Current Research Journal of Biological Sciences 1 (1): 11-13, 2009
ISSN: 2041-0778
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Application of Thiocarbamate Based Chemical for Minimization of Invertase Enzyme Activity in Sugar Cane Juice

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Abstract: The commercially grown Indian cane variety has sucrose content around 12-14% at peak maturity stage. Due to pre and post milling losses average sugar recovery remain in the range of 9.5-10.0%. This is a serious situation, which affect the overall performance and profitability of sugar industry. Therefore, regular use of an effective broad-spectrum chemical is important in improving sugar recovery at mill level. In the view of above facts, the investigation has been done with application of thiocarbamate based chemicals. The comparative activity of thiocarbamate-based chemicals was tested against invertase enzyme and sucrose contain in sugar cane juice. Two different commercial chemicals (1) SUGARBAG PLUS™ and (2) KILLBACT ™ were applied @ 5 ppm, @10 ppm and @ 15 ppm with control. SUGARBA G PLUS™ @ 10 ppm and KILLBACT ™ @ 15 ppm. Dextran, sucrose and reducing sugar were analyzed periodically with thiocarbamate chemicals treatments, and data were analyzed with analysis of variance. SUGARBA G PLUS™ @ 15 ppm was found best treatment because lowest dextran content was observed in this study.

Key words: Dextran, reducing sugar, *Leuconostoc mesenteroids*, sucrose, thiocarbamate, invertase enzyme

INTRODUCTION

Sucrose is a primary sweetening agent, which is commercially derived from sugarcane Puttabasavaish, (1976). Sugarcane plant has capacity to loose stored sucrose with in a few days after harvest. The deterioration of harvested cane is primarily a biochemical process followed by bacterial inversion through the cut ends or damaged sites of stalk. Post harvest sucrose loses due to biochemical or microbiological inversions are a major problem in sugarcane growing countries. Batta and singh (1986) Microorganism found in interior of cane stalk immediately after cutting. The deterioration due to this microorganism is known as bio-deterioration and caused by mainly *Leuconostoc* sp. These organism forms nodular colonies after multiplication under favorable condition by converting sucrose in to polysaccharide such as dextran..Cerutti *et al.* (2003) Dextran causes numerous problems in sugar refinery due to its viscous nature and remains in sugar crystal, which is morphologically abnormal, and effect sugar recovery and processing operation. Biological losses in sugarcane could be minimizing by application of chemical. The use of various chemical and there efficacy has been revised. Clarks (1997) Thiocarbamate based chemicals are new class of organo-sulphur chemical that has been approved by FDA official of USA for food industry sanitation. The thiocarbamates are active against microorganisms as well as invertase enzyme. WHO (1980) further these chemicals are relatively less toxic to higher form of life and more so, they are inactivated or degraded to non-toxic end products when heated to more than 80 °C. this is main reason for not adding such chemicals in maceration/imbibitions water (Solomon, 2000).

MATERIALS AND METHODS

All experiments were done at experimental Research farm of Allahabad Agricultural Institute – Deemed University, Allahabad. Sugar cane variety CoS 95255 (high sugar and early maturing) was evaluated for their quality characteristics. Sample was crushed in 2-roller vertical crusher and juice was collected in the sterile sample bottles. This juice was passed through four folds of muslin cloth to remove bagasse/soil particles. The study was carried out on harvested cane with two Thiocarbamate based chemicals SUGARBAG PLUST™ and KILLBACT™ sprayed over harvested cane and mixed in primary juice @ 5ppm, @ 10ppm and @ 15ppm. Experiments were repeated with 3, 6 and 9 days interval from the time of harvesting. Three replicates were used in each experiments which was donated as R_1, R_2 and R_3. Juice was periodically analyzed for Dextran by Rapid Haze method Madan and shahi (1998) and Sucrose % in Juice- by polarimeter Gurtu (2000) for the microbiological analysis. Sugarcane juice was taken from Cane grown in research farm of biochemistry for this study. Already identified and isolated colony of *Leuconostoc mesenteroides* used for microbiological analysis. SUGA RBA G PLUS™ and KILLBACT ™ was applied @ 5 ppm, @10 ppm and @15 ppm with control.

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Antimicrobial susceptibility testing-Sterile MRS media was pored into sterile petri dishes, which have been inoculated with test organism. Well (10 mm diameter) were made with the help of flamed cork borer on the surface of the MRS plates and different concentration of thiocarbamate chemical were added in different wells. These were incubated at 37 °C for 24 h. The presence of zone of inhibition was regarded as the presence of antimicrobial action (Aneza, 2003).

RESULTS AND DISCUSSION

The results in Table 1 showed that both chemicals have inhibitory effects on growth of dextran producing bacteria *Leuconostoc mesenteroides,* with formation of zone of inhibition. Among the bacteria tested SUGARBAG PLUS™ @ 15 ppm posses greatest inhibitory effect on the growth of *Leuconostoc mesenteroides* followed by SUGARBAG PLUS™ @10 ppm and KILLBACT ™@ 15ppm. Result is supported by finding of Samaraweera who also evaluated the effectiveness of several chemicals in sugar factories Samaraweera (2002). Nicolos reported that dithicarbamate chemicals prevent microbial activity in cane juice Nicolas (2002). The results in Table 2 indicated that the data of biochemical parameters at the time of harvesting. Results indicated in Table 3 that chemicals gave significant impact on dextran formation process. Dextran value at harvesting time was zero. Dextran formation rate is increased with the increment of time period. Different chemicals at different doses also influenced the dextran formation rate. It indicated from data that dextran values was less in chemicals treated juice as compare to control (T_0) the more reduction observed in SUGARBAG PLUS™@15ppm followed by SUGARBAG PLUS™@ 10 ppm and KILLBACT ™@ 15ppm.Therefore, it concluded that there was significant difference between the two chemicals and SUGARBAG PLUS™@ 15ppm was best dose because its mean value was lowest and differs from others. It is quite obvious from the table that dextran content increase in storage condition, this is because the dextran is polymer, which formed due to action of dextransucrase enzyme secreted by *Leuconostoc mesenteroides* bacteria. Thiocarbamate chemicals have capacity to minimize the microbial activity of *Leuconostoc mesenteroides* to a great extant and it reduces the dextran in juice. Bishwas evaluated the control of microbial infection resultant minimization in dextran formation by application of thiocarbamates chemicals (Bishwas, 2003). Data clearly indicated in Table 4 that, highest sucrose content was decreased in control; juice that was treated with thiocarbamate chemicals shows less decrement in sucrose%. It clearly indicated that chemical SUGARBAG PLUS™@ 15ppm gives highest control condition for sucrose deterioration. Thiocarbamate chemicals reduce the sucrose deterioration, it was because of thiocarbamate chemicals reduced the invertase enzyme activity as well as it inhibit the growth of *Leuconostoc mesenteroides* in sugarcane juice. Experiments have conclusively demonstrated that application of thiocarbamate-based chemicals could control the biological losses in harvested cane. Application of SUGARBAG PLUS™ @ 15 ppm suppresses the dextran formation as well as reducing sugar, in cane juice and help in retention of recoverable sugar. KILLBACT™ chemical was less effective at 15 ppm. Thiocarbamate chemical could be useful in sugar mills to minimize sugar losses in juice.

Table 1: Effect of thiocarebamet based chemicals on growth of *Leuconostoc mesenteroides*

Concentrations	Zone of inhibition (cm)
Sugar Bag Plus™ @ 5 ppm	0.3
Sugar Bag Plus™ @ 10 ppm	0.6
Sugar Bag Plus™ @ 15 ppm	0.9
Kill bact™ @ 5 ppm	0.3
Kill bact™ @ 10 ppm	0.5
Kill bact™ @ 15 ppm	0.7

Table 2: Biochemical parameters of Sugarcane juice at the time of harvesting

Biochemical Parameters	Mean
Sucrose %	14.00
Dextran (mg/100 brix)	00.00

Table 3: Days-wise mean performance of thiocarbamate based chemicals on dextran content (mg/100ml)

Treatments/Days	3 Days	6 Days	9 Days
Control For Sugarbag Plus™	301.66	556.66	844.33
Sugarbag Plus™ @ 5ppm	276.66	506.67	718.33
Sugarbag Plus™ @ 10ppm	213.00	377.67	574.33
Sugarbag Plus™ @ 15 ppm	212.33	384.00	536.67
Control for Killbact ™	301.66	556.86	844.83
Killbact™ @ 5ppm	284.00	505.00	720.00
Killbact™ @ 10ppm	277.66	484.00	689.33
Killbact™ @ 15ppm	226.00	397.67	595.00
CD (Chemical)	12.98235	25.13755	26.64127
Result (Chemical)	S	S	S
CD (Doses)	18.35982	35.54986	37.67644
Result (Doses)	S	S	S

Table 4: The days-wise mean performance of thiocarbamate chemicals on sucrose %

Treatments/Days	3days	6 days	9 days
Control For Sugarbag Plus™	12.10	10.10	8.93
Sugarbag Plus™ @ 5ppm	12.23	11.10	9.17
Sugarbag Plus™ @ 10ppm	12.87	11.77	9.67
Sugarbag Plus™ @ 15 ppm	13.53	12.17	10.53
Control for Killbact ™	12.03	10.13	9.00
Killbact™ @ 5ppm	11.93	10.77	8.93
Killbact™ @ 10ppm	12.17	11.23	9.33
Killbact™ @ 15ppm	12.77	11.87	9.73
CD (Chemical)	0.142082	0.190193	0.191527
Result (Chemical)	S	S	S
CD (Doses)	0.200934	0.268973	0.27086
Result (Doses)	S	S	S

ACKNOWLEDGEMENTS

The authors are grateful to Director of Indian institute of Sugarcane Research for providing facilities. Authors are also gratefulacknowledge the department of biochemistry, Allahabad Agricultural institute Deemed University for helping and providing laboratory facilities.

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