

A SIMPLIFIED STARCH DETERMINATION FOR RAW SUGARS

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ABSTRACT

The conventional starch determination (employing precipitation, filtration, re-dissolution, colour development) is compared with a simplified direct colorimetric procedure, which is readily automated. The reproducibility is better than 3%, with a correlation coefficient of 0,87 between the two methods.

Introduction

The method currently used for starch determinations (a modification of that due to Balch¹), the details of which have been published by Matic,² whilst being reasonably specific, is fairly time-consuming and involves many manipulations. In the hands of a relatively inexperienced analyst each of these steps is error-prone. Radford³ has reported a standard deviation of ± 10 ppm for starch values in the 130-175 ppm range, whilst far higher, apparently random differences have often been observed between laboratories. For routine determinations it is preferable to use the simplest method available, with as few opportunities for operator error as possible. Obviously, for quality control purposes, the quicker a result is to hand the more useful is that result. A result available 4-5 hours later can have no bearing on process control and hence is of academic interest only. A simple method also has the advantage of lending itself to automation far more easily than one with several involved steps. Automation of the starch determination has application in the automatic adjustment of enzyme-dosing levels for example. Sang *et al*⁴ have successfully automated a direct, colorimetric method for starch. However, the levels of starch encountered were far higher than those found in local sugars.

Archibald,⁵ Davis (C.S.R. method)⁶ and Charles⁷ have all reported rapid methods which omit the precipitation stage. Archibald and the C.S.R. method both use a hot, mild digestion of the sugar in calcium chloride solution to ensure that the starch is in a form suitable for subsequent reaction with iodine, the C.S.R. absorbance readings being made at 700 nm against potato starch as standard. Charles acidifies an aqueous solution with phosphoric acid and has an iodide concentration in the final solution of 0,05% (compared with 0,1% in the conventional method). His readings are taken at 560 nm against amylopectin as standard. Sang *et al*⁴ employ a direct colorimetric method, using the same reagent concentrations as in the standard method, but using corn starch as standard and reading at 570 nm. These workers have reported that both the iodide and iodate concentrations are statistically significant sources of variation. In order to deviate as little as possible from the conditions of the conventional method, whilst simplifying and automating the determination, we used an aqueous sugar solution with

potato starch as standard and took readings at 600 nm.

We would like to emphasise that the simplified method is not in any way offered as a replacement for the conventional, reasonably specific method, but rather as a process control where rapid, but relative estimates are acceptable, and in cases where automatic monitoring is feasible.

Matic² has recently published a comprehensive review and comparison of the starch methods currently in use and concludes that due to the empirical nature of the starch determination by means of the starch-iodine complex it is essential that the method is followed implicitly if reproducible and meaningful results are to be obtained. Whilst in full agreement with this approach there is perhaps also a place for a more rapid method.

Experimental

The sample preparation was the same whether the colour was developed manually and read in a spectrophotometer (Beckman D B - G) or whether the determination was automated. (Technicon AutoAnalyser II) 25 g of a raw sugar sample were dissolved in about 30 ml of hot distilled water and boiled gently for 2 min ± 10 sec. The cooled solutions were diluted to 100 ml in a volumetric flask and filtered (SS 613 filter paper).

(a) Manual determination:

20 ml aliquots were withdrawn and the colour developed as in the conventional method. 5,0 ml of a potassium iodate-acetic acid solution (0,3567 g KIO_3 (previously dried 2 h. at 105°C) + 57 ml glacial acetic acid made to 1 litre) and 0,5 ml 10% (w/v) potassium iodide (freshly prepared) were added to each flask, the contents swirled, made to 50 ml and well mixed. The colour was read in a 1 cm cell at 600 nm against a blank solution prepared by omitting the sugar solution.

(b) Automated determination:

For the automated determination a Technicon AutoAnalyser II was used. The experimental manifold is detailed in Fig. 1. Samples were run at a rate of 30 h.⁻¹ with a sample : wash ratio of 2 : 1. A series of standards was included about once an hour to compensate for any drift. Reagents were prepared as follows:

- (i) Acetic acid- KIO_3 : 14,3 ml glacial acetic acid + 0,8918 g KIO_3 (previously dried 2 hours at 105°C) were dissolved and diluted to 1 litre. The solution was stored in an amber glass bottle and found to be stable.
- (ii) KI : A 0,5% (w/v) solution was prepared fresh each day. The baseline was adjusted with water in the sample line.

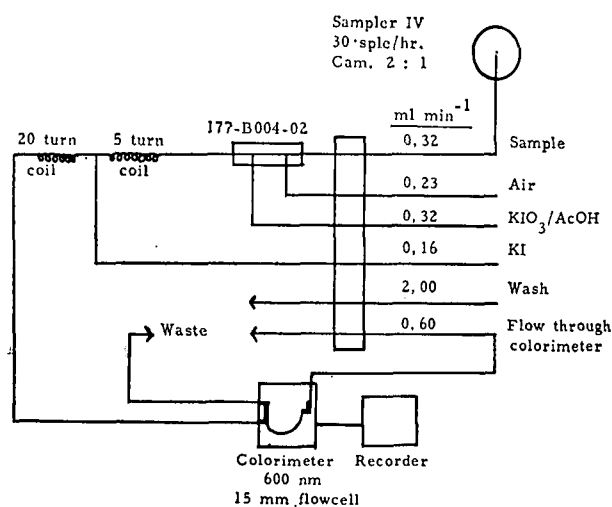


FIGURE 1

The calibration curve was prepared using potato starch (BDH). A stock solution was prepared containing 0.25 mg starch (dry basis)/ml. Working standards were obtained by diluting 5, 10, 15, 20, 25 ml of this stock solution to 100 ml. The stock solution was kept for not more than one week.

Working standards were prepared daily. For the automated determination these solutions were placed directly in the sample cups, whilst 20 ml aliquots were used if the colour was developed manually. This covers the range 0-250 ppm starch in the sugar sample.

Results

Table I gives the reproducibilities obtained on repeatedly analysing three different samples using the automated method.

Table I

Sample	Starch (ppm)	s	%	n
1	69	± 1,5	2,2	23
2	163	± 4,2	2,6	23
3	169	± 3,3	2,0	23

The analysis of 26 samples of unaffinated raw sugars by both the conventional method and the rapid method gave the following regression equation:

$$y = 18,2 + 0,98 x$$

where y represents the results by the rapid method and x the results from the conventional method.

The correlation coefficient was 0,87.

An inter-laboratory comparison of the conventional method gave a regression equation: $y = 7,70 + 1,00 x$ with correlation coefficient 0,97 (26 samples). The results of these comparisons are presented in Table II.

Table II

Comparison of the conventional and rapid methods

Rapid method	Starch (ppm)		Δ (R - L1)
	Conventional method Lab. 1	Lab. 2	
170	140	150	+ 30
165	140	145	+ 25
155	125	140	+ 30
150	115	125	+ 35
145	120	115	+ 25
145	140	140	+ 5
140	120	130	+ 20
140	115	130	+ 25
140	110	120	+ 30
135	115	125	+ 20
130	125	140	+ 5
125	100	110	+ 25
125	125	130	0
125	125	130	0
120	95	105	+ 25
120	105	110	+ 15
115	105	110	+ 10
115	120	130	- 5
105	95	100	+ 10
105	90	100	+ 15
105	100	100	+ 5
100	95	105	+ 5
100	90	105	+ 10
90	55	65	+ 35
85	70	70	+ 15
80	80	85	0

When comparing the inter-laboratory variances with the inter-method variances an F-value of 5,36 was obtained. ($F_{0,05;25;25} = 1,97$ and $F_{0,01;25;25} = 2,60$), indicating that the rapid method shows a greater variance about its fitted line than does the inter-laboratory comparison.

Discussion

It was found necessary to boil the solutions before proceeding with the analysis. If the sugars were dissolved in water at ambient temperature or slightly warmer, the starch results were significantly lower and, furthermore, showed a continued decrease with time of standing (about 13% decrease over 2 hours) due to retrogradation. Provided the initial boiling was used the decrease in starch obtained over a 2-hour period was less than 5%.

To avoid undue amplification of the signal on the AutoAnalyser and hence noisy, unreliable traces, the sample concentration was markedly increased over that used by Sang. The high sucrose levels had no obvious deleterious effect.

It was convenient to incorporate the iodate and acid solutions into a single stable reagent and to prepare fresh iodide solution daily. The hydraulics of the automated system were found to be more stable if the acid reagent was added before the iodide.

The reproducibility of the method is better than 3% and the correlation coefficient for the two methods is 0,87 (determined on 26 samples of VHP sugar). The rapid method gives results which are consistently higher than those given by the conventional method.

The scatter of the results using the rapid method is greater than that obtained with the conventional method, although the reproducibility of a single sample is excellent. There is a tendency for the difference between the two methods to increase as the starch content increases.

A direct colorimetric method such as that proposed is very susceptible to interfering substances in solution (e.g. gums, dextrans, colloidal substances, colour, etc.) and the good correlation may be due, in part, to the fact that only VHP sugars were examined. In the case of lower purity products, assuming colour corrections, the interferences may be large enough to preclude application of the method.

Acknowledgements

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