Two Improved Methods for the Determination of Soluble Carbohydrates

Alan E. Flood* and C. Austen Priestley

East Malling Research Station, Maidstone, Kent, ME19 6BJ, England

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Modifications have been made to a periodate oxidation procedure originally described by Rappaport et al. These have resulted in a procedure suited to the routine determination of a wide range of carbohydrates in solution, including soluble sugars, sugar alcohols, oligosaccharides and the hydrolysis products of reserve polysaccharides. A reasonably accurate estimate of fructose in a mixture of sugars is possible by the determination of the periodate consumption of aliquots before and after the destruction of fructose.

An improved ferricyanide method is suited to the determination of reducing sugars.

Both methods may be applied with equal success to hexose and pentose sugars. When used in conjunction, the two methods enable the estimation of fructose, reducing sugar, sucrose and sugar alcohol in aliquots of the same extract.

1. Introduction

The biochemical study of plants frequently entails the quantitative analysis of mixtures of sugars and sugar alcohols. In general these mixtures may be separated chromatographically and the components determined individually. However, for many purposes such detailed analysis is not necessary and it is often sufficient and more expeditious to make qualitative chromatographic examination together with quantitative determination of the total carbohydrates in the mixture.

For the lack of a better method, total reducing power has long been accepted as a measure of the total soluble carbohydrates in plant extracts. With mixtures of sugars, including perhaps oligosaccharides, the values obtained by such methods may be misleading because of the differing reducing powers of different sugars. Furthermore, sugar alcohols which may form a considerable part of the soluble carbohydrate pool are not determined.

Two procedures to be described in this paper, which have been used principally in the analysis of carbohydrates extracted from the various tissues of apple trees, have general applicability to soluble carbohydrates from other sources. One is a modification of Rappaport's method and may be used to determine total soluble carbohydrates including sugar alcohols by the estimation of residual periodate after their oxidation with sodium metaperiodate in strong acid. It may also be used to determine individual

* Present address: Wye College (University of London), Ashford, Kent, England.
carbohydrates in solution. The other is an improved procedure for using the Hagedorn–Jensen ferricyanide reduction\(^3\) as a simple method for determining reducing sugars individually or in a mixture.

2. Periodate oxidation method

2.1. Experimental

2.1.1 Reagents

Sodium metaperiodate: 0.2\% solution (w/v). Stored in the dark and diluted as required.

Sulphuric acid: approximately 6 N.

Buffer solution I: 80 g of dipotassium phosphate (K\(_2\)HPO\(_4\)) and 30 g of sodium hydroxide/litre.

Potassium iodide: 3\% (w/v) and 2\% (w/v) solutions.

Sodium thiosulphate: 0.005 N accurately standardised.

Starch: 1\% (w/v) solution.

2.1.2 Procedures

2.1.2.1. Total carbohydrates by periodate oxidation (range 50 to 300 µg/ml)

Four lipped test tubes (150 mm \(\times\) 19 mm was a convenient size) were used for each determination. Two tubes received 2 ml of the sample sugar solution and another two tubes 2 ml of water, all followed by 2 ml of 6 N-sulphuric acid. Sodium periodate solution was prepared for use by the addition of 3 parts water to 5 parts 0.2\% stock solution. To one sample tube (A) and one water blank were added 4 ml of periodate and the tubes heated in a boiling water bath for 2 h (20 min is sufficient if no oligosaccharides are present). The second sample tube (B) was heated in the boiling water bath for 3.5 h to destroy fructose, cooled, then 4 ml of periodate added to it and to its water blank prior to heating in the water bath for 20 min.

At the conclusion of the heating with periodate, each tube was treated as follows: approximately 10 ml of water were added and the tube left in the boiling water bath for 2 min, then removed, cooled, the contents washed out into a 250-ml conical flask and 12 ml of buffer solution I added.

To each flask in turn, 10 ml of 2\% potassium iodide were added and the contents immediately titrated with 0.005 N-thiosulphate, using starch near the end point.

If \(a\) and \(b\) are periodate consumptions (as ml 0.005 N-thiosulphate differences from blank values) for tubes A and B, respectively, the weight (in µg) of sugars oxidised is given by 94 \(b + 114\ (a-b)\), i.e. 114\(a - 20b\). The factors 94 and 114 are those given by Rappaport for aldohexoses and fructose, respectively. The theoretical factors are 90 and 112.5. When \(a\) and \(b\) are not too dissimilar in magnitude an error of 10\% in the value of \(b\) would lead to an error of only 3\% in the figure for total carbohydrate.

2.1.2.2. Spectrophotometric modification (range 20 to 100 µg/ml)

The procedure was similar to that for the volumetric method. To tube A, 1 ml of sugar solution, 1 ml of 6 N-sulphuric acid and 2 ml of sodium periodate solution (2 parts 0.2\% stock mixed with 9 parts water) were added. Tube B was treated similarly but with the periodate added after 3.5 h heating. When oxidation was complete, 10 ml of water
were added to each tube and heating continued for a further 2 min. The tubes were then cooled and their contents washed out into 50-ml standard flasks. To each flask, 6 ml of buffer solution I was added, followed by water if necessary to bring the volume to about 35 ml and then, with gentle swirling, 10 ml of 3% potassium iodide. The solutions were made to the mark and their light absorption at 352 nm measured in the spectrophotometer. Calibration curves were prepared using standard sugar solutions or the optical density related to 0.005 N-thiosulphate by mixing duplicate samples using the quantities of reagents given above and comparing the optical density of one solution with the titration values for the duplicate.

2.1.2.3. Single sugars
These were determined as described above, but the fructose destruction as carried out in tube B was omitted. It was convenient, however, to modify the method by decreasing the amount of acid used and adding it already mixed with periodate. For the volumetric method (50 to 280 µg sugar/ml) a mixture of 5 parts of 0.2% NaIO₄ and 3 parts of 6 N-H₂SO₄ was made in sufficient quantity to last for one day’s analytical work. Sugar solution (2 ml) and 4 ml of this acid periodate were mixed and treated as described for tube A except that only 9 ml of buffer were used. For the colorimetric method (20 to 100 µg sugar/ml) a mixture of 8 parts of 0.2% NaIO₄, 3 parts of water and 11 parts of 6 N-acid was made and 1 ml of this mixture heated with 1 ml of sugar solution. The quantity of buffer solution required was 3 ml. Apart from these differences, the methods were exactly as given above.

2.1.3. Technique
It was most important to pay attention to the following details.

1. To prevent excessive evaporation the tubes were covered with glass marbles while being heated in the water bath.
2. At least 20% of the periodate should remain after all the sugar is oxidised otherwise some reduction of iodate to iodide takes place, followed by liberation of iodine.
3. Minute deposits of solid on the sides of the tubes above the liquid level after heating in the bath dissolve only slowly in cold water. The tubes were therefore kept in the bath for a short time after the addition of water.
4. A large excess of potassium iodide is necessary for quantitative liberation of iodine by the periodate.
5. Before the potassium iodide is added:
   (a) the pH of the solution must lie between 6.3 and 6.9;
   (b) the solution must be sufficiently diluted with water to slow down the reaction between iodate and iodide.
When the solution is to be titrated too great a dilution makes observation of the endpoint difficult. Under the conditions given, no blue colour should return 10 min after the titration is complete. Much greater dilution is employed in the spectrophotometric method and under these conditions the iodate–iodide reaction is negligible.

2.1.4. Further modifications
The periodate procedure has been in regular use for the study of carbohydrate resources in fruit trees at East Malling Research Station. The method, chosen because of its
suitability for estimating all the various components of these resources, is applicable to the estimation of acid hydrolysates of polysaccharides as well as individual and total soluble sugars. Sorbitol constitutes a large proportion of the soluble carbohydrate of a number of Rosaceous species grown as temperate fruits and this compound is estimated by the same procedure.

In a later modification, one blank, one standard and eight samples were analysed in a batch. Aliquots of 1.00 ml of the test solution were dispensed into 125 x 12 mm test tubes using a calibrated hypodermic syringe dispenser. These were followed by 1.250 ml of 0.2% sodium metaperiodate solution from a syringe burette and finally 1.20 ml of 5 N-sulphuric acid. The ten tubes were covered with glass marbles and transferred to a vigorously boiling water bath where they remained for 2 h. It was essential to add the sulphuric acid last to ensure adequate mixing in tubes of this size. The heating time could be halved when estimating sugars in acid hydrolysates. At the end of this time, water (approx. 5 ml) was added to the tubes which then remained in the bath while ten 250-ml conical flasks were washed and drained ready to receive their contents. The latter were pored into their respective flasks and the tubes rinsed with cold, distilled water from a plastic wash bottle. Successive rinses were directed down one side of each tube and poured into the flask from the other side. Five such rinses, whose total volumes approximated to 10 ml, ensured quantitative transfer from tubes to flasks. Phosphate buffer (5 ml) was then added to each flask from a burette or plastic dispenser. The concentration of the buffer was increased from the original so that ten flasks could be served from one filling of a 50-ml burette and the solution contained 96 g of dipotassium phosphate and 36 g of sodium hydroxide/litre. Each flask in turn was titrated immediately after the addition of 5 ml of 2% potassium iodide solution, using 0.0025 N-sodium thiosulphate with starch solution as indicator added near the end-point. The accuracy of the titration is limited principally by the size of droplet released from the tip of the burette and half the original strength of thiosulphate was chosen for use in a burette which was graduated and delivered to an accuracy of 0.05 ml. The system described is capable of estimating 50 to 400 µg sucrose equivalents (per ml) to an accuracy of ±3 µg. As a working rule, and apart from specimens of pure fructose, the carbohydrates normally encountered were estimated from the equation:

\[ \text{amount in } \mu g = \frac{100 (\text{ml thiosulphate for blank} - \text{ml for sample})}{2} \]

The titrimetric method of estimating the iodine equivalent to the residual periodate was chosen because, although the manual titration took longer than a colorimetric determination, the whole operation sequence was faster. No time was spent in controlling volumes accurately during transfer of solutions from tubes to flasks and the method of transfer ensured that the tubes were cleaned and ready for the next cycle of operation. Provided the marbles were replaced after the tubes were rinsed this was the only cleaning required. Materials present as dust in new tubes or as deposits from drying cloths could give serious interference. An experienced operator could prepare a set of ten tubes in less than 5 min and complete a titration sequence in under 20 min. It was normally expected that 30 or 40 estimations in duplicate (60 or 80 titrations)
would be made by one person in a day, thus allowing time for sample preparation and extraction together with other essential routines.

Experiments are in progress to modify the system further to make use of mechanical dilution apparatus. One diluter with glass syringes is being used for taking 0.5-ml aliquots of test solution which are displaced from the probe after its transfer to the test tubes by 2.5 ml of a mixture containing equal volumes of 0.2% sodium metaperiodate and 5 N-sulphuric acid. This mixed reagent is unstable in light but satisfactory results have been obtained where it has been kept in darkness in quantities which were used in under one week. A second diluter with nylon or plastic syringes is being used to sample the contents of the test tubes after heating. It has been set to remove 2.0 ml from the tube, originally containing 3.0 ml, and this is displaced into its flask with 10 ml of diluted buffer solution. The latter is one-third the strength of the original buffer in order both to increase the volume of the displacing fluid and to reduce the rate of reaction between iodate and iodide before titration. Still more dilution seems desirable but this is beyond the capacity of the present diluter syringe so the required conditions may be met by adding an increased volume of more dilute potassium iodide solution—10 ml of a 1% solution. The reaction remains stoichiometric under these conditions but it must be noted that titration values bear a different relation to the sugar concentration from those given previously. Provided the syringe which samples the test solution is adjusted to within narrow limits of the desired volume, it is suggested that the remainder should be set approximately only. A calibration series should be run using standard sugar solutions and the results used to adjust the thiosulphate concentration so that 1.00 ml is equivalent to 100 μg sugar in the standard volume. It is not expected that this system will save time compared with the previous manual procedure but the use of diluters should reduce the skill and concentration required of the operator.

2.2. Discussion of results

Rappaport et al.\(^1\) described a method for the determination of monosaccharides and sugar alcohols based on oxidation by periodic acid in acid solutions. This appeared to provide a sounder basis than other methods for total carbohydrate estimation in that the amounts of periodate required corresponded very nearly to the stoichiometric amounts. Aldohexoses and sugar alcohols consumed \((n - 1)\), while ketohexoses\(^2\) consumed \((n - 2)\) molecular proportions of periodate where \(n\) is the number of carbon atoms in the sugar molecule.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Found</th>
<th>Rappaport's value</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>95.7</td>
<td>—</td>
<td>93.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>95.5</td>
<td>94</td>
<td>90.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>110</td>
<td>114</td>
<td>113</td>
</tr>
<tr>
<td>Galactose</td>
<td>93.6</td>
<td>94</td>
<td>90.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>97.9</td>
<td>—</td>
<td>95.0</td>
</tr>
<tr>
<td>Raffinose</td>
<td>90.0</td>
<td>—</td>
<td>90.0</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>92.4</td>
<td>94</td>
<td>91.0</td>
</tr>
</tbody>
</table>
TABLE 2. The destruction of fructose from a sucrose hydrolysate by heating with sulphuric acid (determined by the colorimetric periodate method)

<table>
<thead>
<tr>
<th>Hours</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>3.5</th>
<th>4</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical density at 352 nm—differences from blank values</td>
<td>1.24</td>
<td>0.93</td>
<td>0.77</td>
<td>0.72</td>
<td>0.71</td>
<td>0.68</td>
<td>0.67</td>
<td>0.67</td>
</tr>
</tbody>
</table>

In the oxidation of the sugars the periodate is reduced to iodate, the initial and the excess periodate being determined. Under appropriate conditions periodate is reduced by iodide to iodate, liberating iodine which may be titrated with thiosulphate. To obtain quantitative liberation of the iodine while keeping the rate of further reduction of iodate negligibly low, closer control of the concentration of the reactants and of the pH were necessary than was apparent in Rappaport's memoir. Suitable conditions are specified in our Experimental section. Table 1 gives the weight of various carbohydrates found to be equivalent to 1 ml of 0.005 N-thiosulphate together with Rappaport's values and the calculated amounts. It will be seen that the results are close to theoretical.

We found that by increasing the acid strength so that oxidation took place in a medium approximately 1.5 N with respect to sulphuric acid, di- or oligosaccharides were simultaneously hydrolysed and oxidised, the periodate consumption being equivalent to the component hexoses.

With mixtures of the type under consideration, a complication arose from the fact that each molecule of fructose, free and combined, only consumed four-fifths of the periodate consumed by an aldohexose or sorbitol molecule. It was therefore necessary to make a separate determination of total fructose. For the present purpose, namely, relating periodate consumption to total carbohydrate, an approximate figure for fructose content was sufficient (see Experimental section). Several methods for determining fructose in the presence of aldoses have been described but a sufficiently accurate assessment of total fructose content was obtained by destroying the fructose by boiling with acid and determining the remaining sugars with periodate. The method was based on the knowledge (a) that the rate of destruction by acid is very much greater for fructose than for an aldohexose and (b) that the hydrolysis of the furanose link is very facile. Table 2 shows the fall in periodate consumption (expressed as optical density units—see below) of a sucrose solution when heated with 3 N-sulphuric acid. In about 3 h the value fell to approximately that due to glucose alone, after which further decrease was slow.

TABLE 3. The difference in optical density at 352 nm between water and glucose solutions when treated by the colorimetric periodate method

<table>
<thead>
<tr>
<th>Concentration of glucose (µg/ml)</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference in density</td>
<td>0.331</td>
<td>0.663</td>
<td>0.972</td>
<td>1.302</td>
</tr>
</tbody>
</table>
Determination of soluble carbohydrates

Figure 1. (●), Sucrose; (○), glucose.

Figure 2. (■), Starch; (+), sorbitol; (▲), fructose. For practical purposes the same solid calibration (-----) line serves for sucrose, glucose, starch and sorbitol. Fructose yields the dotted line (- - - -) which differs from the other by an amount near the theoretical.

Figures 1 and 2. Results using the modified periodate procedure with batches of test solution containing appropriate amounts of reagent grade sugars dissolved in water in 100-ml graduated flasks. 1.00-ml aliquots were heated with reagents at 100 °C for 2 h and the total residual periodate was titrated with 0.0025 N-sodium thiosulphate. Some of the values are at the extreme limit of the range using the chosen quantity of periodate.
and so the periodate consumption after, say 3.5 h, provided a measure of the glucose content. Similar results were obtained with a mixture of fructose, glucose, sucrose and raffinose. It should be emphasised that this method, depending as it does on compensation of errors, is not proposed as a method for fructose determination except for the specific purpose of correcting a periodate consumption of a mixture of sugars when calculating total carbohydrates. For this purpose it is sufficient and convenient.

In order to adapt the method to smaller quantities and to increase its convenience, it was found an advantage to determine the iodine liberated by measuring its light absorption at $\lambda = 352$ nm rather than by titration with thiosulphate. Table 3 illustrates the use of this modification in the determination of glucose. The relationship between glucose concentration and optical density is nearly linear.

More recent modifications have been concerned with increasing the number of samples that can be processed in a working day. Advantage has been taken of semi-automatic liquid dispensers and, with certain adjustments to reagent strengths and volumes, satisfactory results have been obtained (Figures 1, 2 and 3).

The disadvantage of the periodate method is that the periodate consumed is obtained as a difference between initial and residual values. This is outweighed by its suitability for use in total carbohydrate determinations. At the same time it provides a useful method for the estimation of single sugars and sugar alcohols. In this case it possesses an advantage over methods using calibration curves in that the sugar content is calculated from titration (or colorimetric) values for iodine liberated using factors very near to the stoichiometric ones. Since calibration curves are not essential, the method may be applied even where reference specimens of pure sugar are not available. Further, the

**Figure 3.** Results using the modified periodate procedure with solutions of fructose, sorbitol and xylose taking 0.50-ml aliquots from solutions of higher concentration than shown in Figures 1 and 2. (▲), Fructose; (+), sorbitol; (△), xylose.
method is applicable in principle to any polysaccharide hydrolysed by 1.5 N-sulphuric acid. The periodate method is insensitive to the organic and amino-acids present in many plant extracts. It is, however, sensitive to glucosides such as phloridzin, which occurs in some apple tissues in similar amounts to the soluble sugars, but these may be readily separated from the sugars by paper partition chromatography.

3. Ferricyanide–arsenomolybdate method

3.1. Experimental

3.1.1 Reagents

Buffer solution II: 140 g of dipotassium phosphate (K$_2$HPO$_4$) and 42 g of tripotassium phosphate (K$_3$PO$_4$)/litre.

Buffer solution III: 84.4 g of dipotassium phosphate (K$_2$HPO$_4$) and 196 g of trisodium phosphate (Na$_3$PO$_4$·12 H$_2$O)/litre.

Potassium ferricyanide: 16.5 g of potassium ferricyanide/litre.

Arsenomolybdate reagent: made as described by Nelson.$^4$

Oxalic acid: 20 g of oxalic acid (C$_2$H$_2$O$_4$·2 H$_2$O)/litre.

3.1.2 Procedure

Potassium ferricyanide solution (10 ml) was mixed with 50 ml of buffer II and made up to 100 ml with water. Schales and Schales$^5$ state that this mixed reagent is stable for 4 months, but Folin$^6$ reported that even slightly alkaline solutions of ferricyanide yielded ferrocyanide—especially when exposed to even diffuse daylight. Our experience suggested that it was better to mix this reagent in small quantities as required.

The sugar solution (2 ml) and 2 ml of the buffered ferricyanide solution were pipetted into a lipped test tube. At the same time a reagent blank was set up with 2 ml of water in place of the sugar solution. The tubes were covered with glass marbles, heated in a boiling water bath for 15 min, cooled and 2 ml of the arsenomolybdate reagent added to each. After at least 5 min the contents were diluted to 25 ml and the colour read at 740 nm against the reagent blank one hour after the addition of the colour reagent.

For the determination of oligosaccharides such as sucrose or raffinose, 10 ml of potassium ferricyanide were mixed with 50 ml of buffer III and diluted to 100 ml with water. The sugar solution (1 ml) and 1 ml of oxalic acid solution were pipetted into a tube, covered with a glass marble and heated in a boiling water bath for 1 h. After cooling, 2 ml of the buffered ferricyanide solution were added and the tube replaced in the bath for 15 min. The determination was then completed as described for monosaccharides. A blank determination was carried out simultaneously.

The precision of the ferricyanide method is satisfactory. In six replicate determinations of glucose at concentrations of 200 and 100 µg/ml, the mean optical densities were 1.441 and 0.711. The respective standard errors were 0.0047 (0.33 µg/ml) and 0.0024 (0.17 µg/ml).

3.2. Discussion of results

In the Hagedorn and Jensen method$^3$ for the determination of reducing sugars by oxidation with alkaline ferricyanide, the consumption of the latter is calculated from the difference between the excess ferricyanide remaining after oxidation of the sugar and
the total ferricyanide found in a blank determination. An obvious advantage would be
 gained by direct determination of the ferrocyanide formed.

One such method has been described by Folin6 based on the formation of Prussian
Blue by the addition of ferric chloride in the presence of a protective colloid such as
gum arabic. Miller and van Slyke7 measured the ferrocyanide by titration with ceric
sulphate. This method, however, is unsuitable for ketoses, as the ceric sulphate reacts
with the α-keto group of one of the oxidation products of such sugars. Shaffer and
Williams8 determined the amount of reduction by measuring the potential at a ferri-
ferrocyanide electrode.

We found that the arsenomolybdate reagent introduced by Nelson4 for the colori-
metric determination of the cuprous oxide formed in the oxidation of sugars by alkaline
copper reagents could be used to determine ferrocyanide in the presence of ferricyanide.

The use of this reagent in conjunction with phosphate-buffered potassium ferri-
ferrocyanide solution provided a simple and reliable method for the colorimetric determi-
 nation of reducing sugars with the following advantages:

(a) no critical heating time factors were involved;
(b) no precautions were necessary against back oxidation by air;
(c) one reading only was required for each determination;
(d) a larger excess of ferricyanide could be used to ensure proportionality between the
    amount of sugar and the extent of oxidation (cf. Miller and van Slyke);7
(e) precise measurement of the ferricyanide and colour reagents added was not neces-
sary.

Colour development on mixing the reagents was practically instantaneous but tended
to intensify somewhat during the next hour, particularly if the solution was diluted too
soon after mixing. After the first hour, further change in intensity was very small.
At pH 1.6, which is that produced by the described method, the colour was stable for
several hours. At higher pH values the initial colour intensity was less and it faded
rapidly on standing.

The density of the colour (λ = 740 nm; pH 1.6) produced by the various sugars was
directly proportional to the amount of sugar in the test solution. Values obtained for
the factor K in the equation

\[
\text{weight of sugar (µg)} = K \times \text{optical density (λ = 740 nm)}
\]

are shown in Table 4.

| Table 4. Factors relating weight of sugar and optical density at 740 nm when treated by the ferricyanide-arseno-
| molybdate method |
|------------------|------------------|
| Sugar            | K    | Sugar            | K    |
| Glucose          | 143  | Sucrose          | 136  |
| Galactose        | 168  | Rhamnose         | 125  |
| Fructose         | 143  | Raffinose        | 187  |
| Arabinose        | 149  | Cellobiose       | 170  |
| Xylose           | 137  | Mannose          | 145  |
| Sorbitol         | 10 000 |                 |      |
Since this factor is the same for glucose and fructose, the determination of a mixture of fructose, glucose and sucrose is possible from measurements made before and after hydrolysis with oxalic acid and a specific determination of glucose using glucose oxidase.  

4. Conclusions

The two techniques described introduce improvements which are valuable for the routine estimation of soluble carbohydrate. The periodate method gives stoichiometric proportion between periodate consumption and carbohydrate concentration for a wide range of compounds. Not only is it useful for the estimation of individual components or mixtures which include sugar alcohols but, since it is carried out in acid solution, polysaccharides which have been extracted by acid hydrolysis may be assayed without prior neutralisation of the extracts. The fact that a somewhat smaller amount of periodate is required to oxidise fructose is not normally inconvenient but the instability of fructose may be turned to advantage in the estimation of its proportion in a mixture by the use of the periodate method before and after boiling with acid. Provided attention is paid to the details of technique, the method gives very reliable results in routine use.

The ferricyanide-arsenomolybdate method was developed from a well-tried procedure for estimating reducing sugars. Its advantage rests in the ability to estimate sugar concentration by direct reading rather than by reference to a blank solution.

Both methods are valuable for the estimation of individual sugars and any choice between them must rest on the type of "sugar" to be estimated. The procedure using ferricyanide is faster than the periodate method but the latter is suited to a wider range of soluble carbohydrates. The two methods may be used in conjunction where it is required to obtain estimates of reducing sugar, sucrose, fructose and sugar alcohol using aliquots of the same sample. This result may then be achieved without a need for detailed chromatographic separations.

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References