A COMPARISON OF THE MICROBIOLOGICAL ACTIVITY ASSOCIATED WITH MILLING AND CANE DIFFUSION

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Abstract

Laboratory tests comparing microbiological activity of juice samples from a milling tandem and a cane diffuser, both located at Maidstone factory, were carried out. Bacterial populations in the juices were enumerated and the contents of sugars and metabolic products monitored. Microbial activity was pronounced in the tandem juices at low temperatures, but minimal in the high temperature diffuser juice. The mesophilic organisms predominating in the tandem samples produced significant quantities of lactic acid, acetic acid and ethanol. A mass ratio of 8 parts sucrose lost to 1 part lactic acid formed was estimated for this low temperature environment. This is much higher than the previously determined ratio of 2 : 1 for thermophilic activity under high temperature conditions. A review of the lactic acid data from routine monitoring programmes indicated a progressive reduction in sugar losses in diffusers which can be attributed to controlling microbial activity by increasing diffuser temperatures to above 80°C. In general it was concluded that sucrose losses due to microbial activity in milling tandems exceed those in diffusers, under current operating conditions in South Africa.

Introduction

In South Africa diffusers are progressively replacing milling tandems for the extraction of juice from sugarcane. The temperature ranges for the two types of extraction units are quite different viz. milling tandems have temperature ranges from ambient to over 60°C at the last mill if hot imbibition is used, while diffusers have the temperature of both juice and cane in excess of 75°C from very soon after the cane enters the extraction unit. The typical temperature profile of each extraction plant has the effect of selecting a different range of micro-organisms. The majority of these micro-organisms consume sucrose as a primary energy source and are therefore responsible for sucrose loss. During the metabolic process many byproducts are formed - the types and proportion depending on the species of micro-organism. *Leuconostoc*, besides producing dextran, is also heterofermentative and products of fermentation are in an equimolar ratio of lactic acid, ethanol and Co_2.

Lactic acid levels have been monitored in juice at various stages of sugar manufacture in Tongaat-Hulett factories for some years. This information has been used to estimate the extent of sucrose losses occurring in process. Results of research done by McMaster and others[1, 2, 3, 4, 5] provided a convenient conversion factor of 2 parts sucrose lost for every part of lactic acid formed. This was, however, only relevant to diffusers and other high temperature systems.

The present study was undertaken to determine experimentally in the laboratory a sucrose loss factor for a milling tandem; and in addition to compare the microbiological activity between diffusers and milling tandems.

Experimental procedure

Determination of microbial counts

Fresh catch samples of crusher juice (Cr.J), mixed juice (MJ), juice from Mill No. 2 and Mill No. 5; draft juice (DJ) and juice from diffuser cells 3, 9 and 14 were collected in sterile glass bottles. The juice temperature was recorded immediately using a digital thermometer. Brix and pH were determined in the laboratory.

After serial ten-fold dilutions in 1% saline diluents, 0,1 cm^3 aliquots were spread plated onto Dextrose Tryptone agar (DTA) and incubated at 30°C, 55°C and 75°C. Other media used were Sabouraud Dextrose agar (SAB) and Sucrose Tryptone agar (STA), both incubated at 30°C for 48 hours. Colonies were then counted and respective titres calculated.

Laboratory juice deterioration experiments

In preliminary experiments, fresh mixed juice samples from the milling tandem and diffuser draft juice samples were screened, pasteurised and then inoculated with MJ and DJ microbial cultures respectively. The MJ was incubated in flasks at 34°C and DJ at 65°C in thermostatically controlled waterbaths. When samples had reached test temperature sub-samples were taken i.e. Time O, and then after 24 hours incubation.

In subsequent experiments fresh samples of MJ and DJ were collected and placed in conical flasks. These flasks were placed in thermostatically controlled waterbaths, MJ at 34°C and DJ at 65°C, where they were allowed to equilibrate to test temperature. Sub-samples denoting Time O were then taken. Further aliquots were taken after 3 and 6 hours. The sub-samples were placed in sachets labelled and frozen immediately.
The analyses performed on the sub-samples were:

- Total titratable acids, by titration with NaOH
- Ethanol, lactic acid and acetic acid by gas chromatography
- Reducing sugars, total sugars and sucrose by the Luff-Schoorl method
- Refractometer brix and pH.

Results and Discussion

Microbial levels

Some hyperthermophilic micro-organisms were isolated from juice from the diffuser. The average titre of hyperthermophiles in DJ was lower than that previously reported.[3] This difference may be attributable to the higher laboratory incubation temperature but the influence of the higher diffuser temperatures adopted in recent years should not be ignored. The results presented in Figure 1 indicate that, on exposure of the diffuser juice to lower temperatures during laboratory incubation, there was a significant increase in bacterial numbers due to the germination of spores. This emphasises the importance of maintaining high temperatures throughout the diffuser to limit the numbers of active micro-organisms. In contrast to the microbial populations of diffusers, the numbers and types of micro-organisms in the milling tandem are large and diverse.

It is well known that the population of *Leuconostoc* spp are implicated in the formation of slime in the mills.[6] The titre of yeasts and *Leuconostoc* spp was high, and showed variation along the milling tandem (Table 1). Figure 1 illustrates the effect of laboratory incubation temperature on the total microbial count, the increase of temperature from 30°C to 55°C resulting in a dramatic reduction in the total count. This suggests that use of hot imbibition water on milling tandems could help control microbiological activity.

TABLE 1

Microbial populations of mill juice

Titre per cm³ juice

Test / Sample	Juice Temperature °C	°Brix	Yeast count SAB at 30°C	*Leuconostoc* spp count STA at 30°C	Total count DTA at 30°C
Cr. J	26	18,3	6×10^{7}	1×10^{9}	2×10^{10}
MJ	29	14,2	5×10^{8}	2×10^{8}	3×10^{9}
Mill 2	34	7,7	3×10^{9}	5×10^{8}	7×10^{9}
Mill 5	35	2,0	5×10^{8}	7×10^{8}	4×10^{9}

Laboratory juice deterioration experiments

High temperature incubation: Early work on the use of lactic acid to estimate sucrose loss referred mainly to beet diffusers.[1, 4, 5] Research done by McMaster and Ravnö[3] established a similar relationship for sucrose loss in sugarcane diffusers, namely 2 parts sucrose lost for every part lactic acid formed.

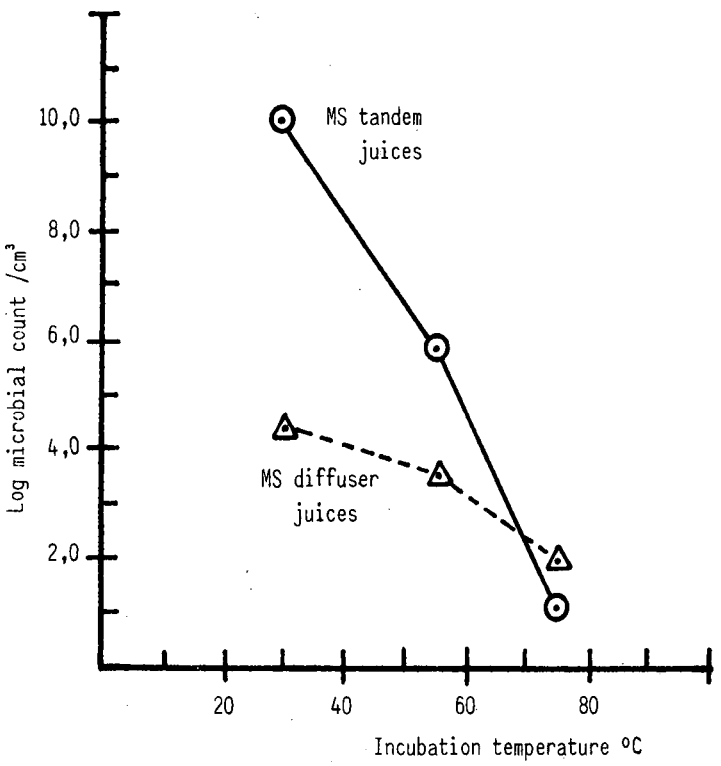

FIGURE 1 Average microbial levels in a diffuser and a mill

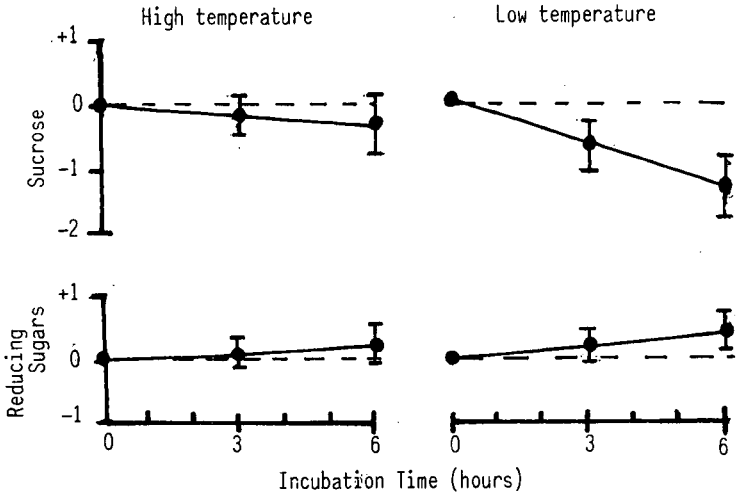

FIGURE 2 Change in sugars content. Units are % juice. Vertical lines show one standard deviation above and below mean.

TABLE 2

Parts sucrose lost for every part lactic acid formed (m/m)

Authors	System	Temperature °C	Range	Mean
Carruthers *et al*[1]	Beet	65°C	1,2 -3,1	2,0
McMaster & Ravnö[3]	Cane	65°C	0,8 -4,3	2,2
Norman & Rorabaugh[4]	Beet	60°-65°C	0,8 -4,4	2,1
Oldfield *et al*[5]	Beet	65°C	1,4 -4,4	2,3

The work of McMaster and Ravnö made use of an added microbial culture. In the present laboratory experiments attempts were made to evaluate the effect of only the natural microbial population on the sucrose/lactic acid ratio. In a series of 14 experiments it was evident that byproduct formation was minimal. This reduced microbiological activity was further confirmed by the low titre of hyperthermophiles in the diffuser juice.

Figure 2 clearly shows this trend. The loss of sucrose and formation of reducing sugars may be attributed to chemical and/or enzymatic inversion, as the change in total sugars was not statistically significant. Thus using only the natural microbial population it was not possible to determine a sucrose/lactic acid ratio.

Many thermophilic organisms are active at the incubation temperature of 65°C used in these tests. The question thus arises of why no significant activity occured. The reason can be found in the elevated temperatures (80°C or more) of the diffuser system from which the samples were drawn. While dormant thermophilic spores can survive under such conditions, the population of these in the incoming cane is low. Consequently the samples would require a relatively long period before sufficient bacteria had been produced to give measurable effects under the test conditions.

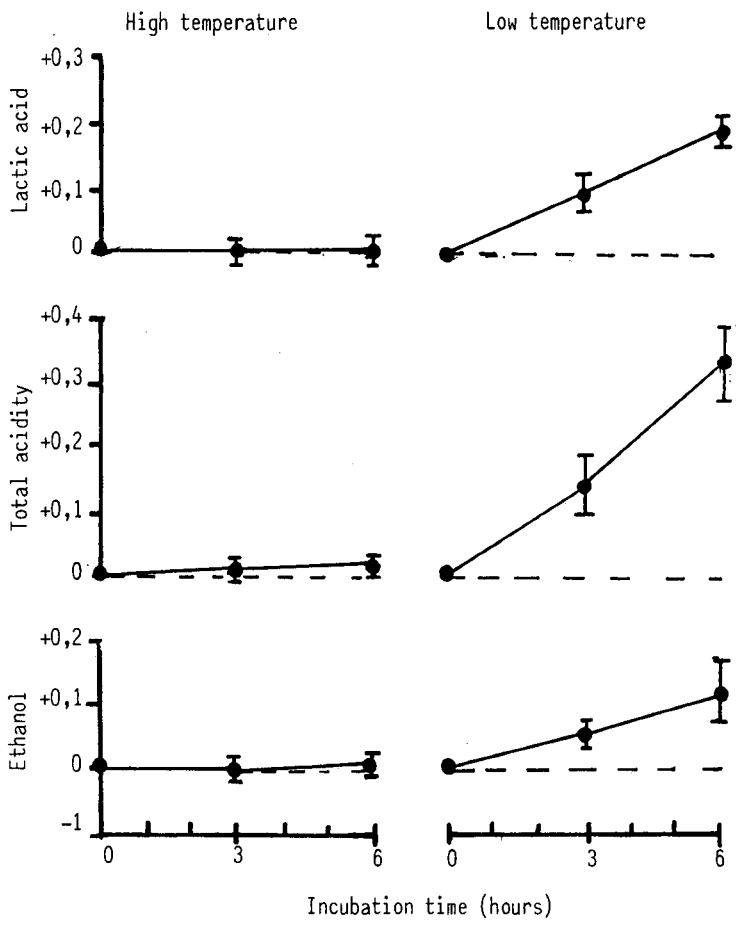

FIGURE 3 Change in metabolic products. Units are % juice. Vertical lines show one standard deviation above and below mean.

Low temperature incubation

Lactic acid has been used as a monitor of sucrose loss in extraction plants. However the ratio of 2 : 1 was established for high temperature systems and not for low temperature systems e.g. milling tandems.

In these experiments it was found that for low temperature systems, lactic acid constituted only 57% of the total acids produced in the system, as opposed to the 90% for diffusers.[3]

This was attributed to the large, diverse microbial populations of mill juice in contrast to the small specialised population of thermophiles present in diffuser juice.

Unlike the corresponding high temperature experiments, significant sugar loss and byproduct formation was observed over the incubation period (Figures 2 & 3). This data was used to calculate a sucrose/lactic acid ratio for the low temperature system (Table 3).

TABLE 3

Sucrose : lactic acid ratio at low temperatures

	Sucrose : lactic acid $^{m}/_{m}$
Mean	7,98
Standard deviation	4,65
Number of data sets	35

On average results from this evaluation gave a mass ratio of 8 parts sucrose for every 1 part lactic acid. Although this value was variable (see Table 3), it has nevertheless been used to provide rough estimates of sucrose loss in milling tandems. This value is much higher than that used for high temperature systems, and again highlights the greater potential and extent of microbial sucrose losses in low temperature milling tandems.

Lactic acid is the major metabolic product of microbial activity at high temperatures and can thus be used alone as the indicator of sucrose loss. The situation at low temperatures is more complex as metabolites such as carbon dioxide, ethanol, other acids and mannitol are also produced. The substantial increase in ethanol and total titratable acidity as well as lactic acid in the low temperature tests is shown in Figure 3.

The data on change in content of sugars and of metabolic products enabled approximate mass balances to be calculated. The average situation is reflected in Table 4. It is clear that other products must have been formed in quantity. Fermentation of sugars to ethanol by yeasts also yields a similar mass of carbon dioxide. In addition, a limited number of acetic acid analyses showed levels approximately equal to lactic acid; which in conjuction with the total acidity data further indicates that no acids other than lactic and acetic were formed in significant quantity.

TABLE 4

g metabolites formed per g sucrose lost

	No. of Samples	Mean	Standard deviation
Reducing sugars	31	0,34	0,18
Lactic acid	31	0,14	0,09
Ethanol	19	0,10	0,07
Unknown	19	0,42	0,08

Factory lactic acid data

Lactic acid analyses of weekly mixed juice composites are performed routinely by the R & D Department of Tongaat-Hulett Sugar Ltd., the trend in lactic acid levels for a diffuser and a milling tandem over the past 5 years is shown in Figure 4.

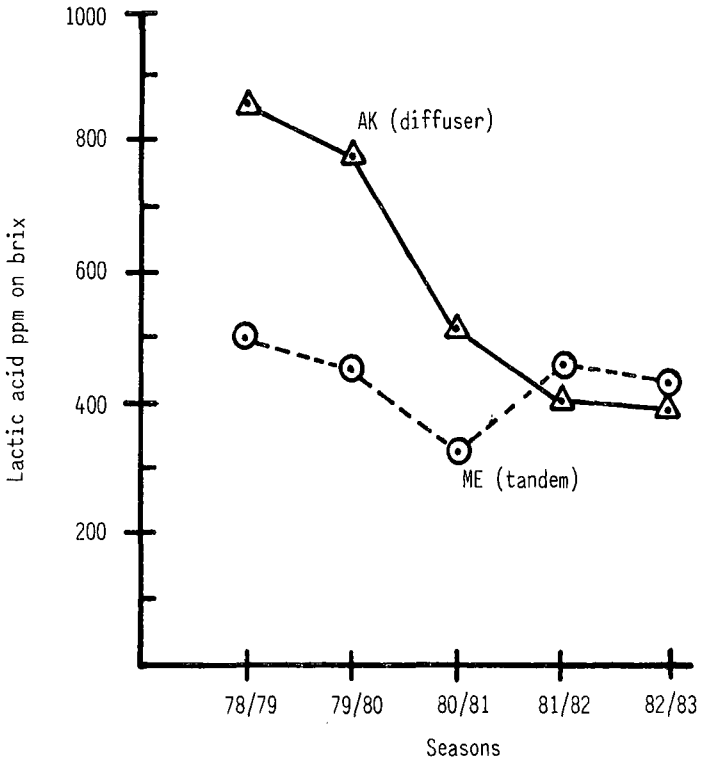

FIGURE 4 Mean annual lactic acid content of mixed juice

It is clear that prior to the 1981/82 season lactic acid content of the Amatikulu diffuser juice was significantly higher than that of the Mount Edgecombe tandem mixed juice. However the increase of diffuser operating temperatures in recent years was responsible for the progressive reduction of lactic acid content of diffuser juice.

TABLE 5

Mean lactic acid content of MJ 1982/83 season

Extraction Plant	Type	Lactic acid ppm on brix
Empangeni	Bagasse diffuser	270
Felixton 1	Tandem	380
Felixton 2	Tandem	570
Amatikulu	Cane diffuser	370
Darnall	Tandem	410
Maidstone 1	Cane diffuser	240
Maidstone 2	Tandem	600
Mount Edgecombe	Tandem	380

Average lactic acid content of juices from all extraction plants in the Tongaat-Hulett Group for the 1982/83 season is shown in Table 5. The results for the Maidstone tandem and Maidstone diffuser, which have the same cane source, highlight the pronounced difference in bacterial activity between high temperature and low temperature systems. In addition figures for all three diffusers are lower than those for any of the milling tandems.

Conclusions

The work reported showed that bacterial activity was minimal in juice samples withdrawn from high temperature diffuser systems and incubated at 65°C in the laboratory. Plate counts were also low, ranging from 10^2 per cm^3 at 75°C to 10^4 at 30°C.

In contrast, incubation of milling tandem juices at 34°C resulted in a significant consumption of sugars. A variety of metabolic products was formed and a ratio of 8 parts sucrose lost to 1 part lactic acid produced was estimated. Plate counts at 30°C revealed large populations (10^{10} per cm^3) of mesophiles.

These findings relate to current diffuser operating temperatures of 80°C or more. The increase in temperature over the last 10 years has been accompanied by a progressive reduction in lactic acid content as routinely monitored in diffusion juices, to the extent that average levels are now below those for milling tandem juices. Taking into account the higher sucrose/lactic acid ratios for tandems, it is evident that microbial sucrose losses are now significantly lower in diffusers than tandems. While this is a welcome change from previous incidences of excessive microbial activity in diffusers,[3] it suggests that more work on the tandem situation is desirable. These should preferably take the form of factory investigations and include the effect of variables such as imbibition water temperature.

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