

# THE DETERMINATION OF NITROGEN FREE ORGANIC ACIDS IN NATAL CANE MOLASSES

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## Abstract

Organic acids in cane molasses from both milling and diffusion factories were quantitatively analysed.

No major differences in the acid composition of the molasses from the two types of factories were found.

## Introduction

With the advent of diffusion in South African sugar factories the first stage of the sugar manufacturing process has changed. Although extraction efficiency and overall yield in comparison with milling has increased in most cases, it is possible that more impurities are formed or extracted during diffusion and that they may differ from those produced or extracted during the milling operation.

Two parameters in the diffusion process are different from the milling process: pH and temperature. During the complete milling operation juice has its natural pH (5,2 or lower in the case of unripe or deteriorated cane) and extraction is carried out at ambient temperature (25-30°C).

The diffusion temperature is kept, in most factories, between 70° — 80°C, sometimes being as high as 85 — 90°C. The pH in diffusion batteries is higher than in a milling train. Some factories lime press water to a pH of 8,0 before returning it to the diffuser, others add lime at several points along the diffuser with the result that the juice pH is kept between 6,4 and 6,8 during the process.

Sucrose and reducing sugars at elevated temperature and pH decompose partly into organic acids. If organic acids are formed during diffusion they are concentrated in the molasses, mainly in the form of calcium salts.

Microbial activity is another possible source of formation of impurities during diffusion and milling. In sugar beet diffusers lactic acid is formed by thermophilic organisms and it has been established<sup>5 7 8</sup> that at temperatures between 70° and 75°C only *Bacillus stearothermophilus* can survive. In this case the amount of lactic acid formed is half the quantity of sucrose decomposed. During milling various mesophilic bacteria grow and the amount of lactic acid formed depends on the type and number of bacteria. However, the retention time of juice and bagasse in a milling train is much shorter than in a diffuser. The purpose of this investigation was to establish if a significant difference exists in the amounts and composition of organic acids in molasses from milling and diffusion factories.

*Analytical Methods for the Determination of Organic Acids*

Organic mono- and dicarboxylic acids have been analysed in cane juice, beet juice and cane molasses by only a few investigators.

Apart from analysis of a few isolated acids a complete survey only became possible with the advent of chromatographic techniques. One of the first complete analyses on cane juice was carried out by Roberts and Martin<sup>20 21</sup> in Louisiana. They separated the organic acids in dried cane juice, without any prepurification, on a silica gel column which was eluted with n-butanol-chloroform mixtures. Fractions were collected and titrated against 0,01 N sodium hydroxide. A similar technique was used by Bose and Datta<sup>3</sup> in India.

Trace quantities in sugar house products were determined by Riddle<sup>19</sup> using gas chromatography after concentrating the acids by steam distillation.

Another possible technique is paper chromatography.<sup>11 12 14 16 23 26 29</sup> Generally, however, paper chromatography is not an easy method for the quantitative measurement of acids. Although the elution from the paper of the components under investigation can be carried out quantitatively it is always a long and involved process.

Ion exchange chromatography has been applied by Stark and co-workers for the determination of organic acids in sugar beet<sup>24</sup>.

A more elegant method of ion exchange chromatography was carried out by Borodkin and Berger<sup>2</sup> for the analysis of organic acids in refinery products. They eluted the acids with a formic acid solution gradually increasing in concentration. The formic acid was removed from the collected fractions by azeotropic distillation with chloroform, followed by coulometric titration of the organic acids under investigation.

Thin layer chromatography was applied by the same authors for the separation of acids but they were unable to find a solvent system that would separate all the acids present in the refinery products.

Gas chromatography has also been used for the separation of acids. Kiely and O'Drisceoil determined volatile acids in beet molasses after extraction with methanol and lactic acid by extracting it in the same way followed by methylation of the acid<sup>13</sup>. The latter technique can be used generally by separating all the organic acids by ion exchange followed by methylation. Although this has not yet been applied to molasses samples, other plant extracts have been successfully analysed in this way. Gee<sup>10</sup> claimed that esterification with methanolic hydrochloric acid was to be preferred to esterification using diazo-methane, as the latter results in undesirable side reactions.

Instead of methyl esters, trimethyl silyl ethers have also been used as suitable derivatives for the G.L.C. analysis of carboxylic acids.<sup>9</sup>

#### *Decomposition of Sucrose and Reducing Sugars at Elevated Temperature and high pH:*

Many articles have been published concerning this. Amongst the final decomposition products of both reducing sugars and sucrose are various organic acids<sup>18 22 25 27 28</sup>. One of the predominant acids formed is lactic acid. Otake<sup>18</sup> claims that the amounts of acids formed are in the following descending order: formic, lactic, acetic, glycolic.

The formation is catalysed by calcium ions as the quantities of acids formed are found to be much higher when calcium hydroxide is used, instead of sodium or potassium hydroxide as the alkaline medium<sup>16</sup>.

It has been found that more acids are produced from sucrose than from glucose or fructose, and this is attributed by Montgomery to the furanoside structure of sucrose as opposed to the pyranoside structure of glucose and fructose<sup>17 30</sup>. The amount of lactic acid has been found to be 60–80% of the total amount of acids formed during the decomposition of sucrose.

### Experimental

#### *(a) Analysis of acids in molasses samples:*

For the analysis of molasses, monthly composite samples were taken. Molasses from the following diffusion factories were analysed: EM, EN, UC and ML. For comparison molasses from the following milling factories in the same area were analysed: FX, DK, JB and PG.

Four to six grams of molasses were weighed accurately and dissolved in approximately 100ml of water. To be able to relate the results to the amount of dry solids the refractometric Brix of the molasses sample was also determined.

The diluted molasses was passed through a column of Amberlite 400 IRA in carbonate form. The amount used was 30–40ml of resin with a bed height of 20cm. The column was subsequently washed with water and eluted with 100ml of ammonium carbonate solution (2N). The eluate was then passed through a cation exchanger in hydrogen form (Amberlite IR 120) with a bed volume of 60–80ml and a height of 20cm. Regeneration of this resin was carried out by hydrochloric acid (1,5 N). The resulting eluate of the second column was concentrated in vacuo at 50°C until a water-free residue was obtained.

Volatile acids are not quantitatively recovered by this treatment as they are partly lost during the concentration in vacuo. For two molasses samples these volatile acids were determined in a separate analysis by gas chromatography.

A silicagel column was packed using Malincrodt silicic acid, which was the only gel which gave good chromatographic separations. The gel was dried at 100°C for 24 hours and sieved through a 100 mesh. Fifteen grams were mixed with 10g of 0,5N sulphuric acid until a free-flowing powder resulted. A slurry was made with chloroform and the mixture was poured into a glass

column (18mm dia.) and compacted by washing with more chloroform until a height of 150–160mm was obtained.

The water-free residue, containing part of the volatile and all the non volatile carboxylic acids of the molasses sample, was mixed with 0,5ml of sulphuric acid (0,5 N) and sufficient dry silicagel was added to obtain a free-flowing powder. This was introduced on top of the silicagel column.

The column was subsequently eluted with 100ml chloroform, followed by 100ml of a mixture of chloroform and n-butanol (95:5) and further portions of 100ml increasing by 5% in n-butanol concentration. The last eluent mixture was chloroform : n-butanol (55:45). As the viscosity of the eluent increased towards the end of the elution air pressure was applied in the later stage (0,1–0,2 bar). All eluent mixtures were equilibrated against 0,5 N sulphuric acid before use, by shaking them with the acid and separating the two layers. Droplets of dilute acid were removed by passing the bottom layer through dry filter paper. The flow rate of the column was 50ml per hour. Fractions were collected (7ml each) and titrated against 0,01 N sodium hydroxide using phenol red as indicator. Carbon dioxide was excluded from the titration by passing a stream of carbon dioxide-free air through the titration vessel. Chromatograms were obtained by plotting the fraction number versus the amount of mg equivalents of acid. The total amount of each acid was determined by integrating the area under the peak.

The fraction numbers of peaks were compared with those obtained using known acid mixtures. The following standard acids were included: formic, acetic, propionic, butyric, fumaric, lactic, succinic, aconitic, glycolic, oxalic, malic and tartaric. A chromatogram of standard acids is shown in Fig. 1. The acids were further identified by qualitative paper chromatography. For volatile acids the method of Kennedy and Barker was used<sup>12</sup>, for non-volatile acids the method of Stark and co-workers<sup>23</sup>.

Further quantitative measurement of volatile acids in molasses was carried out by the method of Kiely and Drisceoil using gas chromatography<sup>13</sup>. This was applied to a sample of FX and EM molasses. The molasses (12,5g) was homogenised with water (10ml) and the pH adjusted to 4,5 with orthophosphoric acid. The mixture was heated under reflux for one hour at 60°C. After cooling to room temperature 70ml methanol was added, the solution was filtered and made up to 100ml with methanol. No methylation of the acids present takes place under these conditions. The prepared sample was subsequently injected into the gas chromatograph (0,5µl). The column used was 3mm stainless steel, 3,30m long packed with 15% diethylene glycol succinate polyester and 2% phosphoric acid on Chromosorb W (80–100 mesh). The column temperature was 95°C, the injection port 200°C. The carrier gas was nitrogen at 50ml per min., the gas chromatograph was a Perkin Elmer F11 with flame ionisation detector.

*(b) Preparation and Analysis of Acids formed by Alkaline Decomposition of Sucrose and Reducing Sugars*

An artificial cane juice, consisting of 15g sucrose, 0,5g glucose and 0,5g fructose per 100ml water was made alkaline with sodium hydroxide (pH = 11). The solution was heated for one hour at 80°C by which time the pH had dropped to 10,0. After cooling, the solution was passed through an anion exchanger, followed by a cation exchanger in the same way as described for the molasses samples. The acids were subsequently separated on a silicagel column. The experiment was carried out in triplicate.

**Results**

In Fig. 2, a chromatogram is shown of acids in molasses from a factory with a diffuser. Fig. 3 shows a chromatogram from a factory in the same area with a milling train.

The accuracy of the method was further investigated by adding a small amount of lithium lactate to the molasses of the latter factory and repeating the analysis. Fig. 4 shows the chromatogram which indicates satisfactory reproducibility and peak enhancement for lactic acid.

Fig. 5 shows the chromatogram obtained after the decomposition of sugars at high pH and elevated temperature.

Further results are listed in Table I.

**TABLE I**

Mol. sample	acid % refract. Brix					
	acetic	succinic + lactic	aconitic	glycolic + oxalic	malic	citric
FX	0,053	0,108	1,353	0,085	0,098	0,094
EM	0,155	0,038	1,121	0,044	0,095	0,098
JB	0,046	0,294	1,860	0,092	0,138	0,165
UC	0,074	0,131	1,954	0,093	0,269	0,319
PG	0,102	0,249	2,047	0,203	0,252	0,305
EN	—	0,146	1,312	0,123	0,122	0,145
DK	0,146	0,168	1,836	0,054	0,084	0,242
ML	0,144	0,317	1,049	0,052	0,080	0,141

The fractions 8-18 are volatile acids. (cf. Fig.1) Those present in EM and FX molasses were analysed by GLC as described before. Only acetic acid could be found in the samples. The peaks obtained in the chromatograms were integrated by cutting the section out of the paper and weighing. The amount of acetic acid was:

FX 0,39% refract. Brix

EM 0,37% refract. Brix

Comparing these results with those in Table I, it is evident that volatile acids were lost during the evaporation stage before the column chromatography.

In fractions 30-40 succinic and lactic acid were found. They are not separated by the chromatographic system used and emerge as one peak. Paper chromatography of the fraction shows that lactic acid was the major component. Both acids were chromatographically identical to standard

samples, using the paper chromatographic method of Stark<sup>24</sup>.

Between fractions 40 and 50 aconitic acid was found, which was identified by paper chromatography and by the violet colour which developed upon heating a few drops of the collected fraction with acetic anhydride<sup>4</sup>.

Between fractions 50 and 75 glycolic and oxalic acid emerged but quantities were too small for further identification.

Between fractions 75 and 85 malic acid was found and confirmed by paper chromatography in a molasses sample from ML.

The next acid was citric (fractions 90-100) which was confirmed by paper chromatography in the samples from DK and ML.

Acids having a higher fraction number than citric acid have not been identified.

In Table II the quantities of acid are listed which resulted from alkaline decomposition of a sucrose solution containing small quantities of reducing sugars.

**TABLE II**

Experiment No.	Acid % solids				
	Acetic	Fumaric	Lactic	Glycolic	Unknown
1	0,030	0,028	0,104	0,146	0,010
2	0,019	0,019	0,080	0,161	0,003
3	0,025	—	0,061	0,146	0,006

**Discussion**

Although G.L.C. analysis of acids is fast in itself, a time-consuming preparation of the sample precedes the analysis in the case of non volatile acids. Experimenting with acid mixtures of known composition further difficulties were encountered in methylating, according to Gee<sup>18</sup>, all the acids present quantitatively. Only a fraction of some acids could be recovered in the analysis and therefore the method was abandoned as being unreliable.

Paper chromatographic methods were also found to be unreliable for quantitative measurement so eventually separation on a silicagel column was adopted. The disadvantage of this method is the amount of time which is required for one analysis.

Although Roberts and Martin<sup>21 22</sup> apparently were able to analyse acids in cane juice using this method without any prepurification it was found that for the analysis of molasses samples pre-treatment by ion exchange was essential for good separation of the organic acids. The acids were isolated from the molasses sample by a modified method Lee and Resnik<sup>15</sup>, who analysed in this way the acids in tobacco leaves.

From Table II and Fig. 5 it is evident that the major decomposition products of sugars are lactic and glycolic acid. If sucrose and reducing sugar destruction takes place during cane diffusion it can be assumed that the decomposition products are carried through the process as calcium salts and will be finally discharged in the molasses. It

is also evident that they will be detected by the methods used for analysis.

If the molasses analyses of those factories operating diffusers are compared with those with milling trains neither the lactic nor the glycolic acid content is higher in the former than in the latter. (Fig. 2 and 3). If an important microbial activity occurs in a diffuser battery this should result in a higher lactic acid content.

In addition, no other organic acids in molasses from diffuser factories are present in significantly higher concentration than in molasses from factories with a milling train. (Table 1)

It can therefore be concluded that the organic acid content of molasses from diffuser factories does not indicate any large sucrose decomposition during diffusion.

In general the amount of organic acids in molasses is an insignificant part of the total amount of impurities, except for the quantities of aconitic acid present, and therefore cannot be responsible for higher molasses production. Apart from lactic acid, which is present in molasses both from factories operating a milling train and those with a diffuser, all other organic acids found are the natural plant acids present in cane juice.

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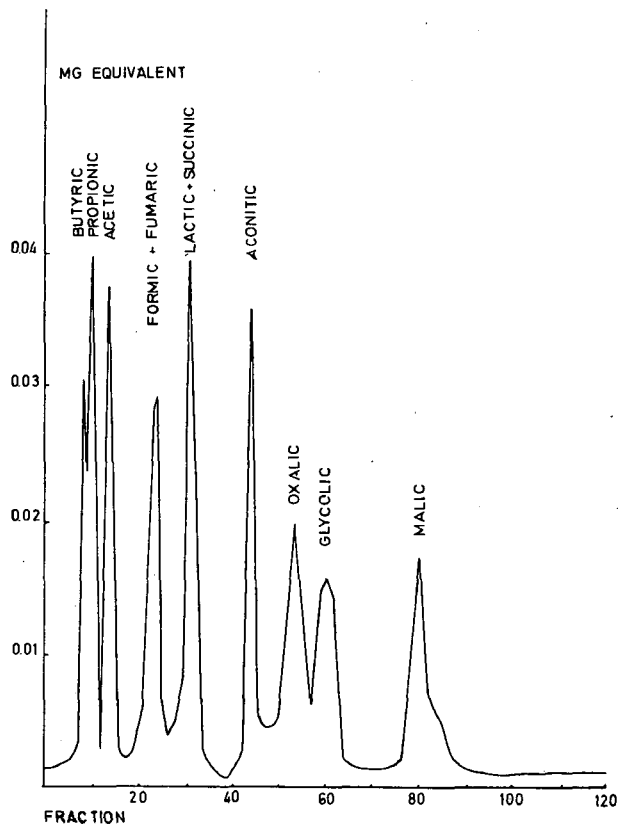


Fig. 1. Chromatogram of a synthetic mixture of carboxylic acids separated on a silicagel column.

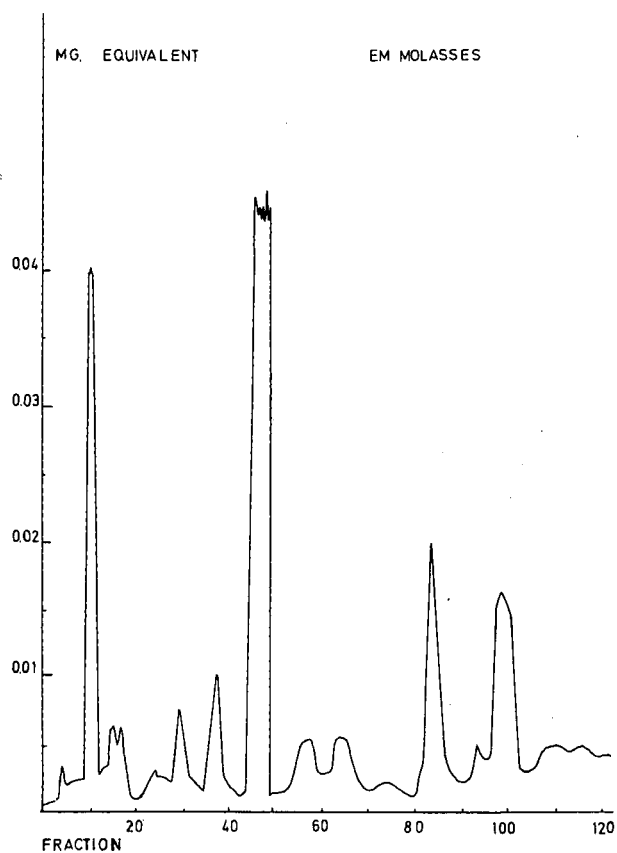


Fig. 2. Chromatogram of acids in EM molasses separated on a silicagel column.

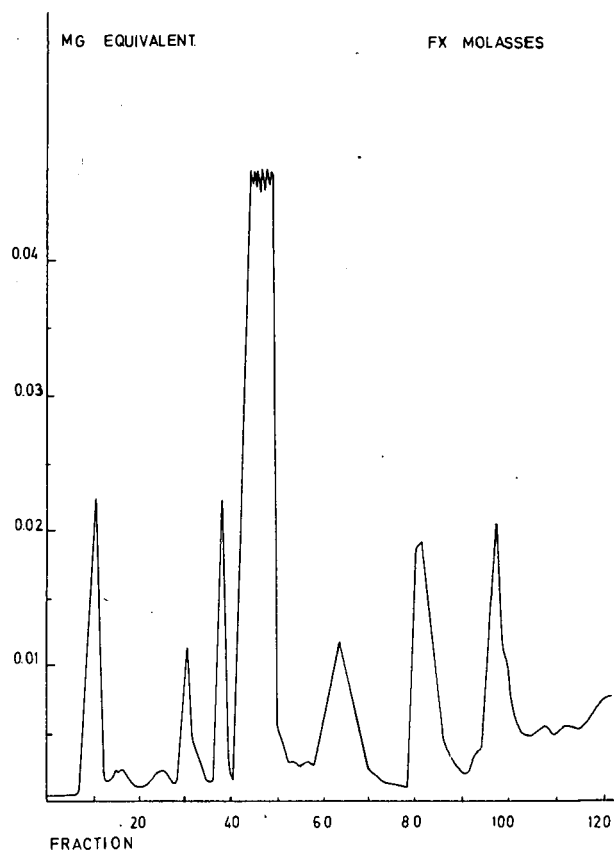


Fig. 3. Chromatogram of acids in FX molasses separated on a silicagel column.

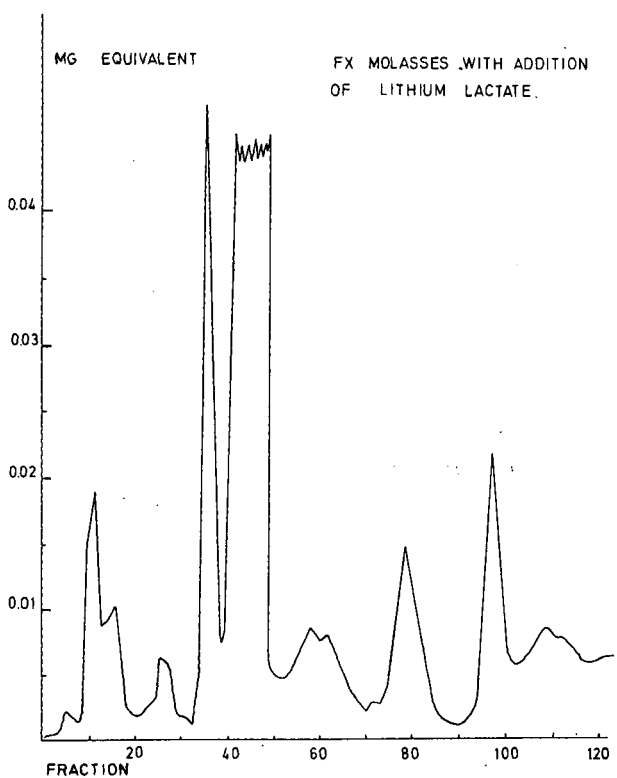


Fig. 4. Chromatogram of acids in FX molasses to which Lithium lactate was added.

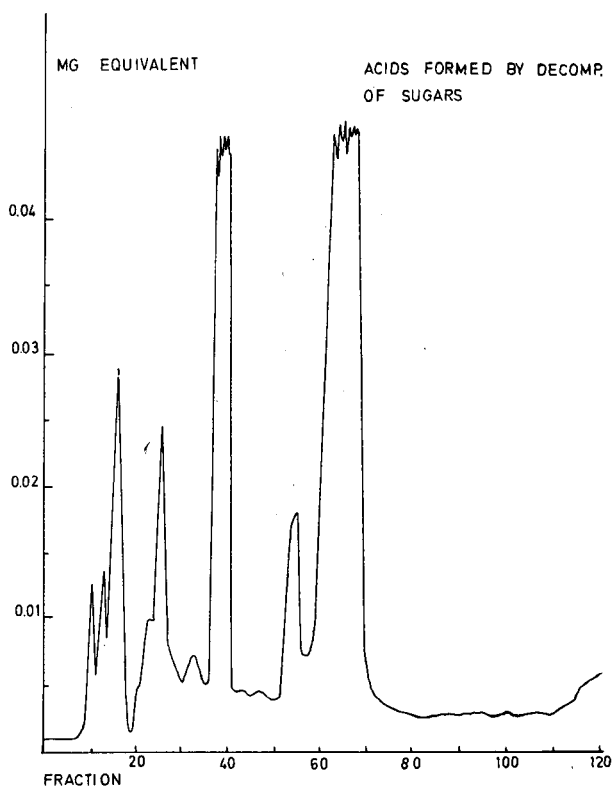


Fig. 5. Chromatogram of acids formed by alkaline decomposition of a mixture of sucrose and reducing sugars.