MEASUREMENT OF SUGAR CONTENT IN FODDER AND SUGAR BEETS

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ABSTRACT

Total fermentable sugars in fodder and sugar beet roots and tops were determined by an automated method involving hydrolysis of the sucrose present in 1M HCl at 68°C followed by colorimetric determination based on the hexacyanoferrate (iii)/(ii) reduction by monosaccharides. Omission of the hydrolysis step permits reducing sugars to be measured, and sucrose is determined by difference.

Results for sucrose determinations are compared with results obtained by polarimetry and refractometry.

INTRODUCTION

Until very recently, sugar analyses on New Zealand beet crops were restricted to polarimetric sucrose determinations on sugar beets (Drewitt, 1976; Greenwood, 1980). Early fodder beet trials investigated only fresh and dry matter yields for stock feed purposes. Sugar beet trials were initiated to investigate the potential for a New Zealand sugar industry, and therefore only sucrose content was of interest.

Following the rapid rise in oil prices during the 1970s, research into the potential of fodder and sugar beets for ethanol fuel production was commenced (NZAEI, 1978, Drewitt, 1979). This necessitated the development of analytical techniques for the determination of total fermentable sugar and reducing sugar as well sucrose. The total fermentable sugar is of prime concern for ethanol production and this includes, in addition to sucrose, the precursors and hydrolysis products of sucrose *viz* glucose and fructose. The possible development of dual purpose sugar/ethanol production plants will be dependent on the use of beets with low total reducing sugar content and it is therefore necessary to be able to distinguish these from beets with high reducing sugar content.

The roots of some fodder beet cultivars, particularly when immature, may contain significant quantities of reducing sugars; beet tops invariably contain a large proportion of their total sugar as reducing sugars in the process of synthesis into sucrose; and the reducing sugar content of roots of all beets, particularly fodder beets, increases during storage as the sucrose reverts to glucose and fructose.

In addition to the implications for processing, the reducing sugar content affects the determination of sucrose by polarimetry. This method is dependent on the degree of rotation of light by sucrose, and the reducing sugars have different angles of rotation. Depending on the proportions of the various reducing sugars present, the angle of rotation and therefore the apparent sucrose content can be changed.

Increasing research into ethanol production has necessitated that methods developed for sugar analyses be both rapid and reliable. This paper describes automated methods developed for the determination of total fermentable sugars, total reducing sugars, and sucrose (by difference) in fodder and sugar beets. The method for the determination of total fermentable sugar was adapted from the molasses analysis method of Janshekar and Mor (1977). Autoanalyser analyses are compared with sucrose measurements by polarimetry and refractometry.

MATERIALS AND METHODS

Sample preparation

Fresh beet roots were cut lengthwise into wedges, each sample consisting of wedges from a number of roots. Gratings were taken off the flat cut surface of each wedge using a cheese grater, single or duplicate 52g samples being collected. If the gratings could not be analysed the same day, they were stored at -5 °C in polypropylene bags.

Leaf samples were chopped into pieces less than 1 cm^2 area then treated as for root gratings.

Samples of roots and leaves from 3 cultivars were used for measurements. These were Vytomo sugar beet, Mono Blanc fodder beet (deep-rooted type) and Yellow Daeno fodder beet (shallow-rooted type).

Extraction of sugar for autoanalyser or polarimetric analysis

The 52 g samples of grated beet roots or chopped leaves were transferred to a blender jar to which was added 144 ml H_2O and 10 ml basic lead acetate suspension (100 g per 300 ml H_2O, kept mixed). This clarifying agent also greatly accelerates dissolution. The mixture was blended at maximum speed in a kitchen blender for 3-5 minutes depending on the efficiency of the blender, then filtered through fluted 15 cm Whatman No 1 paper into a flask containing 1g of sodium oxalate (AR grade) powder. The filtrate was left for 1 hour, to precipitate the lead, before filtering through a second 15 cm No 1 paper.

This extraction technique is based on that described by Le Docte (1927) for sugar beet roots and assumes that the volume of insoluble matter from 52g beet is 6.0 ml, so that the sugar contained in the 52g sample is extracted into a final solution volume of

$$(52 - 6) + 144 + 10 = 200 \text{ ml.}$$

An aqueous sucrose solution, 26 g/100 ml, is used as a 100% standard. The assumption of a final solution volume of 200 ml does not necessarily hold for leaves or for fodder beet roots which may contain smaller proportion of insoluble matter. Relationships were therefore derived for several cultivars between the insoluble matter content and (i) dry matter %, (ii) sugar as percentage of fresh weight, and (iii) sugar as percentage of dry weight. All relationships were highly significant, the best being that between insoluble matter and dry matter. The following relationship between true sugar %, measured sugar % and dry matter was derived:

True sugar % = measured sugar % + 0.002 (24-DM%)

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For dry matter percentages between 19 and 29%, which covers all sugar beets and many fodder beets, less than 1% error is involved and the correction will not generally be necessary.

Autoanalysis

Total fermentable sugar determination. Total fermentable sugar content (sucrose plus reducing sugars) of beet roots and leaves was determined by an adaption of the automated method of Janshekar and Mor (1977). In this method, sucrose was hydrolysed to glucose and fructose by 1M HCl at 68 °C and the resulting monosaccharides measured, along with the other reducing sugars present by the alkaline reduction of the yellow hexacyanoferrate (III) ion to colourless hexacyanoferrate (II) at 95 °C.

The adapted autoanalyser manifold, designed for a Chemlab Continuous Flow System, is shown in Fig. 1. Dialysis, necessary in molasses analysis (Janshekar and Mor 1977), was initially used as a precaution but was found to be unnecessary for fodder and sugar beet analysis.

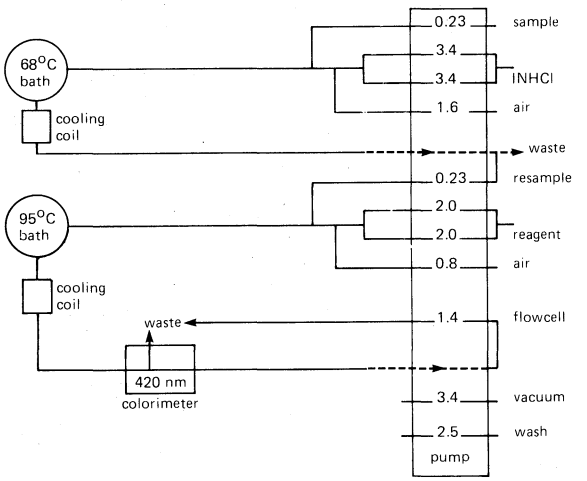

Figure 1: Autoanalyser manifold for total and reducing sugar determination.

Reducing sugar determination. To measure the reducing sugar content (chiefly glucose and fructose) of roots and tops, the acid hydrolysis step was omitted. As shown in Fig. 1, this simply entailed removal of several pump tubes. The resampling tube for the total sugar determination then became the sampling tube for reducing sugar determination. This arrangement increased the proportion of sample for reducing sugar determination and provided the necessary increased sensitivity to determine the low quantities of reducing sugars present in most root samples.

Reagent strength and standard curve. The concentration of $K_4Fe(CN)_6$ reagent (in a 4%w/v NaOH solution) should be varied to suit the required working range, strengths from 0.50 to 1.75 g/l covering most purposes. Standards are diluted as required from a working "100%" standard made by dissolving 26g sucrose in 100 ml H_2O. A typical standard curve for total fermentable sugar is shown in Fig. 2.

Sucrose determination by polarimetry.

The temperature of sample and standard solutions was adjusted to 20 °C (±0.5 °C) before filling the polarimeter tube. The solution was adjusted to give an even field on the eyepiece and the sucrose % read directly from the scale, after checking standards (diluted as required from the "100%" working standard used in the automated procedures).

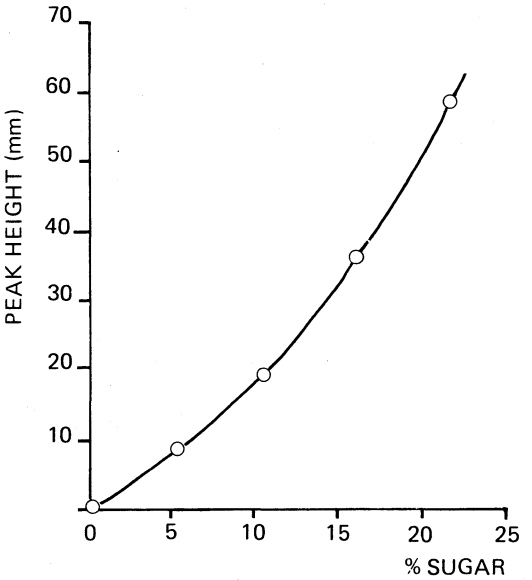

Figure 2: Typical standard curve for full-range analysis.

Sucrose determination by refractometry

A pocket refractometer (Bellingham & Stanley, England) calibrated in sucrose units was used. Juice from the grated beet was squeezed directly on to the prism. In accordance with the manufacturer's instruction for beet measurements, 2.5% was subtracted from the measurements obtained.

RESULTS AND DISCUSSION

Storage of frozen samples

Gratings were stored for up to 3 months at −5 °C with no deterioration or inversion of sucrose (Table 1). If samples were stored as frozen wedges rather than gratings, the initial grating of the thawed wedges had to be discarded, as low sugar content solution (1-3% sugar) moved to the cut edges of the wedges during the cooling period before the samples were properly frozen.

Extraction of sugars

Increasing the blending time and/or temperature did not increase the concentration of sugar and in the sample solution (Table 1). It was therefore concluded that complete extraction was achieved in the procedure described.

Comparison of methods for sucrose determination

Autoanalyser and polarimeter. Sucrose content by autoanalyser (as determined by the difference between total and reducing sugar content) was compared with direct measurement by polarimeter. All three cultivars were characterised by very low concentrations of reducing sugars in the mature roots (Table 1 & 2) and therefore good agreement was obtained (Fig. 3, $r^2 = 0.91$).

Provided the reducing sugar content was 0.5% sucrose or less, determinations by polarimetry and autoanalyser almost invariably agreed to within 0.3% sucrose (Table 2). At higher concentrations of reducing sugars, the polarimetric determination ranged from 1.0% lower to 2.5% higher sucrose than the autoanalyser determination. The wide variation in the discrepancy, which was not correlated with the total reducing sugar content, was presumably caused by variations in the proportions of the various reducing sugars present. The high concentration of reducing sugars in leaves made the polarimetric sucrose measurement meaningless (Table 2).

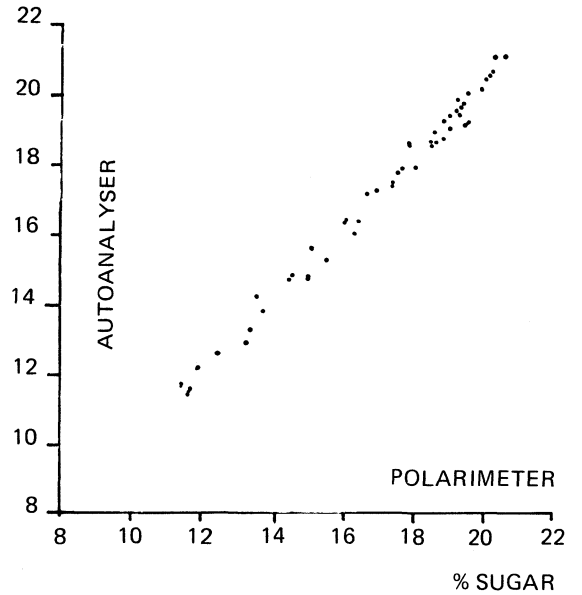

Figure 3: Regression of sucrose analysis by autoanalyser (total sugars less reducing sugars) and polarimeter.

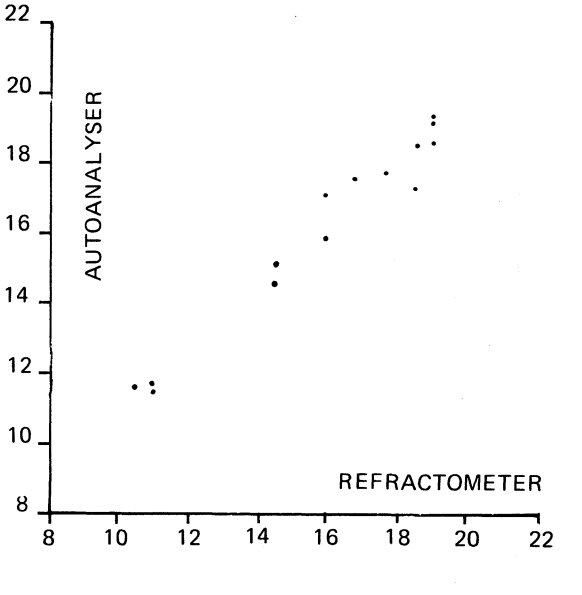

Figure 4: Regression of sucrose analysis by autoanalyser and refractometer.

Autoanalyser and refractometer. Surprisingly good agreement was obtained between sucrose determinations by autoanalyser and refractometer (Fig. 4), but the discrepancy widened as the sucrose content decreased, probably reflecting the effects on the light refraction by the increasing proportion of other constituents in the sugar solution.

Variation of total sugar content within beet root

Top to bottom. Roots were split into 6 discs of equal weight for sugar measurement. None of the 3 cultivars showed any marked trends in total sugar concentration (Table 3).

Outside to centre. Discs from roots were progressively grated into 52g samples, commencing from the outside of each disc. The results (Table 3) show that all 3 cultivars had the lowest total sugar concentration in the outside sample which included the skin. Vytomo sugar beet and Mono Blanc fodder beet showed no trend thereafter but Yellow Daeno fodder beet showed a definite reduction in sugar content towards the centre of the root. Core sampling, by taking a disproportionately large sample from the centre of the root, would therefore under-estimate the true sugar percentage.

Large to small roots. The sugar concentrations decreased with root size with all 3 cultivars, with the variation with size decreasing in the order Mono Blanc fodder beet, Yellow Daeno fodder beet, Vytomo sugar beet (Table 3).

CONCLUSIONS

The automated methods described in this paper permit the rapid and reliable determination of total fermentable sugars and reducing sugars in beet roots and leaves. Sucrose was determined by difference.

Polarimetry gave good agreement with sucrose determination by autoanalyser provided the reducing sugar content was 0.5% or less. Mature roots of the 3 cultivars almost invariably had levels below this limit. At higher concentrations, polarimetric analysis was unreliable.

A pocket refractometer gave reasonable agreement with sucrose determination by autoanalyser, (provided the refractometer reading was corrected as recommended) although the error in the refractometer results increased with decreasing sucrose content.

TABLE 1: Effect of storage time and extraction conditions on measured sugar content in roots.

	Fresh (a)		Frozen 6 months (a)		Total sugar % (b) time (min) and temp. (°C) of extraction			
	Sucrose %	reducing sugar %	sucrose %	reducing sugar %	2/20	5/20	5/60	20/60
V	18.0 (.03)	0.26	18.0 (.03)	0.25	18.3	18.4	18.3	18.4
MB	15.1 (.04)	0.24	15.1 (.05)	0.25	15.4	15.3	15.3	15.4
YD	11.3 (.04)	0.26	11.2 (.06)	0.31	11.6	11.6	11.8	11.7

(a) Mean of 5 duplicate analyses (standard deviation in brackets)
(b) Average of 2 samples.
V Vytomo sugar beet; MB Mono Blanc fodder beet; YD Yellow Daeno fodder beet.

TABLE 2: Comparison of sucrose determination by autoanalyser and polarimeter.

	Autoanalyser			Polarimeter
	% total sugar	% reducing sugar	% sucrose (difference)	% sucrose
Mature Roots (a)				
V	19.2	0.27	18.9	19.0
MB	15.5	0.23	15.3	15.1
YD	12.8	0.24	12.6	12.5
Growing Roots*(b)				
V	12.6	0.81	11.8	10.8
MB	9.2	0.61	8.6	9.6
YD	8.4	0.64	7.8	8.6
Tops (b)				
V	5.7	2.5	3.2	2.1
MB	4.5	2.0	2.5	1.5
YD	4.0	1.8	2.2	1.1

V, MB, YD as for Table 1.
* Only roots containing >0.50% reducing sugars are included in this comparison.
(a) Average of 15 different samples for each cultivar.
(b) Average of 5 different samples for each cultivar.

TABLE 3: Variation in total sugar content within roots.

Top to bottom

	1 (top)	2	3	4	5	6 (bottom)
V	17.4	18.1	18.1	19.1	18.0	18.2
MB	15.7	16.4	15.8	15.8	15.4	16.1
YD	12.3	12.0	12.0	10.8	12.0	12.3

Outside to centre

	1(out)	2	3	4	5	6	7	8	9	10
V	14.8	18.0	18.8	18.6	18.8	18.3	18.5	18.1	18.5	17.9 (centre)
MB	12.7	15.6	16.3	16.2	16.5	16.0	16.6	16.2	(centre)	
YD	11.2	13.6	13.0	12.8	11.9	10.7	10.9	11.3	10.6	(centre)

Large to small roots (same treatment of one trial)

	large	small
V	17.2	19.0
MB	14.0	17.7
YD	10.6	13.2

V, MB, YD as for Table 1.

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