XXVI. THE IODOMETRIC ESTIMATION OF SUGARS.

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IN the course of a series of investigations of the changes that take place in the nature and amount of the carbohydrates of apples during the process of ripening, both in ordinary storage and in cold storage, it has been found that the usual method of estimating fructose by means of the polarimeter is very unsatisfactory. In the first place, the optical activity of apple juice is very low; the sugars present appear to consist mainly of saccharose, glucose, and fructose, the last being in considerable excess, with the result that the juice gives a very small polarimetric reading. Moreover, the juice is always coloured, and is frequently so viscous that it is necessary to dilute it very considerably before it is possible to clear the solution by means of basic lead acetate and alumina cream. The resulting solution seldom contains more than 0.5 % or 1 % of sugars, giving in a 2 decimetre tube a maximum reading of one degree. Obviously the error involved in calculating the proportion of each of the sugars present from such small readings is very large. It becomes clear then that some other method is required.

Several papers dealing with the action of bromine and iodine in alkaline solution on various sugars have been published, some of them quite recently. It has been definitely established that under suitable conditions, both bromine and iodine bring about the oxidation of most sugars, the main product being the corresponding monobasic acid. Bromine in cold aqueous solution slowly oxidises aldoses, and has a still slower action on ketoses. In a mixture of aldose and ketose, however, it is not possible so to control the experiment that only the aldose is attacked.

Bougault [1917] suggests the use of weakly alkaline solutions of iodine. Glucose is then oxidised according to the equation:

According to Bougault, fructose when treated in the same way is not appreciably attacked; he admits however that his results are not consistent, and show many discrepancies.

Colin and Lièvin [1918] have attempted to modify and improve Bougault's method. They point out that the degree of alkalinity of the solution may influence the result obtained. While Bougault used a mixture of sodium carbonate and sodium bicarbonate, Colin and Lièvin prefer a solution containing per litre 35 g. of disodium phosphate and 50 cc. of *N* sodium hydroxide.

Romijn [1897] recommends the use of alkaline iodine solutions for the estimation of glucose in presence of fructose. He describes experiments with sodium carbonate, ammonium carbonate, trisodium phosphate, and borax, and prefers the last, as being only very weakly alkaline, and having a less destructive action on the sugar molecule.

The objections to the use of the borax-iodine solution advocated by Romijn are: (1) below 25°, the action is unduly prolonged; (2) at temperatures above 25°, fructose is attacked; (3) in such weakly alkaline solutions, the alkalinity is appreciably reduced by the formation of acid from the sugar.

According to Romijn, all the aldoses behave like glucose, and such di- and tri-saccharides as saccharose and raffinose, which do not reduce Fehling's solution, are also attacked.

Willstätter and Schudel [1918] comment on Romijn's work, and point out that the course of the reaction depends on the amount and on the concentration of the alkali present. There must be sufficient alkali to neutralise the acid formed as a result of the oxidation, but if this amount is exceeded the reaction is not complete. According to Willstätter and Schudel the reaction goes best with decinormal solutions, and in the proportions required by the equation:

They state that if these conditions are fulfilled the error with glucose is not more than 1 %. They point out however that in the presence of free alkali the sugars undergo the Lobry de Bruyn transformation.

They find that cane sugar is not acted upon. They also give results with invert sugar, which approximate closely to theory; and they state that under the same conditions fructose is not attacked; no figures however are given for fructose alone.

The following experiments have been carried out in order to determine whether any of the methods mentioned above are applicable to the estimation of the amounts of glucose and fructose present in fruit juices. It has been found, as the result of a large number of determinations carried out with solutions of pure sugars, that neither the method of Colin and Lièvin nor that of Willstätter and Schudel is exact, and that in no case is the sugar oxidised quantitatively to the corresponding monobasic acid; on the other hand, there is always a very definite attack on the fructose. The results obtained with invert sugar are misleading since the low result obtained from the glucose is masked by the partial oxidation of the fructose.

It has further been noticed that the reaction mixture always smells strongly of iodoform, suggesting that some secondary change is taking place, Romijn also draws attention to the fact that a large number of organic substances which give the iodoform reaction are readily oxidised by alkaline iodine solutions.

It seems probable however that the chief source of error is the action of dilute alkalis on sugars, investigated by Lobry de Bruyn and van Ekenstein [1895, 1896, 1897]. Since even such weak alkalis as lead hydroxide bring about this change, it is certain that it would occur in a solution containing a considerable excess of sodium hydroxide. Starting therefore with a solution of pure glucose, part of this sugar would be converted by the alkali into mannose (which is also oxidised by iodine) and part into fructose. Consequently the whole of the sugar present would not be oxidisable by iodine, and the result obtained for the glucose would be too low. Conversely, a solution of fructose would yield a certain proportion of both glucose and mannose, and this probably accounts for the apparent partial oxidation of the fructose.

It may be assumed therefore that the behaviour of the above sugars towards alkaline iodine solutions is in some measure dependent on the Lobry de Bruyn transformation, and that in the presence of iodine some definite equilibrium is reached.

The equilibrium obtained in this way must be a very complex one. According to the observations of Lobry de Bruyn and van Ekenstein, the composition of the almost inactive syrup obtained by the action of dilute alkalis on glucose, mannose, or fructose depends on the nature of the sugar which was used at the start. It appears also from the present series of experiments that the removal of the aldoses present by oxidation does not, as would be expected, tend to convert more ketose into aldose.

It is however possible to utilise the method of Willstätter and Schudel for the analysis of mixtures of glucose and fructose. A large number of experiments show that a given weight of glucose always uses up a definite and constant weight of iodine, although not the amount theoretically required. The same statement holds good for fructose. Neither of these reactions is in any way affected by the presence of other sugars. Experiments with mixtures of glucose and fructose in varying proportions show that the iodine equivalent of the mixture calculated from the two constants in all cases shows very close agreement with that found by experiment. This being the case, it is possible to calculate from the copper reducing power of a mixture of glucose and fructose, and from the amount of iodine used up by the mixture, the proportion of each sugar present.

Experimental details.

The solution of sodium thiosulphate used was carefully standardised by Volhard's method against *N*/10 potassium permanganate. The latter had been standardised in the usual way with ferrous ammonium sulphate and also

against oxalic acid; the values obtained by these two methods showed very close agreement.

The concentration of the sugar solutions used was determined by Bertrand's method, using the same permanganate solution, and the purity of the sugar was also tested by means of the polarimeter. The reaction was carried out in well-stoppered wide-mouthed bottles.

1 cc. *N*/10 iodine contained 0.01293 g. iodine.

1 cc. *N*/10 thiosulphate was equivalent to 0.0128 g. iodine.

(20 cc. iodine = 20.2 cc. thiosulphate.)

A. *Method of Willstätter and Schudel.*

(1) 10 cc. glucose solution (0.978 %).

20 „ *N*/10 iodine.

30 „ *N*/10 NaOH.

The mixture was allowed to stand 15–20 minutes at the ordinary temperature, then acidified with dilute sulphuric acid and titrated with thiosulphate.

Titrations: 10.2, 10.1, 10.1, 10.2, 10.15. *Mean*: 10.15.

From this, it follows that 1 g. of glucose requires 1.315 g. of iodine.

(2) 10 cc. fructose solution (1.012 %).

10 „ *N*/10 iodine.

20 „ *N*/10 NaOH.

Titrations: 9.3, 9.3, 9.3, 9.3.

From the above it follows that 1 g. of fructose takes up 0.1028 g. of iodine.

B. *Method of Colin and Lièvin.*

Three different solutions were tried, containing varying amounts of alkali, in order to see whether a concentration could be found at which the glucose would be completely oxidised and the fructose remain unaffected.

Phosphate solution I contained 35 g. of sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) and 50 cc. of *N* NaOH per litre.

Phosphate solution II contained 35 g. of sodium phosphate and 100 cc. of *N* NaOH per litre.

Phosphate solution III contained 35 g. of sodium phosphate and 25 cc. of *N* NaOH per litre.

The mixtures used were:

10 cc. sugar solution.

20 „ *N*/10 iodine.

40 „ phosphate solution.

The mixture was allowed to stand for one hour at the temperature of the laboratory, then acidified with dilute sulphuric acid, and titrated with thiosulphate solution.

The results are given below (Table I). It will be noted that phosphate solutions I and II give results identical with that obtained by Willstätter's method, although solution II contains twice as much sodium hydroxide as

solution I. The third mixture did not contain enough alkali to neutralise the gluconic acid formed. It was repeated, and the results obtained are given under nos. (4), (5) and (6).

(4) Three of the samples were left for two hours to see if the reaction would go further, but the result was negative.

(5) and (6) To each of six other samples was added 10 cc. of *N*/10 sodium hydroxide. Three of them were left for one additional hour, and the rest for two additional hours.

(7) The mixture of glucose and phosphate solution I was heated to 37° for one hour, to see if the reaction would be more complete at a higher temperature. Increase of temperature was however also found to be without influence on the result.

C. *Method of Colin and Lièvin (fructose).*

10 cc. fructose solution (1.012 %).

10 „ *N*/10 iodine.

20 „ phosphate solution.

In all cases the numbers given are the mean of five closely agreeing titrations

Table I.

A. *Willstätter's Method.*

Glucose ... 10.15

Fructose... 9.3

B. *Method of Colin and Lièvin (glucose).*

(1)	Glucose + phosphate I (1 hr.),	10.15
(2)	„ „ II „	10.15
(3)	„ „ III „	12.62
(4)	„ „ III (2 hrs.),	12.65
(5)	„ „ + 10 cc. <i>N</i> /10 NaOH (3 hrs.),	10.18
(6)	„ „ + 10 cc. <i>N</i> /10 NaOH (4 hrs.),	10.18
(7)	„ „ I at 37° (1 hr.),	10.13

C. *Method of Colin and Lièvin (fructose).*

Fructose + phosphate I (50 cc. *N* NaOH per litre), 9.6

„ „ II (100 cc. „ „), 9.32

The titration corresponding to the complete and quantitative oxidation of glucose to gluconic acid is 9.55. The titration required if fructose is completely unchanged is 10.1. It appears from the above results that glucose is oxidised by alkaline iodine solutions to the extent of about 95 %. The reaction proceeds smoothly, provided that sufficient alkali is present to neutralise the gluconic acid formed. Increasing the amount of alkali does not appear to influence the result, which is also unaffected by heating to 37°.

In the case of fructose, phosphate solution I seems to have caused somewhat less change in the fructose than phosphate solution II, which is the more strongly alkaline, or than Willstätter's reagent, the results obtained with the two last being identical.

There is, however, no advantage in using the method of Colin and Lièvin rather than that of Willstätter and Schudel, since the results are not quantitative in either case. Willstätter's method is preferable on account of its greater simplicity, and also because the time required for the reaction is less.

This method has therefore been adopted, and has been tested with mixtures of glucose and fructose in varying proportions.

The results obtained are given below in tabular form (Table II). The percentage of each sugar present is calculated as follows:

$$\begin{aligned} 2.369 &= \text{weight of CuO equivalent to 1 g. of glucose;} \\ 2.369 &= \text{ " " " " " fructose;} \\ 1.315 &= \text{ " " iodine absorbed by 1 g. of glucose;} \\ 0.1028 &= \text{ " " " " " fructose} \\ \text{Let } x &= \text{weight of glucose in a given volume } V; \\ \text{ " } y &= \text{ " fructose " " } \\ \text{Then } 2.369x + 2.369y &= \text{wt. of CuO corresponding to } V; \\ 1.315x + 0.1028y &= \text{wt. of iodine corresponding to } V. \end{aligned}$$

The solutions used were:

$N/10$ iodine; 1 cc. = 0.01293 I;
 $N/10$ thiosulphate; 1 cc. = 0.0128 I;
 $N/10$ KMnO_4 ; 1 cc. = 0.006892 Cu.
 1 cc. glucose = 0.00978 gr. glucose;
 1 cc. fructose = 0.01012 gr. fructose.

Table II.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)		(10)	(11)		(12)
	cc. glucose solution	cc. fructose solution	cc. KMnO_4 (mean)	CuO equivalent of (4)	Titration with $\text{Na}_2\text{S}_2\text{O}_3$ (mean)	Difference from blank	Iodine in (7)	Wt. of glucose			Wt. of fructose		
								taken	calculated		taken	calculated	
1	10	10	54.7	0.4719	9.25	10.95	0.1402	0.0978	0.099		0.1012	0.101	
2	10	5	40.9	0.3527	9.75	10.45	0.1338	0.0978	0.098		0.0506	0.051	
3	10	15	68.5	0.5907	8.95	11.25	0.1440	0.0978	0.098		0.1518	0.151	
4	10	20	82.3	0.7098	8.65	11.55	0.1479	0.0978	0.096		0.2024	0.203	
5	5	10	41.15	0.3548	4.32	5.78	0.0798	0.0489	0.048		0.1012	0.101	
6	15	10	68.25	0.5886	4.33	15.87	0.2032	0.1467	0.147		0.1012	0.102	
7	25	10	95.35	0.8222	4.30	26.0	0.3329	0.2445	0.244		0.1012	0.103	
8	15	5	54.45	0.4696	4.62	15.58	0.1994	0.1467	0.147		0.0506	0.051	

The method has also been tried with a number of other sugars, with results which are of some interest.

The other hexose sugars give results which resemble that obtained with glucose, but are not identical with it. Each sugar appears to have its own characteristic iodine value, and mannose appears to be oxidised with greater difficulty. Of the pentoses tested, arabinose takes up almost the theoretical amount of iodine, while rhamnose is oxidised much less completely. This may

be due to the steric influence of the methyl group. Moreover, the fact that rhamnose does not when oxidised yield the very unstable rhamnonic acid, but a lactone, may influence the reaction.

The results obtained with the disaccharides are remarkable.

The action on saccharose is negligible. In actual experiments the titration differed by 0.1 cc. from the blank, but this is within the limits of experimental error and, further, saccharose almost always contains a trace of reducing sugar.

Maltose behaves quite normally, and the iodine value found by experiment agrees closely with that calculated from the equation:

This is also in agreement with the copper-reducing power of maltose.

Lactose, on the other hand, takes up twice as much iodine as is required by the above equation, which fact suggests that the sugar is hydrolysed, and that both the glucose and the galactose molecules are oxidised.

In this connection it is interesting to note that Lobry de Bruyn and van Ekenstein [1895, 1896, 1897] found that when lactose is subjected to prolonged treatment with dilute alkali, it is hydrolysed, as much galactose being produced as in ordinary inversion, together with a little pseudo-tagatose—but no glucose. They assumed that the latter was split off in the form of an anhydride; this does not appear to be the case when iodine is present.

The numerical results are shown in Table III. The solutions used were approximately 2 %, but the actual concentration of the solutions was estimated by Bertrand's method, and checked by means of the polarimeter, except in the case of rhamnose, of which only a small sample was obtainable. In this case however the specimen was very well crystallised, and appeared to be pure, and its copper-reducing power was quite normal.

Table III.

Sugar	Wt. of sugar in 5 cc.	Titration $N/10 Na_2S_2O_3$ Mean	Diff. from blank	Iodine equivalent of 1 g. sugar	
				Found	Calculated
Mannose ...	0.108	11.83	8.17	0.977	1.509
Galactose ...	0.107	8.25	11.75	1.418	1.509
Arabinose ...	0.1004	6.65	13.35	1.702	1.69
Rhamnose ...	0.1087	12.15	7.85	0.9243	1.509
Saccharose ...	0.100	9.95	0.05	—	—
Maltose ...	0.0462	14.4	5.6	0.7456	0.75
Lactose ...	0.104	7.80	12.2	1.502	0.75

SUMMARY.

In dealing with fruit juices, the ordinary method of estimating fructose by means of the polarimeter is unsatisfactory, on account of the low polarimetric readings obtained.

Various iodometric methods for the estimation of aldoses in presence of ketoses have been tested, and none of them has been found to give quantitative results. Experiments with pure sugars show that while the oxidation of glucose is never complete there is always a partial action on fructose. The attempt to find experimental conditions which will ensure the complete oxidation of the glucose while the fructose remains unchanged has been unsuccessful.

It is suggested that the results obtained are due to the behaviour of sugars towards dilute alkalis, which has been investigated by Lobry de Bruyn and van Ekenstein.

In using either the method of Colin and Lièvin or that of Willstätter and Schudel, a definite and constant weight of iodine always reacts with a given weight of glucose. This weight of iodine is not affected by changes in the amount of alkali present, or by the presence of other sugars in the solution. The above statement is also true of fructose. It is therefore possible, using either of the above methods, to calculate from the copper-reducing power of a solution containing glucose and fructose, and from its action on iodine in alkaline solution, the amount of each sugar present in the solution.

The method has also been applied to other sugars. It is shown that each sugar has its own characteristic iodine value. The behaviour of rhamnose is abnormal. The three disaccharides investigated give very different results. Saccharose is not oxidised under the conditions of the experiment. Lactose and maltose are both oxidised, but lactose requires twice as much iodine as maltose.

The work described above has been undertaken in connection with investigations of Cold Storage problems which are being carried out for the Food Investigation Board of the Department of Scientific and Industrial Research.

REFERENCES.

- Bougault (1917). *J. Pharm. Chim.* (vii), **16**, 97.
Colin and Lièvin (1918). *Bull. Soc. Chim.* **47**, 403.
Lobry de Bruyn and van Ekenstein (1895). *Rec. Trav. Chim.* **14**, 156, 203.
— (1896). *Rev. Trav. Chim.* **15**, 92.
— (1897). *Rec. Trav. Chim.* **16**, 257.
Romijn (1897). *Zeitsch. anal. Chem.* **36**, 18, 349.
Willstätter and Schudel (1918). *Ber.* **51**, 780.