XV. IODIMETRIC DETERMINATION OF REDUCING SUGARS IN THE APPLE.

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It was pointed out by Judd [1920] that the polarimetric method in combination with the copper reduction method for the determination of fructose and glucose in mixtures of these sugars was unsuitable for the analysis of apple extracts, not only because of the low optical rotation of the extracts, but because they are always coloured and frequently viscous. Evans [1928] investigated various methods of clearing the extracts and found that considerable dilution was necessary for satisfactory clarification. In order to obtain a sufficiently large reading on the polarimeter the cleared solutions had to be evaporated under reduced pressure to a suitable concentration, making the analysis very tedious. Both these workers recommended the use of the iodimetric method [Baker and Hulton, 1920; Cajori, 1922; Willstätter and Schüdel, 1918] in combination with copper reduction for the estimation of fructose and glucose in the apple. This method is based on the oxidation of the sugars by alkaline iodine, and some disagreement existed as to the extent of the oxidation until Hinton and Macara [1924] reinvestigated the reaction. They established the fact that glucose was quantitatively oxidised to gluconic acid in 10 minutes at room temperature, provided not more than half the iodine added was reduced and the ratio of iodine to sodium hydroxide in terms of normal concentration was 1:1.25. They also found that fructose was slightly oxidised at 17.5°, the amount of oxidation varying with concentration, amount of alkali present, time of reaction and temperature. Hinton and Macara attribute the discrepant results obtained by earlier workers to the use of impure samples of sugars.

Evans [1928] repeated Hinton and Macara's work on the oxidation of glucose at 17.5° and also made observations at 5°. He found that oxidation of glucose was complete in 45 minutes at 5° and obtained the same iodine value at both temperatures, namely 1.402 g. of iodine reduced per g. glucose. This is slightly lower than the theoretical value 1.410 obtained by Hinton and Macara. Evans obtained somewhat higher values than Hinton and Macara for the amounts of iodine reduced per g. fructose in spite of the lower

temperature, probably owing to the much longer time of reaction. From the oxidation of invert sugar by iodine at 5° Evans found 0.022 g. of iodine reduced per g. of fructose. Hinton and Macara determined the iodine value for fructose in solutions containing large amounts of fructose relative to glucose and deduced from their results that in invert sugar the value would be 0.012 g.

From the results obtained by these observers it seemed clear that before applying the iodimetric method to apple extracts a thorough investigation of the oxidation of fructose under carefully controlled experimental conditions was necessary, since by far the largest proportion of reducing sugar in the apple is fructose. Examination of the methods of purification of glucose used by Hinton and Macara and by Evans showed that the latter did not employ a high temperature for the final drying of the sample of glucose used. As it is difficult to remove the last traces of water from the sugars without applying heat it is possible that the slightly low values Evans obtained for the amount of iodine reduced per g. of glucose were due to insufficient drying. Since the oxidation of fructose by iodine is considerably affected by both temperature and time of reaction, it was thought that more consistent results would be obtained by using the longer time of reaction necessary for complete oxidation of glucose at a low temperature than by using the short time necessary at the temperature employed by Hinton and Macara. Small variations in either time or temperature would result in less difference in the amount of oxidation of fructose at a low than at a high temperature; for this reason Evans worked at a temperature of 5°. The oxidation by alkaline iodine of carefully purified samples of glucose and fructose has now been investigated at 1°.

It has also been necessary to study the effect of clearing and of the use of different deleading agents on the results obtained by the iodimetric method. In the preparation of apple extracts for estimation of sugars by the copper reducing method Haynes and Archbold [1928] found basic lead acetate and potassium oxalate satisfactory as clearing agents, although a correction of 3 % of the sugar estimated was necessary owing to the loss of sugar in the clearing process. They also found that reliable results could be obtained by using the uncleared solution, obtained by diluting either the expressed juice or an alcoholic extract after evaporation of the alcohol.

Preparation of samples of glucose and fructose.

A sample of glucose purchased as pure was recrystallised three times from alcohol and water and then dried *in vacuo* over phosphorus pentoxide for 4 months. It was then dried at 105° to constant weight (2–3 hours). An ash determination was carried out, and the rotatory power and copper reducing power of the sample found. The results were as follows:

$[\alpha]_D^{20}$ (10 % solution) observed 52.73°; calculated 52.74° Ash < 0.01 %

Copper reducing power. Weight of glucose taken 0.4056 g./100 cc.

Weight of glucose found 0.4055 g./100 cc.

Fructose was prepared by the method of Harding [1922] used by Hinton and Macara and by Evans. It was found necessary to use 300 cc. of 75 % alcohol to dissolve 400 g. of sugar instead of 200 cc. stated by Harding. The sample was recrystallised three times and then dried first over sulphuric acid and then over phosphorus pentoxide to constant weight. It was then further dried at 50° till again constant. The rotatory power was then observed at 27° and the copper reducing power determined.

$[\alpha]_D^{27}$ (5.59 % solution) observed – 88.19°; calculated (using Vosburgh's [1920] formula) – 88.15°.

Copper reducing power. Weight of fructose taken 5.59 g./100 cc.

Weight of fructose found 5.52 g./100 cc.

Details of method for determining the iodine value of sugar solutions at 1°.

The procedure advocated by Hinton and Macara [1924] was adopted except that the reaction was allowed to proceed at 1° instead of at 17.5°. In order to keep the reaction mixture at the correct temperature the stock solutions of iodine and alkali were kept in a refrigerator at 1°.

Measured amounts of the sugar solution containing about 0.08 g. of sugar were run into glass bottles and the solutions made up to 100 cc. The bottles were stoppered and placed at 1°; when the solutions had cooled down to this temperature 20 cc. of 0.1 *N* iodine and 5 cc. of 0.5 *N* sodium hydroxide were added. The bottles were then immersed in a water-bath at 1° and rotated slowly during the progress of the reaction. Blank determinations were carried out omitting the sugar solution and adding instead 100 cc. of water. These determinations always agreed with the values obtained by direct titration of iodine (measured at 1°) and thiosulphate. When the reaction was complete the solutions were immediately acidified with 5 cc. of 2 *N* sulphuric acid and the excess iodine titrated with standard sodium thiosulphate (0.05 *N*), using starch as indicator. The starch solution was made up with saturated sodium chloride as first suggested by Hagedorn and Jensen [1923]: 130 g. of sodium chloride were dissolved in 400 cc. of boiling water and 5 g. of soluble starch, mixed to a paste with cold water, added to the boiling salt solution; the mixture was boiled for 5 minutes and then allowed to cool and diluted to 500 cc. This solution keeps indefinitely.

Effect of varying the time of reaction on the oxidation of glucose at 1°.

In order to determine the time necessary for the complete oxidation of glucose to gluconic acid at 1° the alkaline iodine was allowed to react with equal amounts of a glucose solution for various times. The results are shown in Table I. The reaction was complete after 2 hours and the theoretical value 1.410 g. of iodine reduced per g. of glucose was obtained. No appreciable difference in this value was found if the reaction was allowed to continue several hours longer, but after prolonged standing (17 hours) a slight increase in the iodine value was observed. There is therefore probably a slow secondary

Table I. *Effect of time of reaction on the oxidation of glucose by alkaline iodine at 1°.*

Amount of glucose present in each estimation = 0.0811 g.

Blank titration 38.05 cc. of 0.051 *N* sodium thiosulphate = 20 cc. iodine (0.1 *N* approx.).

Time of reaction in hours	cc. thiosulphate = iodine reduced	g. iodine reduced per g. glucose
1	17.55	1.395
1½	17.70	1.408
2	17.75	1.411
2½	17.75	1.411
3	17.70	1.408
4	17.75	1.411
5	17.70	1.408
6	17.75	1.411
7	17.75	1.411
17½	17.80	1.415

oxidation of glucose by alkaline iodine, but for many hours the amount is too small to be detected.

The iodine value for glucose, 1.410 g., was confirmed by further estimations on different amounts of glucose, the time of reaction being 2 hours. One set of results is shown in Table II. Hinton and Macara's result for glucose is thus

Table II. *Oxidation of glucose by alkaline iodine at 1°.*Blank titration 38.05 cc. of 0.051 *N* sodium thiosulphate = 20 cc. iodine.

Sample I			Sample II		
Weight of glucose in each estimation = 0.0643 g.			Weight of glucose in each estimation = 0.0794 g.		
No.	Thiosulphate = iodine reduced cc.	Iodine per g. glucose g.	No.	Thiosulphate = iodine reduced cc.	Iodine per g. glucose g.
1	14.05	1.412	1	17.35	1.409
2	14.10	1.416	2	17.35	1.409
3	14.10	1.416	3	17.35	1.409
4	14.05	1.412			
5	14.00	1.407			

Mean of 8 estimations 1.411.

confirmed for a temperature of 1°, and it seems probable that, as suggested above, Evans's sample of glucose was not quite dry.

The oxidation of fructose at 1° in solutions containing different ratios of fructose to glucose.

The ratio of fructose to glucose in the mature apple varies in general from about 2.5/1 to 4.5/1, but in a few cases ratios as high as 6/1 may occur. Having established the iodine value for glucose at 1° therefore, a series of estimations was carried out on solutions containing proportions of fructose to glucose ranging from 0/1 to 40/1 in order to determine the correct iodine value of fructose in the presence of glucose.

For this purpose a 0.3 % solution of glucose and a 4 % solution of fructose were prepared. 20 cc. of the glucose solution were taken for each estimation and quantities of fructose varying from 8 to 40 cc. All volumes were made

up to 100 cc., and the estimations carried out as described above. Two or more determinations were carried out for each ratio of fructose to glucose. The amount of iodine reduced by fructose was found by subtracting the amount reduced by glucose alone from the total amount reduced by a given solution. The results are shown in Table III. It will be seen that the iodine value for

Table III. *Oxidation of fructose by alkaline iodine at 1° in mixtures of glucose and fructose.*

Time of reaction 2 hours.

cc. of 0.5 *N* NaOH = 5.

Weight of glucose in each estimation = 0.06 g. approx.

Titration of glucose alone = 29.75 cc. of 0.043 *N* thiosulphate.

Ratio fructose glucose (approx.)	Fructose present g.	Thiosulphate = iodine reduced by fructose cc.	Iodine reduced per g. of fructose g.
1 : 1	0.0617	0.15	0.0134
2 : 1	0.1234	0.35	0.0157
3 : 1	0.1850	0.55	0.0163
4 : 1	0.2454	0.75	0.0169
5 : 1	0.3085	0.95	0.0170
6 : 1	0.3927	1.15	0.0162
7 : 1	0.4250	1.20	0.0156
8 : 1	0.4980	1.30	0.0144
9.5 : 1	0.5750	1.45	0.0139
11 : 1	0.6900	1.70	0.0136
24 : 1	1.5671	3.00	0.0106
30 : 1	1.9589	3.60	0.0102
40 : 1	2.7020	4.70	0.0096

fructose increases from 0.013 g. to 0.017 g. as the ratio of fructose to glucose increases from 1/1 to 5/1; and then decreases again slowly reaching a value of 0.0096 when the ratio is 40/1. Over the range of fructose/glucose ratios found in the apple the value is 0.016 to 0.017; 0.017 being the value for ratios 4/1 and 5/1. Since a difference of 0.001 in the iodine value of fructose makes only a negligible difference in the calculation of the amounts of fructose and glucose in their mixtures, the value 0.017 has been adopted for the whole range of fructose/glucose ratios in the apple. The value for equal amounts of the two sugars under these experimental conditions is 0.013 g., nearly the same as that suggested by Hinton and Macara for a temperature of 17.5° and rather lower than that found by Evans at 5°.

Effect of variations in time of reaction and proportion of alkali on the iodine value of a mixture of glucose and fructose at 1°.

Variations in the iodine value of fructose in the presence of glucose with alkali concentration and time of reaction were next studied, to define the precision necessary to obtain accurate results at 1°. Hinton and Macara showed that if the alkali exceeded the amount required for conversion of the iodine to hypoiodite and iodide, slightly low results were obtained for the iodine value of glucose at 17.5°. In Table V the small effect of excess alkali on the glucose has been neglected, so that the time change in amount of iodine

reduced by fructose is probably slightly greater than the recorded value. It will be seen in Tables IV and V that even at the low temperature of 1° the oxidation of fructose is markedly affected by differences in both amount of alkali and time of reaction.

It is thus essential not to exceed the time necessary for complete oxidation of glucose to gluconic acid by more than a few minutes before stopping the reaction, and also to regulate the alkali concentration so that the amount present is just sufficient to convert the iodine to hypoiodite. The results are shown in Tables IV and V.

Table IV. *Effect of variation in the time of reaction on the iodine value of fructose at 1° .*

Ratio fructose/glucose = 2.5/1.

Amount of glucose in each estimation = 0.06 g. (approx.).

Titration of glucose alone = 27.00 cc. of 0.044 *N* sodium thiosulphate.

Time in hours	Thiosulphate = iodine reduced by fructose (cc.)	Iodine reduced per g. fructose (g.)
2	0.50	0.0179
2½	0.60	0.0215
3	0.65	0.0232
5	0.90	0.0321
6	1.00	0.0358
17	1.60	0.0573

Table V. *Effect of varying the proportion of alkali on the iodine value of fructose at 1° .*

Ratio fructose/glucose = 2.5/1.

Amount of glucose in each estimation = 0.06 g. (approx.).

Blank titration 44.20 cc. of 0.044 *N* sodium thiosulphate = 20 cc. iodine.

Titration of glucose alone = 26.60 cc. of 0.044 *N* sodium thiosulphate.

cc. 0.5 <i>N</i> NaOH added	Thiosulphate = iodine reduced by fructose (cc.)	Iodine reduced per g. of fructose (g.)
4	Insufficient alkali for complete oxidation of glucose present	—
5	0.5	0.0174
6	0.6	0.0209

The application of the iodimetric method to the determination of reducing sugars in apple extracts.

In his work on the sugars in the apple Evans [1928] determined the amounts of fructose and glucose present by combination of the iodimetric method and Lane and Eynon's [1923, 1] method of estimating reducing sugars by copper reduction. The values for the two sugars were obtained by solving the simultaneous equations

$$C_1x - C_2y = \text{iodine value per 100 cc. of solution,}$$

$$K_1x - K_2y = \text{copper reducing power of 100 cc. of solution,}$$

where C_1 and C_2 are the g. of iodine reduced per g. of glucose and fructose respectively, and K_1 and K_2 the g. of cuprous oxide formed per g. of glucose

and fructose at the dilution used. Substitution of the more accurate values 1.410 and 0.017 for the g. of iodine reduced per g. of glucose and fructose respectively for those used by Evans makes a difference of less than 1 % in the amount of fructose, and about 0.0005 % in the amount of glucose calculated by solving the above equations. There is therefore no serious error in the results quoted by him. Solutions obtained by diluting the expressed juice of the apple and clearing with basic lead acetate and potassium oxalate were used for the estimations, and Evans stated that potassium oxalate and sodium acetate are not attacked by alkaline iodine (see p. 109).

The clearing of apple extracts with basic lead acetate and potassium oxalate has since been thoroughly investigated by Haynes and Archbold [1928] who found that in cleared solutions the results of sugar estimations were about 3 % lower than in uncleared solutions. They further showed that a similar loss of sugar occurred in clearing artificial mixtures of sugars, acid and pectin, of the same composition as the apple extract. In the estimation of total and reducing sugars by copper reduction clearing of the apple extracts is thus not necessary. Estimations in the dilute uncleared solution must, however, be carried out immediately, as hydrolysis of sucrose occurs on standing. This is not the case in cleared solutions, which remain unchanged for months—a distinct advantage during a series of routine estimations. A comparison between the iodine values of cleared and uncleared apple juices showed differences amounting to 10 %, the uncleared solutions giving the higher values. The clearing was carried out in dilute solution as described by Evans [1928, p. 4] and the uncleared juice was diluted to the same extent for estimation. 75 to 100 cc. of the dilute solutions (containing about 0.06 g. reducing sugar) were used for each estimation, and the reaction was carried out as described on p. 103, except that 25 cc. of iodine and 6 cc. $N/2$ NaOH were used. The volume of sugar solution was always made up to 100 cc. before adding the iodine and alkali. It should be pointed out that this solution is diluted to twice its volume for the determination of copper reducing power. The iodine values must therefore be halved for calculation of the amounts of fructose and glucose by means of the simultaneous equations. The results are shown in Table VI and point to the presence of substances oxidisable by iodine other than sugar in the uncleared extract, since clearing is known to cause a loss of only 3 % in the sugars.

Table VI. *The iodine values of cleared and uncleared apple juices.*

100 cc. of diluted solution used for each estimation.

Blank titration 50.6 cc. of 0.049 N thiosulphate = 25 cc. iodine.

Sample	Thiosulphate = iodine reduced (cc.)		Iodine reduced by 100 cc. of diluted solutions (g.)	
	Cleared juice	Uncleared juice	Cleared juice	Uncleared juice
1	25.65	28.25	0.1595	0.1757
2	21.00	28.80	0.1306	0.1792
3	25.80	27.90	0.1604	0.1736

Hinton and Macara [1927] have suggested the use of chloramine-T and potassium iodide as a weaker oxidising agent than alkaline iodine, for the determination of aldose sugars in cases where oxidisable material other than sugar is present, but it was found on trial that the results of estimations in cleared and uncleared solutions still showed a difference of 10 %. Hence no advantage is obtained by using chloramine-T instead of alkaline iodine for the determination of fructose and glucose in apples. Clearing is therefore essential if the iodimetric method is to be used satisfactorily, and an advantage if only copper reducing values are required, since it avoids the necessity of immediate estimation. The clearing process has in consequence been re-investigated to determine its effect on the iodine values of sugar solutions.

For this purpose the following solutions were prepared: fructose, 20 %; glucose, 8 %; malic acid, 4 %; pectin, 0.3 %. Equal amounts of the four solutions were mixed, giving a solution of these substances in the concentrations in which they occur in the apple. Mixtures were also made omitting the pectin, and omitting both the pectin and the malic acid, and diluting with water to obtain the appropriate concentrations. Two aliquots of each solution were diluted and neutralised in exactly the same way as the apple juice. One aliquot was cleared with basic lead acetate and potassium oxalate, and the iodine value of 100 cc. determined while the iodine value of the other was determined in 100 cc. of uncleared solution. The results are shown in Table VII. In every case a higher value was obtained in the cleared solution.

Table VII. *The iodine values of sugar solutions before and after clearing with basic lead acetate and potassium oxalate.*

Approximate concentrations of constituents in the diluted solutions used for estimations:

Solution F = Fructose 0.2 %.
 „ G = Glucose 0.08 %.
 „ M = Malic acid 0.04 %.
 „ P = Pectin 0.003 %.

Blank titration 51.35 cc. of 0.049 *N* thiosulphate = 25 cc. iodine.

Solution used	Thiosulphate = iodine reduced (cc.)		Iodine reduced by 100 cc. of solution (g.)	
	Uncleared	Cleared	Uncleared	Cleared
Sample 1				
F + G	17.70	18.08	0.1101	0.1124
F + G + M	17.65	17.90	0.1097	0.1114
F + G + M + P	17.75	18.00	0.1104	0.1120
Sample 2				
F + G + M	17.90	18.48	0.1114	0.1149
F + G + M + P	17.90	18.35	0.1114	0.1141
Sample 3				
F + G + M	16.50	16.80	0.1027	0.1045
F + G + M + P	16.48	16.85	0.1024	0.1050

It has been stated by Davis [1916], Deerr [1916] and Geerligs [1909] that the loss of sugar on clearing with basic lead acetate is chiefly a loss of fructose. In that case no marked decrease in the iodine value, similar to that found in

the copper reduction value, would be expected since the amount of oxidation of fructose is so small. The consistently high results for the iodine values found in the cleared solutions, however, suggest that some oxidation by iodine of the potassium oxalate occurs. Consequently the effect of iodine on potassium oxalate alone and in the presence of glucose was investigated. Varying amounts of oxalate alone were allowed to react with iodine under the normal experimental conditions, while in a second set of estimations the solutions were acidified and titrated immediately. In both cases some oxidation occurred, the differences in titration from the blank varying from 0.1 cc. to 0.8 cc. of 0.05*N* sodium thiosulphate as the amount of oxalate increased from 0.5 cc. to 10 cc. In the presence of glucose the amount of iodine used was greater than that required for the complete oxidation to gluconic acid by an amount equivalent to 0.2 cc. of 0.05*N* sodium thiosulphate per cc. of potassium oxalate, a similar amount to that found using oxalate alone. There is therefore a small but definite oxidation of oxalate under these conditions not affected by the presence of sugar, and the use of potassium oxalate as a deleading agent requires a correction of about 0.2 cc. to the titration obtained. Since a correction has also to be made to the values obtained by copper reduction it was decided to try other deleading agents.

*The effect of different clearing and deleading agents on the
iodine values of sugar solutions.*

Solutions of fructose and glucose containing in some cases malic acid and pectin, were cleared with both normal and basic lead acetate and deleading with potassium oxalate, sodium carbonate and sodium phosphate (Na_2HPO_4). Colloidal ferric hydroxide, as suggested by Evans, was also tested but proved less convenient owing to the bulky nature of the precipitate obtained. The method adopted was similar to that already described for testing the effect of clearing and solutions of the same strengths were employed (see Table VII). With both normal and basic lead acetate potassium oxalate gave higher values for the iodine equivalents in the cleared than in the uncleared solutions. Sodium carbonate did not completely remove the lead from solutions cleared with normal lead acetate, and in the course of the iodimetric estimation a precipitate of lead iodide was obtained on the addition of acid to the reaction mixture [see also Meade and Harris, 1916]. With basic lead acetate as a clearing agent, the removal of lead appeared to be complete, but the filtrate was not clear, and the iodine equivalent was about 1.5 % too low in the cleared solution.

With sodium phosphate (Na_2HPO_4) and normal lead acetate a clear filtrate was obtained, but the solution was acid and required neutralising before the addition of alkaline iodine in order that the correct proportion of alkali should be present during the oxidation reaction. Using basic lead acetate a neutral filtrate was obtained, and the solution could readily be filtered at once. In addition, estimations of sugar, both iodimetrically and by copper reduction

showed that by using sodium phosphate as the deleading agent there was no loss of sugar in the clearing process. Norris and Brodie [1918] state that sodium phosphate completely removes lead and has no effect on the reducing power of sugar solutions, and Englis and Tsang [1922] found the smallest loss of sugar with this deleading agent.

Further trials with sodium phosphate as a deleading agent, using both normal and basic lead acetate as clarifying agents, were then carried out. The sugar solutions containing acid and pectin were cleared and deleading, and allowed to stand for various times before filtering. It was found that prolonged standing, 3 days, caused re-solution of some lead, since both with normal and basic acetate a precipitate of lead iodide appeared during the iodimetric estimation. The agreement between the results for shorter times of standing up to 16 hours was good in both cases. In view of the extra process of neutralisation required when normal lead acetate is used, and also the incomplete precipitation of malic acid by this salt, it was decided to continue the use of basic lead acetate for clearing the apple extract, but to use sodium phosphate instead of potassium oxalate as a deleading agent. The results of these trials are shown in Tables VIII and IX.

Table VIII. *The effect of clearing with normal and basic lead acetates, and of using different deleading agents on the iodine values of sugar solutions.*

Solutions as in Table VII.					
Blank titration 57.4 cc. of 0.044 <i>N</i> thiosulphate = 25 cc. iodine.					
Clearing agent	Solutions used	Thiosulphate = iodine reduced (cc.)		Iodine reduced per 100 cc. of solution (g.)	
		Uncleared	Cleared	Uncleared	Cleared
Deleading agent potassium oxalate.					
Sample 4					
Normal lead acetate	F + G	24.00	24.25	0.1330	0.1343
	F + G + M + P	24.05	24.45	0.1335	0.1354
Deleading agent sodium carbonate.					
Normal lead acetate	F + G	24.00	25.00	0.1330	0.1385*
	F + G + M + P	24.00	24.25	0.1330	0.1343*
Sample 5					
Basic lead acetate	F + G + M + P	22.38	22.05	0.1237	0.1222
Deleading agent sodium phosphate (Na ₂ HPO ₄).					
Basic lead acetate	F + G + M + P	22.38	22.40	0.1237	0.1241
Cleared with colloidal ferric hydroxide.					
	F + G	22.25	22.75	0.1233	0.1260
	F + G + M + P	22.30	23.00	0.1237	0.1274

* Lead iodide formed during the reaction.

Lane and Eynon [1923, 2] have objected to the use of sodium phosphate as it does not entirely remove alkaline earth metals, and their presence will affect the copper reduction of a sugar solution. This objection, however, does

Table IX. *The effect of time of standing on the iodine values of solutions cleared with normal and basic lead acetate and deleaded with sodium phosphate.*

Blank titration 57.0 cc. of 0.044 N thiosulphate = 25 cc. iodine.				
Iodine values of 100 cc. of uncleared solutions 0.1327 g. ¹ ; 0.1282 g. ²				
Time of standing	Thiosulphate = iodine reduced (cc.)		Iodine reduced per 100 cc. solution (g.)	
	Clearing agent		Clearing agent	
	Normal lead acetate	Basic lead acetate	Normal lead acetate	Basic lead acetate
Filtered at once	—	23.90	—	0.1324 ¹
2 hours	23.05	23.10	0.1277	0.1280 ²
Overnight	23.20	23.15	0.1285	0.1282 ²
3 days		Lead iodide formed		

not hold for apple extracts since the amounts of calcium present are too small to cause any error.

The use of sodium phosphate as a deleading agent in the clearing of apple extracts.

As the use of basic lead acetate and sodium phosphate had proved quite satisfactory for clarification of mixtures of sugars, malic acid and pectin, this method of clearing was applied to a series of extracts from stored apples. These extracts were prepared both by alcoholic extraction of the fresh tissue [Haynes and Archbold, 1928, p. 976] and by expression of the juice from previously frozen pulp [Haynes, 1925, p. 78]. The clearing method described by Evans was used, but a saturated solution of sodium phosphate was substituted for the potassium oxalate. 2–3 cc. of phosphate were added for each cc. of basic lead acetate solution. The solutions prepared from the alcoholic extractions were found to develop a yellow colour on standing, the colour becoming more intense as the time of standing was prolonged. No improvement was obtained by keeping in the dark instead of exposed to direct sunlight on the bench. The solutions prepared from the expressed juice remained quite colourless on standing. No colour appeared in either case if the solutions were deleaded with potassium oxalate. A comparison of the iodine values showed that the values for the coloured solutions which had been deleaded with sodium phosphate were considerably higher than those for the colourless solutions deleaded with potassium oxalate. Three sets of comparative values are shown in Table X. No correction has been made for the iodine reduced by the potassium oxalate; this correction would of course slightly increase the difference between the two sets of observations. Extracts prepared from very immature fruit were found to be coloured whichever deleading agent was used, and by solving the equations obtained by determining the iodine value and copper reducing power, negative values for fructose were obtained. These results show that in the coloured solutions iodine is reduced by some substance other than sugar.

Table X. *Iodine values of solutions prepared from alcoholic extracts of apple tissue cleared with basic lead acetate and delead with sodium phosphate and potassium oxalate.*

Solution	Solution delead with	
	Sodium phosphate Iodine reduced per 100 cc. (g.)	Potassium oxalate Iodine reduced per 100 cc. (g.)
1	0.1635	0.1513
2	0.1927	0.1722
3	0.1624	0.1465

Since the use of alcoholic extracts is preferable to the use of expressed juice [Haynes and Archbold, 1929] for sugar estimations, and the results obtained when sodium phosphate is used as the deleading agent require no correction for loss of sugars during clearing, the coloured solutions were boiled with charcoal to see if decoloration without loss of sugar was possible.

Table XI. *Iodine values of solutions prepared from alcoholic extracts of apple tissue cleared with basic lead acetate and sodium phosphate and decolorised by charcoal.*

Solution	Coloured solution Iodine reduced per 100 cc. (g.)	Decolorised solution Iodine reduced per 100 cc. of original solution (g.)
1	0.1778	0.1566
2	0.1965	0.1621
3	0.1637	0.1491

Trials were made with animal charcoal and a sample of "suchar¹." The "suchar" was found to be much more efficient for decolorising the solutions and was used for all subsequent work.

The iodine values of the colourless solutions obtained by boiling with charcoal were next determined. 200 cc. of the neutral cleared, coloured solution were boiled with 0.5 g. of charcoal for 1 minute. The solution was filtered and the charcoal thoroughly washed with boiling water. The volume of filtrate was then made up to 250 cc., and the iodine value determined in the usual way. In every case a decrease in the iodine value was found as compared with the coloured solutions. Three sets of results are shown in Table XI.

In some cases one boiling was not sufficient to decolorise the solution, if this was so the solution was filtered and again boiled for 1 minute with fresh charcoal. This process was repeated until the solution was colourless. The iodine value decreased with each successive boiling until the solution was colourless, after which further boilings had no effect. It was found necessary to boil once more after the solution was apparently colourless in order to ensure a constant result. The effects of increasing the quantity of charcoal used and the time of boiling were tried, but no advantage was obtained in

¹ Suchar is a charcoal specially prepared for sugar refining by the British Suchar Processes, Ltd., and kindly supplied by them.

either case. The results are shown in Table XII. Tests were then carried out with invert sugar solutions to which sucrose had been added to see if adsorption occurred during the boiling process. No change was found in the iodine

Table XII. *Iodine values of a cleared extract of apple tissue after different times of boiling with varying amounts of charcoal.*

No. of boilings	Time of each boiling (mins.)	Wt of charcoal (g.)	Iodine reduced per 100 cc. of original solution (g.)
4 (colourless after 3rd boiling)	1	0.5	0.302
4 (left to stand $\frac{1}{2}$ hour before filtering off charcoal)	1	0.5	0.298
3	1	1.0	0.301
3 (colourless after 2nd boiling)	5	0.5	0.299

value after boiling once with charcoal, and after six boilings a loss of only 0.8 % was observed. The reducing power estimated by copper reduction however decreased 1 % after one boiling and 5 % after six boilings. This loss of sugar occurred in both total and reducing sugars and suggests that a slight adsorption of fructose and sucrose occurs during the boiling, while glucose is not affected. Consequently no change is found in the iodine value after one boiling with charcoal.

The iodine value and reducing sugars determined by copper reduction are shown in Table XIII.

Table XIII. *The effect of boiling with charcoal on the reducing power and iodine values of an invert sugar solution containing sucrose.*

No. of boilings	Reducing sugar (by copper reduction)		Sucrose g./100 cc.	Iodine reduced per 100 cc. (g.)
	g./100 cc.	Total sugar g./100 cc.		
Unboiled	0.720	1.032	0.312	0.0753
1	0.713	1.026	0.313	0.0753
2	0.711	1.006	0.295	—
6	0.685	0.936	0.251	0.0747

The copper reducing power can be satisfactorily determined in the uncleared, or in the cleared but coloured solution, and the results combined with iodine values determined in the decolorised solution. The loss of reducing power during decoloration is therefore not a serious drawback to the use of sodium phosphate as a deleading agent. Since the cleared solutions darken considerably on standing, the effect of the more intense colour on the iodine value was next examined. An alcoholic extract of apple tissue was prepared and after evaporation of the alcohol the solution was diluted and cleared with basic lead acetate and sodium phosphate. The solution was colourless immediately after filtering, and an estimation of the iodine value was carried out at once, both with and without a preliminary boiling with charcoal. The

cleared solution was allowed to stand and again estimated after intervals of 3 and 8 months. More than one boiling was necessary to decolorise the solutions after long standing.

The results are shown in Table XIV.

Table XIV. *Iodine values of apple extract cleared and dealed with sodium phosphate estimated after different periods of standing both in coloured and decolorised solutions.*

	Cleared coloured solutions estimated			Cleared decolorised solutions estimated		
	At once	After 3 months	After 8 months	At once	After 3 months	After 8 months
No. of boilings required	—	—	—	1	2	4
Sample 1	0.2089	0.2089	0.2067	0.1796	0.1750	0.1767
Sample 2	—	0.1635	0.1625	0.1442	0.1448	—
Sample 3	0.1927	0.1907	—	0.1703	0.1739	—

No change was found in the iodine values of either the coloured or decolorised solutions after 8 months. Thus depth of colour does not in itself tend to increase the iodine value. In order to test the validity of the figures obtained by this method a comparison was made with iodine values of solutions dealed with potassium oxalate; since it was possible that the colourless solution obtained in this way might also contain the oxidisable material, estimations were carried out on solutions which had been boiled with charcoal in addition to the ordinary estimation. In this case the iodine value increased considerably during the boiling, and a solution boiled without the addition of charcoal showed an even greater increase. A solution of fructose and basic lead acetate and potassium oxalate showed the same increase in iodine value after boiling with or without the addition of charcoal, while the iodine value of a solution of potassium oxalate alone was unaffected by boiling. This increase appears to be due to some action of the excess of potassium oxalate on the sugars during boiling. Potassium oxalate is therefore unsuitable as a deleading agent for solutions prepared from immature apples. It has already been stated that such solutions are coloured even if dealed with oxalate, and the above results make it clear that decolorising by boiling is not practicable.

Table XV. *Iodine values, g. iodine reduced per 100 cc., of solutions dealed with sodium phosphate and potassium oxalate before and after boiling.*

Sample	Solution dealed with			
	Sodium phosphate		Potassium oxalate*	
	Not boiled	Boiled	Not boiled	Boiled
1	0.1635	0.1442	0.1513	0.1754
2	0.1927	0.1703	0.1722	0.2040
3	0.1624	0.1423	0.1465	0.1703

* No correction has been made for the oxidation of potassium oxalate. The results are therefore about 0.5 % too high.

The parallel experiments with extracts of mature apples (Table XV) using the two deleading agents showed that nearly the same result is obtained in the solution deleading with sodium phosphate after decoloration as in that deleading with potassium oxalate but not boiled. By varying the amounts of phosphate added during clearing it was found that the development of colour was largely dependent on the amount of phosphate present. Three solutions were prepared, one containing insufficient phosphate to remove all the lead, one with a slight excess of phosphate and one with a large excess. After standing for 3 months the first solution was still colourless, the second pale yellow and the third deeper yellow. The one containing excess lead was then completely deleading, and iodine values were determined on all three. Similar values were obtained for the two solutions which had stood with excess of phosphate, but the third gave a high value indicating a conversion of fructose to glucose in the presence of lead.

From the foregoing results it was concluded that for extracts prepared from mature apples satisfactory results could be obtained by using either potassium oxalate or sodium phosphate as the deleading agent, but that oxalate was inadmissible unless the cleared solution was colourless. The use of oxalate also has the disadvantage of requiring corrections both in the iodine and copper reduction values to give the correct results. The use of sodium phosphate requires no such corrections, but is more tedious since the additional process of decoloration is necessary. Since a good deal of work on immature fruit is in progress in this laboratory sodium phosphate has been adopted for routine work.

It has been found that the iodimetric method can be used satisfactorily for the estimation of much smaller amounts of sugar than those used in the work described here. It is now being employed in combination with the Hanes [1929] modification of Hagedorn and Jensen's method of sugar estimation for the determination of the small amounts of sugar in the developing apple. These results will be published shortly.

[*Note*, added 13th Feb.] The polarimetric method is not only tedious but there are indications that it is liable to give untrustworthy results in the case of apple extracts. Evans's [1928] results show quite close agreement between the actual polarimeter readings and those expected from the iodine and copper values of the solutions. Examination of his paper shows that he has assumed that the specific rotation of fructose is independent of concentration. If his data are recalculated using the formula relating specific rotation and concentration of fructose given by Vosburgh [1920, 1921] it is found that the polarimeter readings are uniformly lower than would be expected from the copper and iodine values. We have made observations which confirm this result, and there is thus some indication of the presence of a laevo-rotatory substance other than sugar in the cleared apple extract.

SUMMARY.

The oxidation of glucose and fructose by alkaline iodine at 1° and methods of preparation of apple extracts for iodimetric estimations have been investigated. Glucose is quantitatively oxidised to gluconic acid in two hours at 1° and some oxidation of fructose also occurs.

In mixtures of glucose and fructose the amount of iodine reduced per g. of fructose increases from 0.013 g. to 0.017 g. as the ratio of fructose to glucose increases from 1/1 to 5/1 and then decreases slowly as this ratio is further increased. The value 0.017 can be used for determination of fructose and glucose in apples by combination of the iodimetric and copper reduction methods, since the ratio of fructose to glucose in the apple is about 4/1.

The presence of oxidisable material other than sugar makes it necessary to clear the apple extracts before carrying out iodimetric determinations. Basic lead acetate with either sodium phosphate (Na_2HPO_4) or potassium oxalate as the deleading agent was found to give satisfactory results with extracts prepared from mature apples.

The loss of sugar during clearing and the slight action of iodine on potassium oxalate make corrections necessary for both the copper reducing value and the iodine value if oxalate is used as the deleading agent. If sodium phosphate is used there is no loss of sugar, but the cleared solution is yellow and still contains some non-sugar substance oxidisable by iodine. The solution can be decolorised by boiling with charcoal and satisfactory results obtained. Copper reduction determinations can be carried out on the yellow solution.

With very immature apples coloured solutions are also obtained when potassium oxalate is used, and these solutions cannot be boiled, as an increase in the iodine value occurs owing to some action of oxalate on the sugars. Sodium phosphate has therefore been adopted as the deleading agent for routine work.

REFERENCES.

- Baker and Hulton (1920). *Biochem. J.* **14**, 754.
 Cajori (1922). *J. Biol. Chem.* **54**, 617.
 Davis (1916). *J. Agric. Sci.* **8**, 7.
 Deerr (1916). *Int. Sugar J.* **18**, 166.
 Englis and Tsang (1922). *J. Amer. Chem. Soc.* **43**, 865.
 Evans (1928). *Ann. Bot.* **42**, 1.
 Geerligs (1909). *Int. Sugar J.* **11**, 276.
 Hagedorn and Jensen (1923). *Biochem. Z.* **135**, 46.
 Hanes (1929). *Biochem. J.* **23**, 99.
 Harding (1922). *J. Amer. Chem. Soc.* **44**, 1765.
 Haynes (1925). *Ann. Bot.* **39**, 77.
 ——— and Archbold (1928). *Ann. Bot.* **42**, 965.
 ——— (1929). *Reports of the Food Investigation Board for 1928–29*.
 Hinton and Macara (1924). *Analyst*, **49**, 2.
 ——— (1927). *Analyst*, **52**, 668.
 Judd (1920). *Biochem. J.* **14**, 255.
 Lane and Eynon (1923, 1). *J. Soc. Chem. Ind.* **42**, 132 T.
 ——— (1923, 2). *J. Soc. Chem. Ind.* **42**, 143 T.
 Meade and Harris (1916). *J. Ind. Eng. Chem.* **8**, 504.
 Norris and Brodie (1918). *Int. Sugar J.* **20**, 238.
 Vosburgh (1920). *J. Amer. Chem. Soc.* **42**, 1696.
 ——— (1921). *J. Amer. Chem. Soc.* **43**, 219.
 Willstätter and Schüdel (1918). *Ber. deutsch. chem. Ges.* **51**, 780.