LIX. THE APPLICATION OF THE IODIMETRIC METHOD TO THE ESTIMATION OF SMALL AMOUNTS OF ALDOSES.

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One of the most interesting facts emerging from the study of the carbohydrate-phosphoric esters formed during alcoholic fermentation is the rapid conversion of glucose into derivatives of fructose and *vice versa*. The fermentation of either glucose or fructose by yeast juice, dried yeast or zymin may give rise to phosphoric esters of glucose, γ-fructose, trehalose and possibly of other sugars, in proportions which vary within wide limits according to the type of yeast and other conditions of the fermentation. For the investigation of these products a reliable method for distinguishing quantitatively between aldoses and ketoses is much to be desired.

The reaction between aldoses and alkaline solutions of iodine

$$R.CHO + I_2 + 3NaOH = R.COONa + 2NaI + 2H_2O$$

was first applied to the estimation of sugars by Romijn [1897] who, after comparative experiments, selected borax as the most suitable form of alkali. At 25° oxidation of glucose and other aldoses was found to be complete in 16–22 hours, but ketoses and non-reducing sugars were also oxidised to a varying extent. Bland and Lloyd [1914] employed a solution containing iodine and sodium hydroxide in equimolecular proportions, and claimed that, at room temperature, oxidation of glucose, maltose and lactose was complete in 5 minutes, while fructose and sucrose were not attacked. Bougault [1917] and Cajori [1922] used sodium carbonate as the alkali and allowed the reaction to proceed for 20–30 minutes at room temperature. Both emphasise the necessity of employing a large excess of iodine (three times the theoretical quantity). Colin and Liévin [1918] replaced the carbonate by disodium hydrogen phosphate without any obvious advantage. In Willstätter and Schudel's [1918] modification sodium hydroxide is used and is added last, in the proportion of 3 mols. to 2 mols. of iodine—as required by the equation. The oxidation is carried out at room temperature during 12–20 minutes. This modification has been most frequently employed in biochemical investigations but the authors' claim that under the specified conditions fructose and sucrose are not attacked has been disputed by Judd [1920] and Bruhns [1923], who have obtained values for fructose varying between 7 % and 11 %

in terms of the reducing power of glucose. Baker and Hulton [1920] found that oxidation of aldoses was complete in 3–5 minutes, but even under these conditions fructose was oxidised to the extent of 7 %. According to Judd this partial oxidation is the result of the Lobry de Bruyn and van Ekenstein transformation but the experimental evidence brought forward by Baker and Hulton does not bear out this suggestion.

The investigations of Kolthoff [1923] and of Hinton and Macara [1924] show that the degree of oxidation both of aldoses and of ketoses is influenced to a marked extent by the relative proportions of iodine and alkali, and that satisfactory results are only to be obtained by careful regulation of these and other factors. In a recent paper Goebel [1927] states that the oxidation of glucose is more complete if the alkali be added gradually over a period of several minutes. In all these investigations the estimations were carried out for the most part on relatively large amounts of the sugars. As we wished to apply the method to the estimation of quantities of the order of 1 mg. it seemed advisable to study first the effect of the various factors under the conditions involved in such micro-estimations.

The following is the general procedure adopted for these tests.

1 or 2 cc. of the sugar solution (0·1 %) was measured into a 50 cc. Erlenmeyer flask, followed by 3 cc. of 0·02 *N* iodine and distilled water to make up the volume to 6 cc., this last serving to rinse down the sides of the flask. The alkali solution was then added slowly and the flask immediately closed with a rubber stopper and immersed in a water-bath at the specified temperature. The use of "room temperature" was given up as the variations were found to be sufficient to produce irregular results. At the end of the given time the contents of the flask were acidified with 1 cc. 0·5 *N* H_2SO_4 and titrated with 0·005 *N* sodium thiosulphate using as indicator 3 drops of a 1 % solution of soluble starch in a saturated solution of sodium chloride. All estimations were made in duplicate and blank tests, also in duplicate, were simultaneously carried out, using distilled water in place of the sugar solutions. These blanks were further checked by direct titration of 3 cc. of the iodine solution with the thiosulphate. The difference between the blanks and the direct titration did not as a rule exceed 0·1 cc., and was due to loss of iodine by volatilisation, impurities in the alkali, etc. The usual precautions were taken as regards calibration of the Ostwald pipettes and the burette. The sugars used in these tests gave values for the specific rotation and reducing power by the Hagedorn-Jensen method as shown in Table I. The values for the reducing power are given in terms of glucose and for the other sugars are of limited significance, but the very low value for galactose, which is similar to that recorded by Pucher and Finch [1928], is of interest in relation to the results obtained by the iodimetric method.

The results given in Tables II and III show the influence of varying quantities of sodium hydroxide and sodium carbonate respectively on the oxidation of glucose and fructose.

Table I.

Sugar	$[\alpha]_{5461}^{20°}$ $c=10$ %	Reducing power %
Glucose (Kahlbaum)	+ 62·8° (21°)	99·9
Fructose (Kerfoot)	−108·7°	96·2
Galactose (Kahlbaum)	+ 96·9°	73·0
Lactose	+ 64·6° (19°)	67·0
Maltose hydrate (Kahlbaum)	+154·8°	68·5
Sucrose	+ 78·6°	—

Table II.

Alkali: 0·1 *N* NaOH, in amount varying from 1 to 1·66 times the equivalent of iodine.

Vol. of 0·1 *N* NaOH cc.	Temp.	Time (mins.)	Glucose (1 mg.)	Glucose (2 mg.)	Fructose (1 mg.)
0·6	17°	20	− 0·5	− 8·7	3·2
			− 0·5	− 8·7	3·2
0·75	„	„	− 0·5	−13·0	3·6
			− 1·0	− 8·7	3·6
0·9	„	„	+ 0·5	− 2·2	4·0
			+ 0·5	−10·0	4·0
1·0	„	„	+ 0·5	− 2·2	4·0
			0	− 5·7	4·0
0·6	21°	5	−15·7	—	0·4
			−15·7	—	1·2
0·75	„	„	−20·9	—	0·8
			−13·1	—	0·8
0·9	„	„	−12·1	—	0·8
			−13·1	—	0·8
1·0	„	„	−12·2	—	1·2
			−12·2	—	1·2
0·6	21°	20	− 0·5	−11·1	5·9
			+ 1·2	−11·1	7·5
0·75	„	„	− 0·5	−13·7	7·5
			− 0·5	−13·7	10·0
0·9	„	„	+ 0·9	− 2·4	8·8
			− 1·3	− 2·0	8·8
1·0	„	„	+ 0·4	− 1·3	9·2
			− 1·3	− 0·9	9·2

For glucose the results are stated as percentage deviations from the theoretical values for complete oxidation to gluconic acid.

For fructose the results are stated as the percentage oxidised, calculated on the basis of the theoretical value for glucose.

The amount of iodine used was in all cases 3 cc. 0·02 *N* solution, equivalent to 5·4 mg. glucose. The titration thus required (for the blank) about 12 cc. 0·005 *N* thiosulphate. 2·25 cc. is equivalent to 1 mg. glucose. The results, though calculated to the first decimal place, are only significant to about 0·5 %.

These results show that:

(1) With sodium hydroxide (0·9 cc.) in the proportion of 3 mols. to 2 mols. of iodine, as recommended by Willstätter and Schudel, the oxidation of 1 mg. glucose is complete in 20 minutes at 17° or 21°, but not in 5 minutes at 21° (iodine in 5-fold excess). With 2 mg. glucose the results for 17° are irregular but are more satisfactory for 20 minutes at 21°.

Table III.

Alkali: 5 % Na_2CO_3 solution (0·94 *N*).

Vol. of 5 % Na_2CO_3 cc.	Temp.	Time (mins.)		Glucose 1 mg.	Glucose 2 mg.	Glucose 3 mg.	Fructose 1 mg.	Fructose 2 mg.	Fructose 3 mg.
0·1	21°	30		+0·4	−23·1	−61·7	—	—	—
,,	,,	,,		0	−33·3	−57·5	—	—	—
,,	,,	,,		−4·1	—	—	—	—	—
,,	,,	,,		−9·8	—	—	—	—	—
			Av.	−4·5	−28·2	−59·6			
0·2	21°	30		+1·0	− 1·2	−18·5	0·4	0·8	1·4
,,	,,	,,		+1·0	− 1·2	−23·4	0·4	2·5	2·2
,,	,,	,,		−1·5	− 3·1	−33·3	1·7	—	—
,,	,,	,,		−2·4	− 3·1	−33·3	0·9	—	—
,,	,,	,,		−0·2	—	—	—	—	—
,,	,,	,,		−0·2	− 2·2	—	—	—	—
,,	,,	,,		−0·2	—	—	—	—	—
,,	,,	,,		−0·2	− 2·2	—	—	—	—
,,	,,	,,		+2·3	—	—	—	—	—
,,	,,	,,		+2·3	—	—	—	—	—
,,	,,	,,		+2·6	—	—	—	—	—
,,	,,	,,		+2·6	—	—	—	—	—
			Av.	+0·6	− 2·2	−27·1	0·8	1·6	1·8
0·4	21°	30		+0·7	− 1·7	−10·3	0·4	0	—
,,	,,	,,		+0·7	—	—	0·4	0	—
,,	,,	,,		+0·7	− 3·0	− 8·4	0	—	—
,,	,,	,,		−2·0	—	—	0	—	—
,,	,,	,,		−1·5	− 1·8	−11·6	0·4	—	—
,,	,,	,,		+0·3	—	—	—	—	—
,,	,,	,,		+0·7	− 0·7	—	0·4	—	—
			Av.	+0·1	− 1·8	−10·1	0·3	0	—
0·4	25°	30					0	0·7	—
,,	,,	,,		+0·3	− 2·4	− 8·3	0	0·4	—
,,	,,	,,		+0·7	− 2·2	− 7·9	0·4	—	—
,,	,,	,,		0	—	—	0·4	—	—
,,	,,	,,		0	—	—	0	—	—
,,	,,	,,		0	—	—	0	—	—
,,	,,	,,		0	—	—			
			Av.	+0·2	− 2·3	− 8·1	0·1	0·5	—
0·8	21°	30		−8·8	—	—	—	—	—
,,	,,	,,		−6·7	−20·3	−30·8	0·9	0·9	—
,,	,,	,,		−8·8	—	—	—	—	—
,,	,,	,,		−8·8	—	—	—	—	—
,,	,,	,,		−9·7	−20·5	—	2·6	0·9	—
			Av.	−8·6	−20·4	−30·8	1·7	0·9	—

(2) Under the conditions necessary for the complete oxidation of 1 mg. glucose, fructose is also oxidised to a significant extent, which may amount to 9 % in 20 minutes at 21°. This disadvantage is not overcome by using other proportions of sodium hydroxide.

(3) With sodium carbonate in suitable amounts (0·2 cc. or 0·4 cc. 5 % Na_2CO_3), oxidation of 1 mg. glucose is complete in 30 minutes at 21°; 2 mg. glucose are oxidised to the extent of 98 %, but with larger amounts the results are low and irregular. Fructose is oxidised only to a very small extent, the values shown (0–2 %) lying close to the limits of titration errors by this method.

(4) With amounts of 5 % sodium carbonate less than 0·2 cc. or more than 0·4 cc. the oxidation of glucose is incomplete.

From other tests it was found that variations in the temperature between 21° and 25°, or in the time between 20 and 45 minutes did not affect the results. The use of sodium carbonate therefore offers a greater latitude as regards amount of alkali, temperature etc. than is permissible with sodium hydroxide.

For routine estimations the conditions adopted were, for amounts of sugar equivalent to 1–1·5 mg. glucose, 3 cc. 0·02 *N* iodine; 0·2 cc. 5 % Na_2CO_3; 21° and 30 minutes. These correspond closely with the conditions recommended by Bougault for larger quantities of sugar.

Table IV gives the average results obtained with various sugars under the above conditions. For the aldoses the results are stated as percentages of the respective compounds calculated from the iodine reduced, according to the equation. For the ketoses and sucrose the results are given in terms of the equivalent quantity of glucose. The results for galactose were persistently low and were not improved by increasing the time or by adding the alkali over a period of 6 minutes[1]. In this table are also included values obtained for barium glucosemonophosphate, barium fructosemonophosphate (Neuberg) and barium fructosediphosphate, those for the fructose derivatives being calculated as percentage glucose. In spite of the low value for the glucosemonophosphate there is good reason for believing that the specimen was free from fructosemonophosphate (Robison and King, unpublished work).

It should perhaps be emphasised that great caution is necessary in interpreting the results obtained by this method with crude biochemical products since many compounds other than aldoses may react with iodine under these conditions.

Table IV.

Sugar	1 mg.	2 mg.	3 mg.	4 mg.
Glucose	100·6	97·8	78·1	—
Galactose	97·0	96·5	85·6	—
Maltose	102·8	100·9	—	78·4
Lactose	102·3	98·3	—	75·5
Fructose	0·8	1·6	1·8	—
Sucrose	2·0	0·8	1·7	—
Barium glucosemonophosphate	—	90·2	—	—
Barium fructosemonophosphate	—	—	—	(15 mg.) 3·0
Barium fructosediphosphate ...	—	—	—	0–2·0

Some experiments were carried out to determine to what extent the Lobry de Bruyn and van Ekenstein transformation may account for the high values obtained for fructose using sodium hydroxide. In order to increase the effect, the ratio of sodium hydroxide to iodine was raised to 10 : 3 but the concentration of alkali was only 0·03 *N*. The results are set out in Table V.

[1] *Note (added 7th June* 1929). Satisfactory results have, however, since been obtained with galactose, prepared by the hydrolysis of a highly purified specimen of α-methyl-*d*-galactoside, for which we have to thank Mr J. A. Pryde.

Column A. Reducing power of fructose by the usual carbonate method (in this case, 0·4 cc. 5 % Na_2CO_3 was used) 30 minutes at the specified temperatures.

Column B. Reducing power using 2 cc. 0·1 *N* NaOH, 30 minutes at the specified temperature.

Column C. Fructose solution allowed to remain for 30 minutes at the specified temperature in presence of 2 cc. 0·1 *N* NaOH, then neutralised and reducing power estimated by the carbonate method (30 minutes, 21°).

Column D. Fructose solution allowed to remain for 30 minutes at the specified temperature in presence of 2 cc. 0·1 *N* NaOH. Iodine then added and solution left for further 30 minutes at the specified temperature.

Table V.

Amount of fructose mg.	Temp.	*A*	*B*	*C*	*D*
1	21°	0·4	13·1	5·3	24·9
5	21°	0·6	16·8	4·4	16·9
1	25°	0·4	17·8	11·4	25·8
5	25°	0·9	17·2	6·2	22·2
1	38°	4·0	68·0	22·7	84·1
5	38°	2·5	30·4	14·7	39·4

Comparison of columns *A* and *C*, as also of columns *B* and *D*, shows that some change is produced by the action of the alkali alone, but this could at most account for only a fraction (30–40 %) of the total oxidation effected by the combined action of the iodine and alkali, shown in column *B*, unless the nett rate of the transformation of fructose into glucose is greatly increased in presence of the iodine owing to the rapid removal of the aldose by oxidation.

Summary.

The conditions for the estimation of very small amounts of aldoses by the iodimetric method have been investigated. Satisfactory results are obtained using 3–4 times the theoretical quantity of iodine with sodium carbonate as the alkali, and allowing the oxidation to proceed during 30 minutes at 21°. Under these conditions the oxidation of glucose is complete while fructose and sucrose are only oxidised to a very small extent.

Values obtained for other sugars and for hexosemono- and hexosediphosphates are also given.

The extent to which fructose is oxidised by iodine in presence of excess of sodium hydroxide is much greater than can be explained by the Lobry de Bruyn and van Ekenstein transformation unless it is assumed that the change from ketose to aldose is largely increased owing to the rapid and continuous removal of the latter by oxidation.

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