within 0·1° by means of a thermostat. When the action was prolonged over more than a few hours, toluene was added in sufficient quantity to prevent the growth of micro-organisms; it was thoroughly mixed with the solution by stirring and from time to time fresh quantities were added so as to maintain the material in a sterile state. Chloroform of course cannot be used as it destroys the maltase present in taka-diastase (compare Fischer, *Ber.*, 1895, **28**, 1429, and Morris, *J. Fed. Inst. Brewing*, 1896, 350). Ultimately, the solution was boiled to destroy the diastase or a trace of sodium hydroxide was added; it was then diluted to 500 c.c. and the reducing power estimated in 25 c.c.

The observations of rotatory power were made in a 200 (or 400) mm. tube at 20·00°; the specific rotatory power of anhydrous maltose[2] is $[\alpha]_D^{20^\circ} = 137{\cdot}6^\circ$, that of dextrose in dilute solution $[\alpha]_D^{20^\circ}$ being taken as 52·7°.

From Table I it is seen that when the time of action of the taka-diastase at 38° under the conditions given above exceeds six hours, the whole of the starch is converted into a mixture of maltose and dextrose, the proportion of dextrose steadily increasing as time proceeds, so that the ratio of dextrose to maltose present, which after six hours is approximately 0·1, increases to about 6·2 after 72 hours. At this point, under the conditions existing in these experiments (namely starting with 0·1 grm. only of taka-diastase), no further conversion of maltose into dextrose occurs.

The curves (Fig. 1, p. 156) show the results graphically.

[1] We have used the commercial product manufactured by Messrs Parke Davis & Co.

[2] Applying Meissl's temperature correction to the value $[\alpha]_D^{15\cdot5^\circ} = 137{\cdot}93^\circ$ obtained by Brown, Morris and Millar (*Trans. Chem. Soc.*, 1897, **71**, p. 112) as the specific rotatory power of pure maltose.

TABLE I. *Action of* 0·1 *grm. Taka-Diastase on Potato Starch at* 38°.

Time in hours	Weight of starch dried in vacuo at 120°	CuO from 25 c.c. of 500 c.c.	α_D in 200 mm. tube at 20·00°	Dextrose in 500 c.c.	Maltose in 500 c.c.	Total starch	°/o starch found	Dextrose / Maltose	Remarks
3	2·0061	0·1321	1·192°	—	—	—	—	—	Dextrin still present.
	1·9978	0·1380	1·273	—	—	—	—	—	No alumina cream added
6	2·0099	0·1581	1·103	0·2204	1·892	1·9914	99·55	0·116	No alumina cream
	1·9914	0·1535	1·097	0·1640	1·932	1·9776	99·34	0·085	,, ,, ,,
12	1·9752	0·1780	0·991	0·5328	1·5930	1·9884	100·7	0·334	,, ,, ,,
	1·9940	0·1800	0·973	0·5748	1·5430	1·9792	99·37	0·372	5 c.c. alumina cream
24	2·0148	0·2189	0·808	1·1596	1·0214	2·0112	99·80	1·135	,, ,, ,,
	1·9876	0·2205	0·764	1·2302	0·9148	1·9739	99·33	1·345	,, ,, ,,
	2·0145	0·2218	0·783	1·2204	0·9534	2·0018	99·37	1·280	No alumina cream
	2·0046	0·2212	0·777	1·2218	0·9414	1·9916	99·39	1·298	5 c.c. alumina cream
	2·0024	0·2167	0·794	1·1546	0·9982	1·9846	99·11	1·157	5 c.c. basic lead added and this pptd. by solid Na_2CO_3
48	2·0000	0·2561	0·594	1·8106	0·3852	1·9942	99·71	4·700	No alumina cream
	2·0000	0·2544	0·616	1·7674	0·4416	2·0086	100·40	4·002	5 c.c. alumina cream
72	2·0016	0·2588	0·556	1·886	0·2874	1·9703	98·45	6·563	,, ,, ,,
	1·9961	0·2562	0·562	1·852	0·3113	1·9620	98·28	5·950	,, ,, ,,
	2·0084	0·2571	0·569	1·8536	0·3231	1·9741	98·33	5·737	,, ,, ,,
	2·0046	0·2568	0·566	1·8538	0·3178	1·9696	98·27	5·833	,, ,, ,,
96	2·0000	0·2592	0·562	1·8838	0·2992	1·9790	98·95	6·296	,, ,, ,,
	2·0048	0·2590	0·572	1·8694	0·3226	1·9879	99·15	5·794	Nothing added
	2·0054	0·2569	0·562	1·8600	0·3082	1·9660	98·05	6·034	5 c.c. alumina cream

TABLE II. *Action of* 0·1 *grm. Taka-Diastase on Potato Starch at* 55°.

Time in hours	Weight of vacuum dried starch	CuO from 25 c.c. of 500 c.c.	α_D in 200 mm. tube at 20·00°	Dextrose in 500 c.c.	Maltose in 500 c.c.	Total starch	°/o starch found	Dextrose / Maltose	Remarks
12	2·0030	0·1578	1·074°	0·2366	1·8564	1·9716	98·46	0·127	Nothing added
24	1·9617	0·1597	1·059	0·2738	1·8146	1·9655	100·2	0·151	,, ,,
48	2·0171	0·1718	1·026	0·4506	1·6576	1·9758	98·0	0·272	5 c.c. alumina cream

The dextrose curve represents the dextrose formed from 100 grms. of starch, *calculated as starch* (by multiplying the dextrose figure at each time by 0·9); the maltose curve similarly represents the maltose as starch (dividing the maltose figure by 1·055). This system of

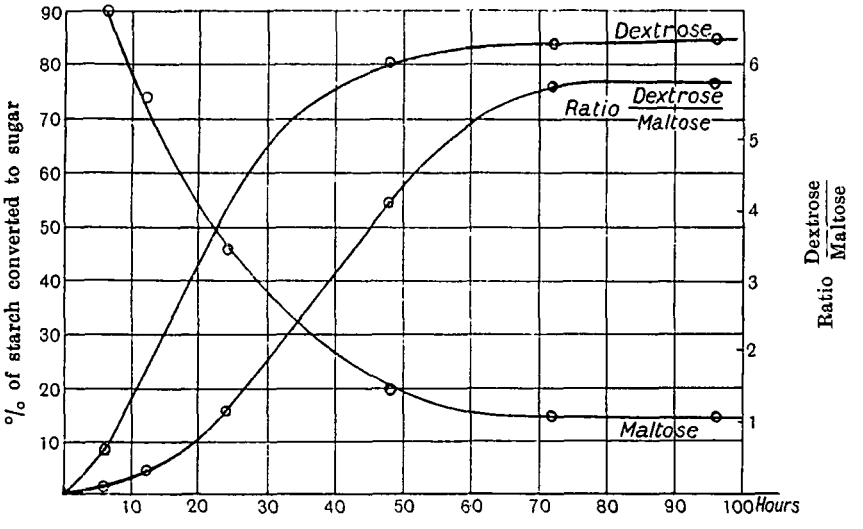

FIG. 1.

plotting the results has the advantage of showing for every moment the *proportion of the original starch which is present either as maltose or dextrose*, the sum of the maltose and dextrose values at each point being approximately 100, so that the two curves are complementary. The actual values plotted are as follows, being the mean values calculated for each time from the series in Table I.

TABLE III.

Time in hours	Starch present as dextrose	Starch present as maltose	Maltose starch / Dextrose starch
6	8·8 %	90·8 %	0·1
12	25·0	74·5	0·33
24	54·0	45·8	1·18
48	80·5	19·6	4·10
72	83·9	14·8	5·66
96	84·2	14·7	5·72

From the shape of the curves, coupled with the results found after three hours, it is probable that the first action of the taka-diastase is to break down the starch to dextrin and maltose, just as in the case of

ordinary diastase; the maltase comes into action comparatively slowly, so that after six hours only $\frac{1}{10}$th of the original starch is present as dextrose. Subsequently, however, the rate at which dextrose is formed increases, following very nearly a straight line curve between six hours and 28 hours, when about 60°/₀ of the starch is present as dextrose; the rate of formation of dextrose then rapidly slows down until a nearly constant value is reached in the neighbourhood of 84°/₀.

This arrest-point (possibly an equilibrium position) is very similar to the arrest-point which is reached in the case of ordinary diastase, at 55°, when about 80°/₀ of the starch has been converted into maltose and 20°/₀ remains in the form of dextrin. It might be thought from the coincidence of the numbers, that with taka-diastase the same proportion of a resistant dextrin is formed which then remains unchanged whilst the maltose is converted into dextrose, but such an assumption does not accord with the values actually found for the reducing power and polarisation at different times given in Table I. Dextrin does not appear to be present after about four hours.

That the product of conversion consists solely or almost solely of the sugars dextrose and maltose, as seems highly probable from the results of Table I, is borne out by the following actual estimations of maltose by the use of maltase-free yeasts (Davis and Daish, *J. Agric. Sci*, 1913, **5**, 462).

2·1671 *grms. vacuum dried starch digested* 36 *hours at* 38° *with* 0·1 *grm. taka-diastase; made up to* 500 *c.c.*

CuO from 50 c.c.	α_D in 400 mm. tube at 20°	CuO from 50 c.c. after fermentation with		Maltose calculated	
		S. Exiguus	*S. Marxianus*	from direct CuO and rotation	from CuO after fermentation
0·4496	1·317°	0·1243	0·1210	0·0833	0·0893

From Table II it is seen that *the action of the enzyme maltase which converts maltose into dextrose is very much restricted at* 55°; on comparing the numbers showing the ratio of dextrose to maltose with those for the same periods at 38° (Table I), it would appear that the enzyme is gradually destroyed at the higher temperature. The destruction, however, does not take place very rapidly, and although the action is decidedly limited after 24 hours, the increase in the ratio of dextrose to

maltose from 0·15 to 0·27, which occurs after 48 hours, shows that maltase still persists and exercises some activity.

As regards the applicability of taka-diastase to the analytical estimation of starch, the values given for the percentage of pure starch found in the conversions from six to 48 hours at 38° agree fairly closely among themselves, although they cover a wide variation in the ratio of dextrose to maltose (namely from 0·1 to 4·7); it thus seems highly probable that maltose and dextrose are the sole products of the conversion. For analytical purposes at least the error likely to be incurred by this assumption is small. It is noteworthy that the average value for the starch present in the purified potato starch found on using taka-diastase in the conversions of six to 48 hours is 99·65, whereas with ordinary diastase a value generally 0·5 per cent. higher was obtained (see p. 165). It is probable that the numbers obtained with taka-diastase more nearly represent the true starch values, as the calculations are based on the constants for two pure sugars only; they do not involve any assumption with regard to specific rotatory power for the "dextrin" existing in solution, which is generally taken at 202°, but some doubt may still be entertained as to the exactness of this value. It is probable too that the purified potato starch contains a small proportion of foreign material; hence the low value 99·6%.

When the conversions with taka-diastase are prolonged beyond 48 hours, somewhat lower starch values are generally obtained, as is seen in Table I; it is possible that some slight destruction of the sugars may occur during these prolonged conversions, but the lower values may also be due to the fact that a larger proportional error is incurred in the reading of the rotation. In Table I, the actual readings in a tube of 200 mm. for the longer conversions range only from about 0·5 to 0·6°; an error of 0·005° in the reading would therefore represent an error of 1%. That there is the actual closeness of concordance between the results for starch given in Table I with such small angles of rotation is due to the fact that with the instrument we have in use it is possible to read the rotation with a probable error not exceeding 0·005°. The use of taka-diastase in starch estimations has the advantage that it gives rise to two *sugars*, maltose and dextrose, the rotatory powers of which have been carefully determined; the temperature coefficients for these are exceedingly small so that no very special precautions to ensure exact constancy of temperature are necessary in ordinary work.

In actual analytical practice it is an easy matter to arrange the

quantities so that considerably higher rotations are observed and the proportional error in this direction minimised; examples of this are given on p. 162. That the addition of precipitating agents such as alumina cream and basic lead acetate, and the formation of heavy precipitates, such as are obtained with lead and sodium carbonate, do not in the least influence the results is shown by the examples in Table I, and by numerous other analyses which we need not quote.

Application of Taka-Diastase to Plant Material.

In estimating starch and the other carbohydrates in plant material, it is most essential that *the enzymes should be destroyed immediately after the sample* is taken, so as to prevent subsequent change in the relative and absolute proportions of the carbohydrates present. Unless this precaution be observed results are obtained which do not in the least represent the amount of actual carbohydrates present in the original tissue. Thus, for example, if the leaf tissue be dried in a drying oven before destroying the enzymes, during the slow loss of water from the leaf material and before the enzymes are paralysed considerable change may occur.

By dropping the leaf or other material directly into a large volume of boiling 95% alcohol to which 1% of its volume of 0·880 ammonia has been added, all plant enzymes seem to be immediately destroyed; no change in the proportions of the sugars present in the extract takes place subsequently even if the solutions are kept several weeks, when proper precautions are taken to exclude air and a little toluene is added to prevent the growth of micro-organisms.

Before the starch can be estimated in the leaf or other tissue, it is necessary to remove the sugars completely; for this purpose the leaf tissue, after killing the enzymes, is extracted for 18 to 24 hours with boiling alcohol in a special large form of Soxhlet extractor which will be described in a subsequent paper. Experiments have shown that the extraction of the sugars from leaves is complete as soon as the green colour of the chlorophyll has been removed. After the extraction is finished, the leaf material is pressed free from alcohol in a powerful screw hydraulic press (for example, of the Buchner type), and is thus obtained free from traces of the sugars, in a form in which it can be rapidly dried. The pressed cake is broken up into shreds and the material so obtained dried in a steam oven for 18 hours. It is then rapidly ground in a small mill and bottled for analysis

Our starch analyses in plant material are always referred to *vacuum dried weights.* For this purpose about 10 grams of the oven dried extracted material is dried to constant weight at 100 or 110° *in vacuo* over phosphorus pentoxide in the apparatus referred to on p. 154. It is generally necessary to heat the material for 24 hours or even longer.

To estimate starch, the dry material (free from sugars and, if necessary, previously extracted with water to remove gums, amylans, etc., see p. 162) is gelatinised with 200 c.c. of water in a beaker flask heated for ½ hour in a water bath at 100°. The solution is cooled to 38°, 0·1 grm. taka-diastase added, together with 2 c.c. of toluene and the mixture left 24 hours in order that the conversion may take place; it is then heated in a boiling water bath to destroy the diastase and the clear solution above the residual leaf material is filtered through a fluted filter paper into a 500 c.c. measuring flask; the leaf residue is thoroughly washed several times by decantation, the washings being passed through the filter paper until the volume of liquid in the flask amounts to about 475 c.c. The necessary quantity of basic lead acetate is then added to precipitate the tannins, etc. present in the solution; the amount required varies considerably with different leaves, generally ranging from 5 c.c. to 25 c.c. A large excess of lead should be avoided and tests should be made after each small addition of lead acetate in order to ascertain when the precipitation is complete. When this is the case the solution is made up to 500 c.c. at 15°, and filtered; 100 c.c. of the filtrate is placed in a 110 c.c. measuring flask, the slight excess of lead precipitated by adding solid sodium carbonate and the volume adjusted to 110 c.c. at 15°. 50 c.c. of the filtrate from the lead carbonate is used for the reduction and another portion polarised in a 400 mm. tube. The following example shows the method of calculation:

Weight of extracted leaf material (*Tropaeolum majus*) after drying in steam oven	= 10·4122 grms.
Weight of leaf material dried in vacuo at 100°	= 9·4059
CuO from 50 c.c. of the final 110 c.c.	= 0·4492 grm.
Polarisation of this solution in 400 mm. tube at 20·00°	= 1·995°

If x = grms. dextrose in 50 c.c. of this solution

y = grms. maltose ,, ,, ,, ,,

we have, using the values of CuO corresponding to 1 grm. of dextrose and maltose for the weight 0·4492 CuO in the tables of Brown, Morris and Millar:

$$2{\cdot}369x + 1{\cdot}362y = 0{\cdot}4492 \quad \ldots \quad (1)$$

For the 400 mm. tube, employing the values $[\alpha]_D^{20^\circ} = 137{\cdot}6$ and $[\alpha]_D^{20^\circ} = 52{\cdot}7$ for maltose and dextrose we have also

$$4{\cdot}216x + 11{\cdot}008y = 1{\cdot}995^\circ \quad \ldots \quad (2)$$

Solving equations 1 and 2 for x and y

$x = 0{\cdot}1095$ grm. dextrose in 50 c.c.
$y = 0{\cdot}1394$ grm. maltose in 50 c.c.

Total dextrose in 500 c.c. original solution $= 0{\cdot}1095 \times \frac{110}{50} \times \frac{500}{100} = 1{\cdot}2045$ grm.

,, maltose in ... ... $= 0\ 1394 \times \frac{110}{50} \times \frac{500}{100} = 1{\cdot}5334$ grm.

Starch corresponding to dextrose $= 0{\cdot}90 \times 1{\cdot}2045 = 1{\cdot}0840$ grm.
,, ,, ,, maltose $= 1{\cdot}5334 \div 1{\cdot}055 = 1{\cdot}4535$,,
Total starch $= 2{\cdot}5375$ grms.

$\therefore$ % of starch in vacuum dried extracted leaf material

$$2{\cdot}5375 \times \frac{100}{9{\cdot}4059} = 26{\cdot}97\ \%.$$

Precautions necessary in taking Leaf Samples for Analysis.

If the dried, ground leaf material is bottled before analysis, it is absolutely necessary when each sample is taken for the analysis, *to turn out the whole of the material on to a sheet of paper and thoroughly mix it before sampling.* If this precaution be not observed and successive samples are taken directly from the bottle, it is found that the proportion of starch present in the material increases towards the bottom of the bottle. This is no doubt due to the fact that the heavier starch grains, set free from the tissue by grinding, sink to the bottom of the bottle, whilst the lighter fibrous material of the leaf rises to the top. This is well shown by the following successive analyses made with potato-leaves (previously freed from sugars by extraction):

1	sample	from	top of bottle,	starch	= 7·54 %	on vacuum dried matter
2	,,	,,	middle of bottle		= 9·19 %	,,	,,
3	,,	,,	,,	,,	= 9·23 %	,,	,,
4	,,	,,	bottom	,,	= 12·29 %	,,	,,

Several similar results were obtained with the leaves of turnips, *Tropaeolum*, etc. which we need not quote, before we became aware of the necessity of special care in the sampling of the finely ground leaf material. When, however, the sampling is carried out in the way described, the agreement between different individual determinations is as satisfactory as could be expected in this class of work. We append a few examples.

TABLE IV. *Turnip leaves. Sample taken 4.45 p.m., July 9th, 1913, bright but cool day. Starch in vacuum dried leaf after extraction of sugars.*

Weight of vacuum dried material	CuO from 50 c.c. of 110 c.c.	a_D in 400 mm. tube at 20·00°	Total maltose	Total dextrose	Total starch	°/₀ starch in vacuum dried leaf	Dextrose / Maltose	Remarks
10·0705	0·3033	1·661°	1·4487	0·5412	1·8601	18·47	0·374	24 hrs. conversion. Required 12·5 c.c. basic lead acetate
9·8300	0·3456	1·439	1·0736	0·9454	1·8690	19·01	0·880	27 hrs. conversion. 12·5 c.c. basic lead acetate
9·2455	0·3000	1·448	1·1880	0·6689	1·7290	18·71	0·563	
					Average	18·73		

TABLE V. *Nasturtium leaves* (Tropæolum majus). *Dry leaf after extraction of sugars. Sample taken July* 11, 1913, 5.15 *p.m. after fairly sunny day.*

Weight of vacuum dried material	CuO from 50 c.c. of 110 c.c.	a_D in 400 mm. tube at 20·00°	Total maltose	Total dextrose	Total starch	°/₀ starch in vacuum dried leaf	Dextrose / Maltose	Remarks
9·4059	0·4492	1·995°	1·5334	1·2045	2·538	26·97	0·786	24 hrs. conversion. 15 c.c. lead acetate required
9·0404	0·4368	1·823	1·3398	1·2518	2·397	26·53	0·934	
					Average	26·75		

Mangold Leaves. Very numerous analyses made with mangold leaves sampled at various periods of the night and day have shown that during the period when cane sugar is being actively stored by the roots, starch is entirely absent from the leaf.

The Necessity of removing Substances soluble in Water other than Sugars which are optically active.

One of the principal difficulties in estimating starch in plant material is due to the presence of gummy substances, tannins, proteins, etc. which pass into solution during the hydrolysis and exercise an effect on the rotatory and reducing power of the solution. These substances are very largely removed by the use of basic lead acetate, but sufficient impurity remains, even after this treatment, to falsify the

analyses in some cases, as shown by the following results obtained with mangold leaves free from starch[1]:

9·6205 grms. vacuum dried leaf was treated with 0·1 grm. taka-diastase as in an ordinary starch estimation; after digestion for 36 hours at 38°, the solution was filtered and washed to 470 c.c., basic lead acetate being then added until no further precipitate was obtained. The solution was made up to 500 c.c., filtered and 100 c.c. deprived of lead by sodium carbonate and the solution made up to 110 c.c.

50 c.c. of filtrate gave CuO = *nil*.
Polarisation in 400 mm. tube = − 0·130°.

This laevo-rotation is very considerable, representing about 7 % of the actual rotation usually measured in an analysis of leaf material. It is probably due to the presence of a gum which forms a lead salt which is not absolutely insoluble in water, so that the solution after filtration from the bulk of the precipitate is saturated with it; on precipitation of the lead with sodium carbonate, a soluble sodium salt is formed which is laevo-rotatory.

That this is the case appears on evaporating 300 c.c. of the 500 c.c. of clear solution obtained after precipitating with basic lead, to about 40 c.c. when a considerable quantity of gummy matter separates; the solution was filtered, washed to about 70 c.c. and sodium carbonate added so as exactly to precipitate the lead. The solution was filtered, evaporated to a syrup, which was then taken up with 50 c.c. of 95 % alcohol, the extract filtered from the residue of gum, sodium acetate, etc., and the alcohol evaporated; the residue was dissolved in water and made up to 100 c.c.

50·00 c.c. gave CuO = *nil*.
Polarisation = − 0·130° in 400 mm. tube at 20°.

In this case the treatment with alcohol has removed approximately two-thirds of the gummy matter (bearing in mind that 300 c.c. of the original solution was taken); it is however extremely difficult, if not impossible, completely to eliminate the gum in this way by repeated treatment with alcohol, even when followed by addition of alumina cream to the aqueous solution finally obtained. The final solution always shows more or less laevo-rotatory power.

In working with leaf and plant material it is generally possible completely to extract the disturbing gummy material prior to the starch

[1] That this was the case was found not only by analysis but by microscopical examination by the chloral hydrate-iodine method; even the guard cells were free from starch.

conversion by a preliminary treatment with water. Thus in the case of the mangold leaf, by adding 200 c.c. of water and 5 c.c. of toluene to the leaf material and extracting for 24 hours at 38°, decanting and washing with a little water and subsequently converting with taka-diastase, in the ordinary way, a solution is finally obtained (after the usual treatment with basic lead and sodium carbonate) which in a 400 mm. tube shows a laevo-rotation of not more than 0·01°. It is noteworthy that the preliminary treatment with water fails to remove the greater part of the material precipitable by basic lead acetate, so that this treatment is necessary even after the preliminary extraction with water.

In the case of plant material from which gummy matter is extracted with extreme difficulty, it would probably be sufficient to introduce a correction for any active substances present by carrying out a control experiment or "blank" in which the diastase is omitted but the material otherwise treated exactly as in the actual estimation of starch.

The application of taka-diastase to the estimation of starch in cereals such as wheat, barley, etc., which present special difficulties, is being studied.

Appendix.

The ordinary diastase method and the error caused by the co-precipitation of dextrin.

To ascertain the degree of accuracy of this method under the most favourable conditions, a series of analyses were made, using purified potato starch dried *in vacuo* at 120° (see p. 154) until constant in weight; it was then gelatinised by heating with 50 c.c. of water in a boiling water bath during 15 minutes, cooled to 55° and subjected to the action of the diastase (0·100 grm. of the alcohol-precipitated enzyme) until all the starch was converted. This was generally the case after two or three hours. The diastase was then destroyed by heating the solution in boiling water during 15 minutes and the solution made up to 250 c.c. at 15°. The reducing power was estimated in 25 c.c. under Brown, Morris and Millar conditions and the rotatory power determined in a 400 mm. tube, the values assumed being maltose $[\alpha]_D = 138°$, dextrin $[\alpha]_D = 202°$.

TABLE VI. *Purified Potato Starch, Diastase Method.*

Conditions	Air dried starch taken	Weight dried in vacuo at 120°	% water in starch	CuO from 25 c.c. *	α_D in 400 mm. tube	Maltose total	Dextrin total	Starch found	% starch in vacuum dried material
5 hours	2·5017	1·9958	20·22	0·2211	5·090°	1·619	0·469	2·004	100·4
4 ,,	2·5110	2·0031	20·22	0·2191	5·125	1·604	0·4895	2·0095	100·3
5½ ,,	—	2·0732	—	0·2273	5·246	1·664	0·486	2·066	99·7
4 ,,	2·5110	2·003	20·22	0·2180	5·125	1·596	0·495	2·008	100·25
23 ,,	2·5002	1·9942	20·24	0·2401	4·968	1·758	0·3360	2·003	100·4
23 ,,	2·5002	1·994	20·24	0·2347	4·968	1·718	0·3636	1·992	99·9
15½ ,,	2·5118	2·0042	20·21	0·2291	5·040	1·677	0·414	2·003	100·0
								Average	100·1
Malt extract about 5 c.c. Made to 100 c.c.	3·0163	2·4220	19·70	0·2727 (ex. 10 c.c.)	15·24	2·000	0·5190	2·414	99·6

* The CuO in all cases is the average of two closely concordant values. Allowance has been always made for the reduction and polarisation due to diastase or malt extract; this was ascertained by a control experiment carried out under exactly the same conditions as in the corresponding experiment with starch. The experiment with malt extract was made on a different sample of starch.

These results (Table VI) are moderately satisfactory, but the following experiments (Tables VII to X) show that a great loss of dextrin occurs in the ordinary diastase method when any kind of precipitate is produced in the solution obtained from the hydrolysis; in these experiments basic lead acetate was added in different proportions and the lead was subsequently precipitated by sodium carbonate or hydrogen sulphide. The amount of dextrin removed depends on the quantity of lead precipitated, so that the lowest results for starch were obtained in the experiments in which the largest quantity of lead was used.

A. *Precipitation of the excess of lead by Sodium Carbonate.*

The starch was gelatinised with 50 c.c. of water and hydrolysed by diastase in the usual way at 55°; after conversion, the solution was washed into a 100 c.c. flask, and 5 c.c. of basic lead acetate added. Sodium carbonate was then added to precipitate the lead exactly and the solution diluted to 100 c.c.; it was then filtered and 10 c.c. used for reduction.

TABLE VII.

Air dried starch taken	Weight after drying in vacuo at 120°	% water in starch	CuO from 10 c.c.*	Maltose in 100 c.c.	α_D in 200 mm. tube	Dextrin in 100 c.c.	Total starch	% starch found in vacuum dried material
—	2·1843	—	0·2375	1·7395	5·42°	0·1550	1·804	82·6
2·0361	1·8601	19·34	0·1922	1·405	4·41	0·1329	1·464	78·7

B. *Precipitation of the excess of lead by Hydrogen Sulphide.*

1. *Using 5 c.c. basic lead acetate.* Procedure as in A, but the lead added to the starch conversion was removed by passing hydrogen sulphide through the solution after diluting to about 100 c.c. The solution was filtered and the precipitate thoroughly washed until the washings amounted exactly to 200 c.c. 20 c.c. used for reduction.

TABLE VIII.

Starch taken	Weight after drying in vacuo at 120°	% water	CuO from 20 c.c.*	Maltose in 200 c.c.	α_D in 200 mm. tube	Dextrin in 200 c.c.	Total starch	% starch in vacuum dried material
2·5008	2·0175	19·32	0·2002	1·464	2·69°	0·3316	1·719	85·23
2·5004	2·0162	19·36	0·2036	1·489	2·69	0·3158	1·727	85·70
2·4962	2·0132	19·38	0·2115	1·547	2·71	0·2862	1·753	87·0

2. Using 5 c.c. basic lead as in 1, but washing the precipitate of lead sulphide more thoroughly, viz. to 250 c.c., so as to ensure that the low results were not due to insufficient washing. 25 c.c. used for reduction.

TABLE IX.

Starch taken	Weight after drying in vacuo at 120°	% water	CuO from 25 c.c.	Maltose in 250 c.c.	α_D in 200 mm. tube	Dextrin in 250 c.c.	Total starch	% starch found in vacuum dried material
2·4783	1·9948	19·42	0·1874	1·376	2·05°	0·3285	1·634	81·89

3. Using 2 c.c. basic lead only; remaining procedure as in 1. Washing to 200 c.c.; 20 c.c. used for reduction.

TABLE X.

Starch taken	Weight after drying in vacuo at 120°	% water	CuO from 20 c.c.	Maltose in 200 c.c.	a_D in 200 mm tube	Dextrin in 200 c.c.	Total starch	% starch found in vacuum dried material
2·4990	2·0157	19·33	0·2080	1·5215	3·04°	0·4672	1·9092	94·7
2·4950	2·0130	19·32	0·2087	1·5260	3·02	0·4504	1·898	94·3

TABLE XI. *Results obtained with purified Potato Starch (vacuum dried) by the modified Sachsse method (A.O.A.C.).*

Weight of starch dried in vacuo at 120—130° till constant	CuO ex 25 c.c.	Dextrose in 25 c.c.	% starch found
2·0239	0·2684	0·1059	94·2
2·0239	0·2680	0·1058	94·1
2·0187	0·2680	0·1058	94·3
2·0187	0·2666	0·1052	93·8
		Average.....	94·1

In this method the material is heated during 2½ hours with 200 c.c of water and 20 c.c. of hydrochloric acid of sp. gr. 1·125, so that hydrolysis is actually effected by 2·52 per cent. hydrochoric acid. After heating, the solution is neutralised with sodium hydroxide, made to 250 c.c. and the dextrose in 25 c.c. estimated by means of Fehling solution.

The values given show that the modified Sachsse method gives results which are 6 per cent. low as compared with those obtained by using diastase.

SUMMARY.

1. The Sachsse method of estimating starch is unreliable in the case of plant material; not only does the presence of pentosans falsify the results as pentoses are formed during the hydrolysis, but actual destruction of dextrose occurs during the prolonged treatment with dilute acid.

2. O'Sullivan's method gives low results owing to the loss of dextrin which occurs during the purification of the solution after the conversion by diastase.

3. A method is described for estimating starch based on the use of taka-diastase; under suitable conditions this converts the starch into maltose and dextrose only and no loss of these sugars occurs when the solution is treated with clearing agents such as basic lead acetate.

4. The necessity of removing substances soluble in water, such as gums, etc., which are optically active and thus cause error in the estimation of starch in plant material is emphasised. Special care is also necessary in sampling.