

# DETERMINATION OF SUCROSE IN CANE JUICE AND MOLASSES BY ISOTOPE DILUTION

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## Abstract

Sucrose in first expressed juice and mixed juice was determined by isotope dilution and the results were compared with the conventional pol determination. It was found that the sucrose content on the average was 0,25% higher using the ID method. Molasses samples, on the contrary, showed a sucrose content which was 0,71% lower than the value found by the Mackay method.

The same molasses samples were also analysed by gas chromatography which gave a value 2,24% lower than the Mackay method.

## 1. Introduction

Most conventional analytical methods for the determination of sucrose in factory products depend upon polarisation. These measurements are unreliable in the presence of optically active non-sucrose constituents.<sup>1-4</sup>

Although many modifications in the polarisation methods have been introduced in order to minimise interference, none is completely reliable, according to Hirschmüller.<sup>4</sup>

The latter author<sup>5</sup> in 1959 introduced a method for sucrose determination in sugar beet based on isotope dilution and this is free from the above-mentioned types of interference.

In isotope dilution methods, a measured quantity of labelled sucrose is added to the sample under investigation, after which sufficient pure sucrose is isolated from the sample to establish a radio-active count.

From the ratio between the radio activity of the standard added and of the finally recovered sucrose, the original sucrose concentration in the sample can be calculated, taking into account the amount of labelled sucrose which was added to the sample.

In the present investigation the isotope dilution method was applied to cane juice and cane molasses. The methods which have been used for sugar beet and beet sugar products were studied and modified for application to cane sugar products. The values for sucrose content in juices and molasses as obtained by the isotope dilution method were compared with those obtained by conventional methods.

## 2. Technique of radio active measurement

In the case of a labelled sucrose molecule, either the C-atom or the H-atom can be labelled. The C-atom has the advantage that <sup>14</sup>C has a long half life (5 730 years) and no corrections have to be made for radio-active decay during the determination. In addition to this the C-atom is built into the skeleton of the sucrose molecule, while the H-atom can wander through the molecule and be lost by dissociation during the determination.

The radiation of <sup>14</sup>C is very weak — <sup>14</sup>C → <sup>14</sup>N + β<sup>-</sup> (0,154 MeV) — and special measuring techniques have to be used.

Hirschmüller and Hoerning<sup>5</sup> used a proportional gas flow counter, operated with methane. In this type of counter, there is no mica window separating the sample from the Geiger-Müller (G.M.) tube, as the sample is placed inside the G.M. tube. In this way a reasonable counting efficiency is obtainable.

Higher counting efficiencies can be obtained in a liquid scintillation counter. In this system, the radio-active material is dissolved or dispersed in a mixture of organic components which emit photons in the ultra violet region of the spectrum when it is energised by radio-active radiation. These photons are counted by a photo multiplier tube.

With the earlier scintillation counters reasonable efficiency was only obtained at low temperature, which limited the application of this technique. Present instruments, however, are less sensitive to thermal excitation and counting at ambient temperature is standard practice. Nevertheless it was found during this investigation that background counts started to increase above 25°C. For this reason the temperature of the laboratory which housed the scintillation counter was kept below 25°C.

## 3. Methods for the isolation of sucrose from the sample

As mentioned in the introduction, the ID method requires that a certain quantity of 100% pure sucrose is isolated from the sample.

Hirschmüller and Hoerning<sup>5</sup> made use of the sucrose-copper complex for this isolation. The sucrose was extracted from beet with di-ethylamine and was crystallised from this solvent by addition of ethanol. The crude sucrose was dissolved in a copper sulphate solution and after one hour the copper-sucrose complex was separated from this mixture. The complex dissociates in hot water and the copper was removed by the addition of collidine, resulting in an insoluble copper-collidine complex. The filtrate of this solution was colourless and clear and pure sucrose was crystallised by the addition of ethanol.

This method was modified in 1970 by Gelen.<sup>6</sup> The beet pulp was extracted with a basic lead acetate solution and filtered. The filtrate was passed through a cation and anion exchanger, concentrated under vacuum and the sucrose crystallised by the addition of ethanol. This crude sucrose was then purified as described previously by forming a copper-sucrose complex after which the copper was removed by collidine. This modification was claimed to be less time-consuming and it gave results identical to the original method developed by Hirschmüller. Gelen also modified the counting technique by using a

liquid scintillation counter, which was applied by McGagin and Eis in 1968<sup>7</sup> for counting <sup>14</sup>C sucrose and is at present generally used for counting radio-active carbon and hydrogen.

A different method of purification was proposed by Sibley and co-workers.<sup>8</sup> They purified the sucrose by preparing the barium saccharate and then decomposed the latter by bubbling carbon dioxide through the barium saccharide suspension. The suspension was subsequently filtered through sintered glass to remove the barium carbonate formed. The sucrose was further purified in the same way described for the other methods.

Mauch<sup>9</sup> compared the method of Hirschmüller and Hoerning with that of Sibley and co-workers for the determination of sucrose in beet. During the initial tests Mauch found that the method of Hirschmüller gave higher sucrose values in beet than the method of Sibley. Additional experiments showed that in Sibley's method the sucrose was not completely extracted from the beet pulp. After increasing the amount of water in the mixture used for the extraction of the beet pulp the two methods gave sucrose values which agreed within the errors to be expected.

## 4. Experimental

### 4.1 PREPARATION OF THE STANDARD SUCROSE

In the earlier part of this investigation the standard sucrose was prepared as described by McGagin and Eis.<sup>7</sup> At a later stage a modification of Lew<sup>10</sup> was followed.

The <sup>14</sup>C sucrose used was Calatomic\* sucrose — U-C-14 50 $\mu$ c dissolved in 0,125 ml 25% ethanol. Using these vials procedure 1, described below (4.1.1) was followed. If after a number of analyses sufficient radio-active sucrose had been collected this was reprocessed into standard sucrose following procedure 2.

#### 4.1.1 Procedure 1

Fifty grams of AR sucrose were dissolved in 12,5 ml distilled water. The mixture was heated to 85-90°C and maintained at that temperature. When all the sugar was dissolved the contents of two vials <sup>14</sup>C sucrose (100  $\mu$ c) were added and the mixture was stirred for 15 minutes after which it was filtered through a millipore filter (0,1  $\mu$ c). The filter was washed and the wash water was combined with the residues of previous determinations and reprocessed according to procedure 2.

To the filtrate ethanol was added and the crystals so recovered were again recrystallised twice from ethanol. The final product was dried under vacuum at 65-70°C. The number of counts per minute on 0,2 000 g were determined and if the value was not between 380 000 — 420 000 the radio-activity was adjusted by adding to the redissolved crystals either more non radio-active sucrose or <sup>14</sup>C-sucrose as required.

#### 4.1.2 Procedure 2

The residues of previous determinations, all having a radio activity of about 200 000 counts per minute

per 0,2 000 g, were added to 50 g of AR sucrose dissolved in 12,5 ml of distilled water. Sufficient <sup>14</sup>C-sucrose was added to raise the activity to the required level and the solution was processed as described under 4.1.1.

## 4.2 PURIFICATION OF SUCROSE

### 4.2.1 Isolation and purification of sucrose from cane juice

In the present investigation the method of Hirschmüller was tried first. It was, however, found difficult to obtain a completely copper-free solution after the precipitation of the copper-collidine complex. In addition, the method was rather elaborate.

Subsequently the juice was first clarified by the addition of dry basic lead acetate. After filtration the lead was precipitated by hydrogen sulphide followed by a second filtration. The clear filtrate was further purified by passing it successively through a cation and anion exchange column.

The deionised solution was concentrated under vacuum in a rotary evaporator to a syrup, absolute ethanol was added and the solution was cooled in a refrigerator. The crystallised sucrose was dissolved and recrystallised after which it was finally dried under vacuum at 65-70°C. This purification method using lead sub-acetate did not result in the same reproducibility as the barium saccharate method described below. In addition to this it became evident that the precipitation of the sucrose as barium saccharate was no more complicated than the purification of the juice with lead sub-acetate.

The method finally adopted was a modification of that published by Sibley.<sup>8</sup> For the determinations in cane juice it was found advantageous to incorporate a prepurification stage as follows. To 30 g of cane juice 1,5 g <sup>14</sup>C sucrose were added and made up to 50 ml with distilled water. This mixture was heated to 60°C and 0,5 g barium hydroxide was added. The small amount of barium and the low temperature make precipitation of barium saccharate impossible and the precipitate found is similar to that obtained in normal lime defecation.

The precipitate was centrifuged. From the clear supernatant sucrose was isolated by heating the solution to 76-80°C and adding 9,5 g of barium hydroxide. The mixture was stirred for 15 minutes. The precipitated barium saccharate was slurried in 40 ml distilled water and carbon dioxide was passed through the slurry at 70°C until neutral to phenolphthalein (external indicator). The mixture was filtered hot and the clear filtrate was made to 50 ml after which the barium saccharate precipitation was repeated.

The filtrate of the second carbonatation was acidified to pH 5,8-6,0 with dilute sulphuric acid. This step was incorporated to remove small quantities of residual barium salts. It was found that these barium salts, probably in colloidal form, were not removed in the subsequent ion exchange treatment.

The precipitated barium sulphate was filtered through a millipore filter (0,1  $\mu$ m) and the filtrate was

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passed through a column packed with 25 ml mixed bed ion exchange resin (Amberlite MB1).

The deionised sucrose solution was concentrated to a syrup under vacuum in a rotary evaporator and the sucrose was crystallised by the addition of ethanol and by cooling. The recovered product was twice recrystallised from ethanol and dried under vacuum at 65-70°C.

#### 4.2.2 Isolation and purification of sucrose from molasses

When the same method as described above was applied to the sucrose determination in molasses it was found that pure sucrose could not be isolated.

Analysis of the final product showed that starch passed the various purification stages and therefore the procedure for sucrose in molasses was modified as follows.

Five grams of molasses, 1,5 g of <sup>14</sup>C sucrose and 10-12 ml distilled water were thoroughly mixed while being gently heated. The mixture was cooled to ambient temperature and 90 ml of absolute ethanol were added. The mixture was stirred and allowed to stand for one hour. It was then filtered on a Buchner filter through Whatman No. 5 filter paper. The clear filtrate was concentrated in a rotary evaporator to remove the ethanol and the concentrate made up to 50 ml with distilled water. No precipitation with barium hydroxide was applied to the molasses solution. The further purification was carried out by three consecutive precipitations as barium saccharate in the same way as described for juice samples.

The subsequent recrystallisation from ethanol was also identical to that described for cane juice samples.

### 4.3 COUNTING

#### 4.3.1 Instrument

The counting instrument used in the present investigation was a Beckman  $\beta$ -Mate II scintillation counter.

#### 4.3.2 Scintillation liquid

In the earlier stages of the investigation a scintillation mixture described by Bray<sup>11</sup> was used. As sucrose is not soluble in nonpolar organic liquids, which are used for scintillation measurement, dioxane-based scintillation cocktails have to be utilized. Dioxane is miscible with water and the following mixture allows sufficient water to dissolve 0,2 g of sucrose, resulting in a transparent mixture.

1,4-di-2-(5-phenyl-oxazolyl)-benzene (POPOP)	0,2 g*
2,5 diphenyl oxazole (PPO)	4,0 g*
methanol (absolute)	100 ml†
ethylene glycol	20 ml ††
naphthalene	60 g**
dioxane to	1 000 ml **

For counting 200 mg of the sucrose were dissolved in 1 ml of water and mixed with 4 ml of the scintillation liquid. This resulted in a completely transparent mixture.

It was found that often high counts were recorded at the initial measurement, gradually decreasing with time. This was caused by the mixture showing a high phosphorescence<sup>12</sup> and if samples were exposed to daylight it required keeping for more than 12 hours in the dark before stable counts were obtained.

Apart from phosphorescence another interference is caused by chemiluminescence, an emission of light by chemical reaction. This latter mechanism is also enhanced by light.

The effect of chemiluminescence can be overcome by heating the sample + scintillation mixture in the vial for 45 minutes at 40°C before handling. This treatment, however, is not always practical.

Peroxides in particular are known to cause chemiluminescence and although peroxide-free dioxane is commercially available or can be prepared, peroxides will form again when left standing in the presence of atmospheric oxygen.

For more than one reason therefore toluene is to be preferred to dioxane. Toluene shows a higher counting efficiency and it does not form peroxides, its only disadvantage being its immiscibility with water. More recently this difficulty has been overcome by the use of suitable surface active agents which can disperse a certain amount of water in toluene in such a way that a transparent mixture is obtained. A new group of solubilisers has been marketed as Bio-Solvs.\* According to Pollay and Stevens<sup>13</sup> these Bio-Solvs are superior to any other presently available agents.

The scintillation mixture finally adopted was made up as follows:

PPO	0,8 g
POPOP	0,04 g
Bio-Solv BBS3	15,00 ml
Toluene to	100,00 ml†

To this mixture 10 ml of water was added. This quantity of water was experimentally found adequate to dissolve the 0,2 000 g sucrose and result in a clear solution.

For the counting procedure 0,2 000 g of sucrose was weighed accurately into a counting vial and subsequently dissolved in 10 ml of the above scintillation mixture.

A blank count was measured on 10 ml of the scintillation mixture and this background count was subtracted from the sample count.

#### 4.3.2 Counting vials

Two types of counting vials are available for counting samples: glass and polyethylene. Due to potassium salts in the glass the background of glass vials is slightly higher than that of polythene vials. For low level counting the latter are preferable. The statement of Lew<sup>10</sup> that plastic vials were unsuitable could not be confirmed. The polythene vials were found to give a lower count than glass and were uniform. Within the expected accuracy the counts per minute of the standard were not dependent on the vial.

\*BDH Scintillator  
†BDH Karl Fischer

††BDH AR  
\*\*Riedel-de Haan

\*Beckman Corp

†BDH Analar

Although the vials are disposable it was found that it required little labour to decontaminate them for re-use. The counting rate of a blank was checked after the decontamination of a batch of vials. For decontamination the vials were boiled eight times in a solution of "Contrad"\*. After the final boiling the vials were rinsed with distilled water.

#### 4.4 COUNTING ACCURACY

The amount of radio-active material in the pure sucrose recovered from the sample results in a count of about 200 000 counts per minute per 0,2 g. The standard sample shows about 400 000 counts per minute.

Counting a sample and a standard the results shown in Table I were obtained.

TABLE I

Standard counts/5 min	Sample counts/5 min
2 222 315	1 068 616
2 220 396	1 068 961
2 215 755	1 069 162
2 218 157	1 069 183
2 217 422	1 070 343
2 213 855	1 069 231
mean 2 217 982	1 069 249

The standard deviation is 3 064 in the standard and in the sample 582. The errors occurring in the background count can be neglected as this is a considerably lower value and in addition frequently determined in between sample counts.

The total standard deviation in the example above will be

$$\sigma_{\text{total}} = \sqrt{\sigma^2_{\text{standard}} + \sigma^2_{\text{sample}}} = 3 118$$

This error on a count per 5 minutes of 1 000 000 was considered to be sufficiently small for the purpose (0,3% relative).

By making the standard sample more radio active this error could have been reduced, but every determination would have been more expensive.

### 5. Results

#### 5.1 JUICE SAMPLES

A number of first expressed and mixed juice samples were analysed by the ID method and by pol determination. The results are shown in Table II.

#### 5.2 MOLASSES

Sucrose in final molasses was also determined by ID and the results were compared with those obtained by the Mackay chemical method.<sup>14</sup> In addition the sucrose content was determined in a number of samples by quantitative gas chromatography.\*

The results including two molasses samples supplied are listed in connection with investigations for ICUMSA in Table III.

### 6. Discussion

#### 6.1 ACCURACY OF THE METHOD

In 4.4 it has been shown that the errors in counting result in a relative error of 0,3% in the final value.

\*BDH

TABLE II  
Sucrose by ID and pol in juice samples

Sample	Pol	% Sucrose by ID
F.E.J.	18,43	18,97
F.E.J.	19,35	19,44
		19,48
F.E.J.	18,94	19,16
		19,20
M.J.	16,79	16,97
		16,98
M.J.	16,11	16,18
		16,26
Extract D.A.C.	8,59	8,66
		8,70
Extract D.A.C.	8,13	8,20
		8,23
M.J.	17,28	17,50
		17,54
F.E.J.	20,22	20,30
M.J.	17,47	17,95

\*Analyses were carried out by Hulett's R & D

TABLE III  
% Sucrose in molasses as determined by chemical analysis, Isotope dilution and gas chromatography

Molasses sample	% sucrose		G.L.C.†
	chemical	Isotope dilution	
JB	35,50	34,95	
		35,60	
SZ	35,72	35,29	34,3
		34,79	
		35,24	
TS	33,46	31,93	31,0
		32,21	
PG	34,54	33,75	33,3
		33,79	
GH	36,19	37,26	35,5
	35,94	36,99	
	36,23	36,99	
		36,75	
ICUMSA I (cane)	35,35	32,83	30,3
		32,94	
SZ	36,28	36,18	
EM	32,83	32,58	31,8
	33,06	32,04	
UK	31,46	29,12	
	31,09	28,31	
ICUMSA II (beet)	52,4 (Dutton)	51,30	
	53,47	57,07	

†Values are the mean of 5 determinations

Observation of the scatter in the results of Tables II and III indicate that other errors e.g. in weighing, pipetting, etc., increase this error.

If we consider the figures in Table III for GH we find a mean of 37,00 with a standard deviation of 0,21 for the four determinations.

If we apply  $\frac{\sigma}{V_n} \times t$  we find the error for the 95% confidence limit to be  $\pm 0,29$  or 0,8% relative.

#### 6.2 JUICE SAMPLES

From Table II it can be calculated that for juice samples the mean difference between the values

determined by ID and those by pol measurement is 0,25 % absolute. Calculation of the t value reveals that this difference cannot be caused by random errors in both determinations but is very significant.

Mahoney and Lucas<sup>15</sup> recently published results on the gas chromatographic determination of sucrose in syrups and liquors. The mean difference between sucrose by GLC in syrup and by single pol was found to be 0,50%, while this difference for double pol increased to 2,1%. This difference was checked in a number of cases by ID and it was confirmed that polarisation methods underestimate sucrose in syrups. The present results show the same trend for juice samples.

Most of the analyses in Table II were carried out using the dioxane-containing scintillation liquid. The scatter in the results would have been less if the cocktail described in 4.3.1 had been used.

## 6.2 MOLASSES SAMPLE

Contrary to the sucrose content in juices it was found that in molasses the conventional methods overestimate the true sucrose content.

The results in Table II show that with one exception the sucrose content by ID is lower than that found by chemical determination.

The average difference between the two methods was (GH sample excluded) 0,71, which amounts to a relative percentage of 2,15.

The average difference between the chemical method and the GLC determination was 2,24% or a relative percentage of 6,8%.

The only abnormal result in this series was a sample of molasses from GH, which gave a higher

sucrose value by the ID method. For this reason four independent ID determinations were carried out, but all gave a higher value than that obtained by the Mackay method.

The gas chromatographic determination, however, was lower, although the difference compared to the Mackay method (0,6%) was less than the average difference.

## 7. Acknowledgements

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