JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Use of Glutaraldehyde and Benzalkonium Chloride for Minimizing Post-Harvest Physio-Chemical and Microbial Changes Responsible for Sucrose Losses in Sugar Cane

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Sugar cane is sensitive to enormous sucrose losses induced by physio-chemical and microbial changes, the severity being increased during the time lag between harvest and crushing in the mills. Minimization of the sucrose losses in the field is essential for better sugar recovery and prevention of sucrose losses. An experiment was conducted to evaluate the efficacy of glutaraldehyde and benzalkonium chloride for their effects on the microbial counts and physio-chemical changes responsible for sucrose losses. Glutaraldehyde and benzalkonium chloride (1000 + 250 ppm) reduced the losses in sucrose content to 7.1% as compared to the 30.8% loss in the control, thus improving the performance by 76.9%. The application of chemicals reduced the acid invertase activity (by 60%), lowered weight loss, titrable acidity, reducing sugars content, dextran, ethanol, and ethylene production and respiration rates. The application led to the reduction in the total bacterial, fungal, *Leuconostoc*, and yeast counts by 67.92, 51.3%, 26.08, and 51.2%, respectively.

KEYWORDS: Sugar cane; glutaraldehyde; benzalkonium chloride; microbial counts; acid invertase activity; juice quality

INTRODUCTION

Sugar cane is the major source of sugar production worldwide with an area of above 4.2 million hectares in India, having average productivity of 70 tonnes ha^{-1}. The millable canes of sugar cane are harvested after 10−12 months of its growth and are then crushed in the mills for sugar crystallization (*1*). In between the harvest of the cane and its crushing in the mills, because of its high sugar and moisture contents, it is highly prone to post-harvest bacterial invasion (*2–8*). The sucrose losses further gets aggravated because of the usual delay in the harvest to milling stage. The sugar recovery thus hovers between 8.5−9.5% cane during the peak crushing period, while it is expected to be as high as 14−15%. A delay of just 3 days in crushing the harvested cane results in a loss of nearly 1.0−3.0 units pol cane during normal crushing season and also results in a perceptible reduction in the performance of the factory and the sugar quality (*8–12*). These losses further escalate depending upon the duration of storage. Thus, the sucrose losses in the sugar cane field continues to be one of the avenues adversely affecting sugar recovery to as low as 8 to 9.5% in subtropical India as compared to 13−14% when the fresh cane is milled (*3, 8*).

The post-harvest deterioration in cane is essentially the results of the action of the bacteria *Leuconostoc*, *Lactobacillus saccharomyces*, *Rodotorula* genera and so forth, which lead to the inversion of sucrose and a large production of dextran, acids, and ethanol affecting the kinetics of sucrose crystallization (*2, 8*). Several strategies involving the use of chemicals/biocides have been tried in the recent past to minimize bacterial inversion and have been successful too in preventing sugar losses (*5, 6, 13–15*). But applications of these chemicals have been limited just to the mills. Frequent spraying of solution containing potassium permanganate (0.1% or 5 ppm) and dimethyl dicarbonate (DMDC) along with sodium meta silicate (SMS, 1%) on harvested cane had been reported to be effective in minimizing invertase activity and retaining the juice quality in the mills (*16–19*). However, even with all the precautions taken in terms of hygiene and sanitation in the mills, it has been found that the time lag between harvesting and crushing in mills is still the major bottleneck miserably affecting sugar recovery.

The present experiment was planned with an objective of evaluating the extent of physio-chemical and microbial changes inducing sucrose losses in sugar cane and the effects of cost-effective and easily available disinfectants glutaraldehyde and benzalkonium chloride (BKC) on the minimization of the physio-chemical and microbial changes for minimizing post-harvest sucrose losses. Gluteraldehyde is known to be found in

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10.1021/jf801394j CCC: $40.75
Published on Web 07/29/2008

usage as a disinfectant and sterilent of endoscopes and surgical equipment, and as a fixative in electron microscopy (*20, 21*). It has a broad-spectrum activity against bacteria, fungi, and viruses (*22, 23*). BKC is a well-known quaternary ammonium, powerful surfactant and disinfectant. It is also a hard surface cleaning and deodoring agent possessing strong bactericidal and fungicidal activities (*24, 25*). The chemicals were used keeping in mind the safety it offers in sugar cane, where the final product, that is, sucrose, will not be affected as it is obtained in the crystalline form. The main objectives of the study were (i) to assess the post-harvest physio-chemical and microbial changes affecting sucrose losses in post-harvest sugar cane; (ii) to study the interaction among the different physio-chemical and microbial parameters affecting post-harvest sugar cane juice quality; and (iii) to study the effects of glutaraldehyde and BKC on the minimization of post-harvest physio-chemical and microbial changes in post-harvest sugar cane.

MATERIALS AND METHODS

Site and Climate. The experiment was conducted at the Indian Institute of Sugar cane Research, Lucknow, India, located at 26° 56′ N, 80° 52′E and 111 m above sea level, which falls in agro-ecoregion 4 (Northern plain and Central highlands, hot semiarid ecoregion with the Alluvial-derived (N8D2) soils of India (*26*)).

Experiment Setup. Twelve month mature cane of field grown sugar cane variety Co Se 92423 were harvested manually at the bottom of the cane with Gadasa (a specialized device for harvesting sugar cane) in February, 2005−2006. Canes were immediately shifted to a clean concrete floor under an asbestos-roofed shed, where the stalks were detrashed and bundled having 20 canes of approximately equal height. There were 4 treatments: T_1, control; T_2, H_2O spray; T_3, glutaraldehyde (1000 ppm, w/v) solution spray; and T_4, glutaraldehyde + benzalkonium chloride (1000 + 250 ppm, respectively, w/v) solution spray. The commercial formulations of glutaraldehyde and benzalkonium chloride were purchased from the local market. Bundles were sprayed using a mist sprayer and 1 L of each solution was used for treating a single bundle. Each treatment was replicated thrice, and all cane bundles after spraying were covered with sugar cane dry leaves called trash. The 12 cane bundles (4 treatments × 3 replications) were completely randomized and kept in a clean shed for 10 days. Two sets of experiments were maintained, and the data set presented is the mean of two experiments.

Sampling, Juice Extraction, and Analysis. At intervals of 2 days (0, 2, 4, 6, 8, and 10 days), 3 canes were sampled from each bundle and were crushed in a clean laboratory roller crusher to extract juice. Before crushing, the roller crusher was surface cleaned with 0.01% $HgCl_2$ solution and subsequently washed 3 times with hot sterile water. Juice was then filtered through a four-layer muslin cloth, collected in sterilized glass bottles (500 mL), and was processed for physical, chemical, and microbiological analysis on the same day.

Loss in Weight, Color, pH, and Titrable Acidity. Cumulative weight losses in sugar cane were recoded at different sampling dates and were expressed as percentage loss of the original weight ($n = 3$). The filtered juice was used for determining the color, pH, and titrable acidity. The pH of the juice was recorded in the pH meter (Systronics pH system 362, India), and then the titrable acidity was determined using the potentiometric titration using 0.1 N NaOH up to pH 8.1, using 1 mL of the diluted juice in 25 mL of distilled water. The results are the means ± SE expressed as g of citric acid equivalent per 100 g Fwt. The color of juice was recorded by measuring the optical density at 720 nm in a PC based double beam spectrophotometer (Systronics, India).

Ethylene Production and Respiration Rate. Ethylene production was measured using a Hewlett-Packard model 5890A GC-FID (Wilmington DE), while the respiration rate was quantified using a Shimadzu 14A GC-TDC (*27*). Results are the mean ± individual determination for each cane and are expressed as μmol $kg^{-1}\cdot h^{-1}$ for ethylene and $mgkg^{-1}$ $Fwth^{-1}$ for CO_2 production

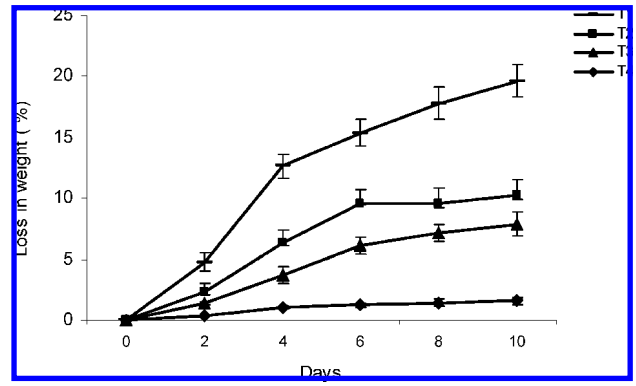

Figure 1. Percentage of weight loss in sugar cane during post-harvest storage at different days. Results are the mean. Vertical bars indicate ± SE. T1—: Control (untreated). T2■: H_2O spray. T3▲: (glutaraldehyde, 1000 ppm). T4◆: (glutaraldehyde + benzalkonium chloride, 1000 + 250 ppm).

Estimations of Sucrose, Reducing Sugars, and Phenols. Sucrose content was estimated by the resorcinol-thiourea method as described by Roe and Papadopoulos (*28*). An aliquot of 0.01 mL of 10% diluted juice was taken in a test tube. Distilled water (1.99 mL) was added to make the final volume of 2 mL, to which 2.0 mL of resorcinol thiourea was added. After mixing properly, 6 mL of conc. HCl was added, and the tube was shaken carefully. The tubes were then transferred to a water bath maintained at 80 °C for 20 min and then cooled under running water. The absorbance was measured within 30 min at 490 nm against a reagent blank. The results are expressed as mg mL^{-1} juice.

The reducing sugars were estimated by the method of Nelson (*29*). An aliquot of 0.5 mL of 10% diluted juice was taken in sugar tubes. Distilled water (1.5 mL) was added to make the final volume of 2 mL, to which 2 mL of copper reagent was mixed and kept in a boiling water bath for 20 min. After cooling, 2 mL arsenomolybdate reagent was added, the volume was made up to 25 mL with distilled water, and the absorbance was measured at 540 nm. The results are expressed as mg mL^{-1}. For phenol estimation (*30*), 0.5 mL of cane juice was mixed with 0.5 mL of Folin−Ciocalteu reagent (CDH, New Delhi) and 1.0 mL of supersaturated carbonate solution, and the volume of the reaction mixture was made up to 10 mL with distilled water. After 1 h, the color intensity was measured at 725 nm in a spectrophotometer (Systronics −2202, Ahmedabad, India), and the results are expressed as mg mL^{-1}.

Estimation of Soluble Protein and Acid Invertase Activity. Protein was precipitated with 10% (w/v) chilled trichloroacetic acid (TCA), and the clear residue was dissolved in 1.0 mL of 0.1 N NaOH at 80 °C for 10 min. Protein was estimated in 0.5 mL of aliquot, according to Lowry et al. (*31*). The intensity of the blue color was measured at 660 nm using bovine serum as the calibration standard.

The activity of the acid invertase was determined in the juice through extraction with 1.0 mL of citrate buffer (pH 5.4) by the method of Hatch and Glasziou (*32*) with some modifications. The reaction was initiated by adding 1 mL of 0.2 M sucrose to a reaction mixture containing 1.5 mL of 0.1 M-citrate buffer (pH 5.4) and 1 mL of juice. The reaction was set at 37 °C for 1 h. The reaction was stopped by heating the tubes in boiling water bath for 5 min. Tubes were centrifuged at 8000*g* for 15 min. The amount of invert sugars was estimated in a 0.5 mL aliquot of the supernatant according to Nelson (*29*). The acid invertase activity was expressed as mmol invert sugars/mg protein/min.

Estimations of Dextran and Ethanol. It was estimated by modified reported by Gupta and Nigam (*33*). To 10 mL of sugar cane juice, 1 mL of trichloroacetic acid (10%), 5 mL of methanol, and 0.5 mL of conc. hydrochloric acid was added. The contents were centrifuged at 8000*g* for 20 min. The supernatant obtained after centrifugation was mixed with 2 mL of absolute ethanol. The ethanol haze formed was measured colorimetrically at 720 nm, and the results were expressed as mg L^{-1}.

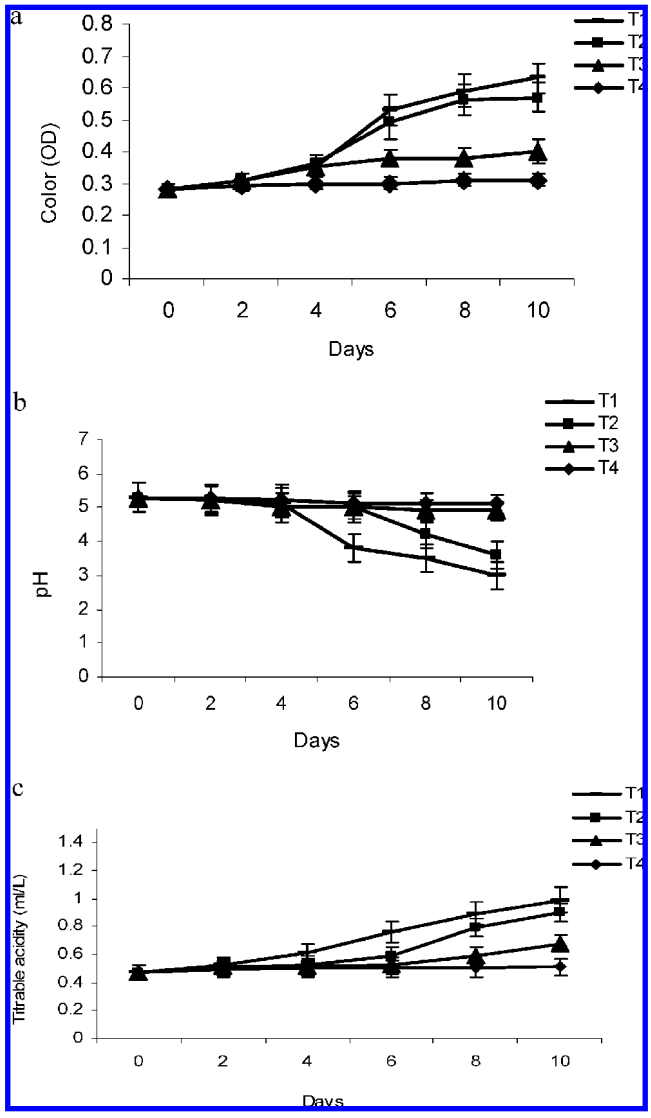

Figure 2. Changes in (**a**) color, pH (**b**), and titrable acidity (**c**) in post-harvest sugar cane under storage at different days. Results are the mean of triplicate samples, and vertical bars represent ± S.E, T1—: control (untreated). T2■: H_2O spray. T3▲: (glutaraldehyde, 1000 ppm). T4◆: (glutaraldehyde + benzalkonium chloride, 1000 + 250 ppm).

For ethanol estimation (*34*), an aliquot of 1 mL sugar cane juice was diluted with distilled water and added into 25 mL of potassium dichromate solution (34 g of potassium dichromate was dissolved in 325 mL of conc. sulfuric acid, and the volume was made up to 1000 mL with distill water) by distillation. After distillation, the flasks were incubated at 60 °C for 20 min in a water bath. The contents were cooled, and the final volume was made up to 50 mL with distilled water. The contents were mixed, and absorbance was measured at 660 nm. The results are expressed as mL L^{-1}.

Microbial Population in Juice. Cane juice was serially diluted using saline water blanks, and different dilutions were plated for total bacterial on NA and TYM medium, fungi on chapadox medium, yeast on MRS medium, and *Leuconostoc* on leuconostoc agar medium plates. Each dilution was plated in triplicates and were incubated at 30 °C for 7−10 days for counting the microbial populations. Colonies of yeast and *Leuconostoc* were ensured after microscopy and tests as per Bergey's Manual of Systematic Bacteriology (*35*). The counts were presented as cell forming units mL^{-1} of juice.

Statistical Analysis. The experiment was conducted in a completely randomized design (CRD) with three replications. Analysis of variance (ANOVA) and linear regression for a completely randomized design were used, and Duncan's multiple range tests as a post hoc analysis was used to compare the means (*36*). The term significant has been used to indicate the differences for which $P \leq 0.05$. Correlation coefficient and regression equations were calculated using MS Excel statistical tools to assess the interrelationships between the different parameters measured.

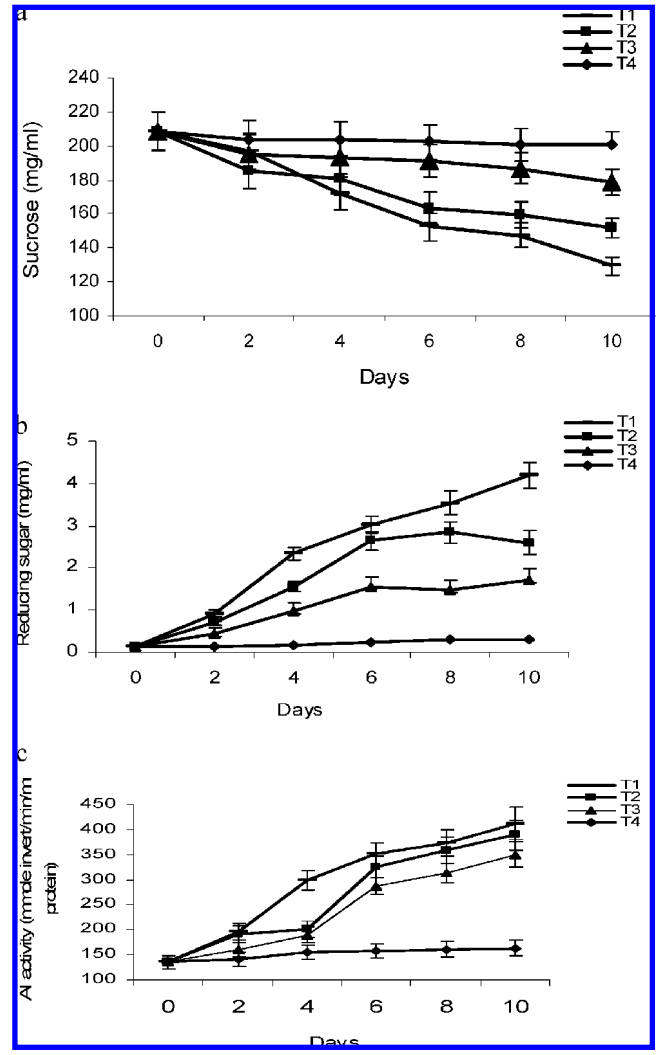

Figure 3. Changes in (**a**) sucrose content (**b**) reducing sugars, and (**c**) acid invertase activities in post-harvest sugar cane under storage at different days. Results are the mean of triplicate samples, and vertical bars represent ± S.E. T1—: control (untreated). T2■: H_2O spray, T3▲: (glutaraldehyde, 1000 ppm). T4◆: (glutaraldehyde + benzalkonium chloride, 1000 + 250 ppm).

RESULTS AND DISCUSSION

Loss in Weight, pH, Color, and Titrable Acidity. At harvest, sugar cane juice was light greenish in color ($OD_{720\ nm}$, 0.28) with pH of 5.29 and titrable acidity of 0.42 mg mL^{-1}. It contained 208.5 mg mL^{-1} sucrose and 0.13 mg mL^{-1} reducing sugars. The acid invertase specific activity was 135.45 μg invert sugar/mg/min. while the phenols, ethanol, dextran, and the total bacterial, fungal, yeast, and *Leuconostoc* counts were minimal (**Figure 6**). The total bacterial and fungal count in the fresh cane juice were approximately 4.7 log CFU mL^{-1} and 1.1 log CFU mL^{-1}, respectively, and a manifold increase with the storage days was observed (378.6% and 131.3%, respectively) on the 10th day of storage. The total *Leuconostoc* and yeast counts also increased significantly (by 99.0 and 50.01%, respectively). The bacterial, fungal, *Leuconostoc*, and yeast counts increased with days of storage as in T_1, and an increasing trend was observed in the microbial counts with storage. Foster

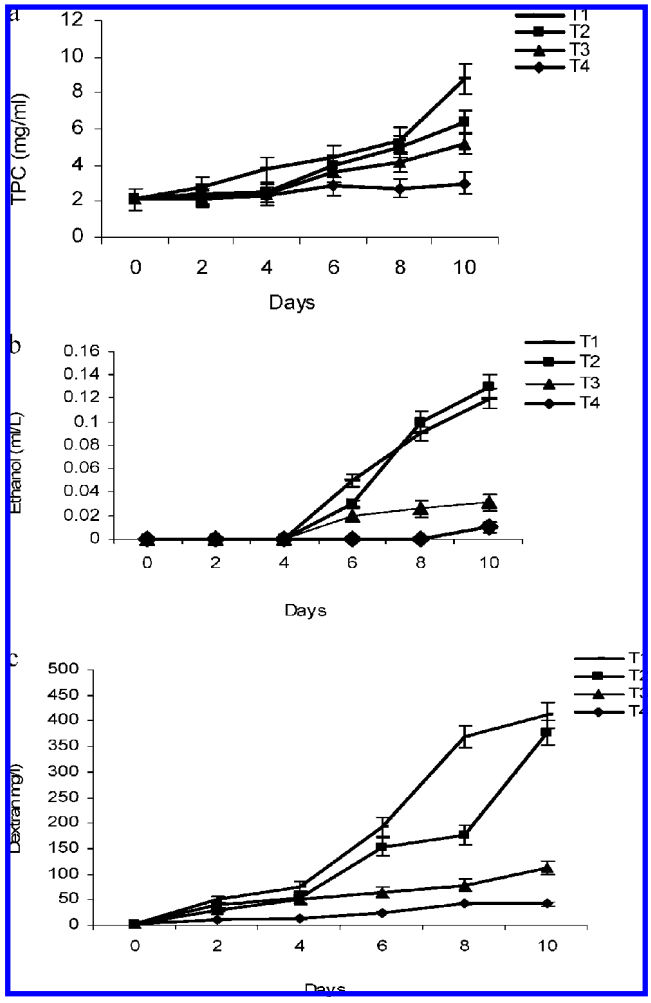

Figure 4. Changes in (**a**) total phenol content (**b**) ethanol, and (**c**) dextran content in post-harvest sugar cane under storage at different days. Results are the mean of triplicate samples, and vertical bars represent ± S.E. T1—: control (untreated), T2■: H_2O spray, T3▲: (glutaraldehyde, 1000 ppm). T4◆: (glutaraldehyde + benzalkonium chloride, 1000 + 250 ppm).

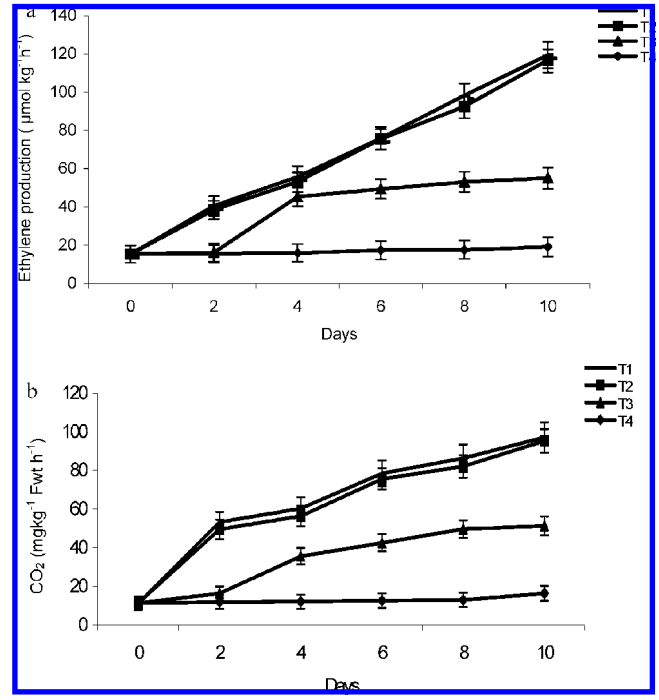

Figure 5. Ethylene production (**a**) and respiration rates (**b**) in post-harvest sugar cane under storage at different days. Results are the mean of triplicate samples, and vertical bars represent ± S.E. T1—: control (untreated). T2■: H_2O spray. T3°: (glutaraldehyde, 1000 ppm). T4◆: (glutaraldehyde + benzalkonium chloride, 1000 + 250 ppm).

et al. (*2*) have reported earlier that post-harvest sugar cane is prone to microbial inversion with bacteria of *Leuconostoc* and *Lactobacillus* genera and yeasts of genera *Saccharomyces* and *Rhodoturula*.

There was an increase in the loss of weight in the stored cane (**Figure 1**), and the weight loss was maximum on the 10th day (19.93%) in T_1. The color of the cane juice darkened as indicated by the increasing OD with days in the untreated canes (**Figure 2a**). However, a declining trend was observed in pH with days of storage (**Figure 2b**). It was found that titrable acidity increased significantly with time period (**Figure 2c**). There was strong positive correlation (**Table 1**) between weight loss, pH, and titrable acidity ($R^2 = 0.99$ and 0.96, respectively).

Loss of weight in cane during its storage has been positively correlated with its drying (*2, 7, 8, 12, 37*). The loss of weight of vegetable and fruits during storage is caused by water exchange between the internal and external atmosphere (*38*). Thus, the higher microbial population in the post-harvest sugar cane would have led to tissue disruption and in turn might have been responsible for the higher weight loss found in the untreated canes. Weight losses have been correlated with microbial spoilage in several lettuces and grapes (*39–41*).

Ethylene Production and Respiration Rate. Ethylene production increased with days of storage, and an increasing trend followed the linear regression equation $y = 20.403x - 3.8933$ (**Figure 5a**). With regard to the respiration rate, it was found that CO_2 increased significantly (88.3%) in the untreated cane, and a strong positive correlation was observed with days of storage ($R^2 = 0.99$). Significant increase in ethylene production is attributed to the increase in the total fungal count. *Bortytis cinerea* is known to produce large amounts of ethylene in tomatoes (*41*). In our study, a strong correlation between total fungal count and ethylene existed ($R^2 = 0.93$). Our results were concurrent with the increase in concentration of inoculated conidia in climacteric tomato fruit (*42*) and to the incidence of deterioration in nonclimacteric table grapes (*27, 43*). The total *Leuconostoc* count during storage was significantly higher than that in the fresh cane juice. A strong positive correlation between total leuconostoc count and acid invertase activity existed ($R^2 = 1.0$). Increase in total *Leuconostoc* count and acid invertase activity rendered the reduction in sucrose content (**Figure 7b**) and increases in the reducing sugar contents via hydrolysis of sucrose to glucose and fructose (**Figure 7a**).

Sucrose Losses, Reducing Sugars, and Acid Invertase Activity. A rapid decline in sucrose content was observed with time (**Figure 3a**). The declining trend followed the linear regression equation $y = -13.171x + 221.23$ ($R^2 = 0.97$, **Figure 3**). Almost opposite to the pattern of sucrose content, the reducing sugars increased rapidly with duration of storage. Reducing sugars increased several fold (3130.76%) from 0.13 to 4.2 mg/mL on the 10th day of storage (**Figure 3b**). A strong negative correlation is observed between the sucrose content and reducing sugar content ($R^2 = -0.99$, **Table 1**).

Acid invertase activity exhibited an increasing trend with the maximum activity on the 10th day (**Figure 3c**). An increase of 87.15% was observed on the 10th day as compared to fresh cane juice in untreated cane. A strong positive correlation

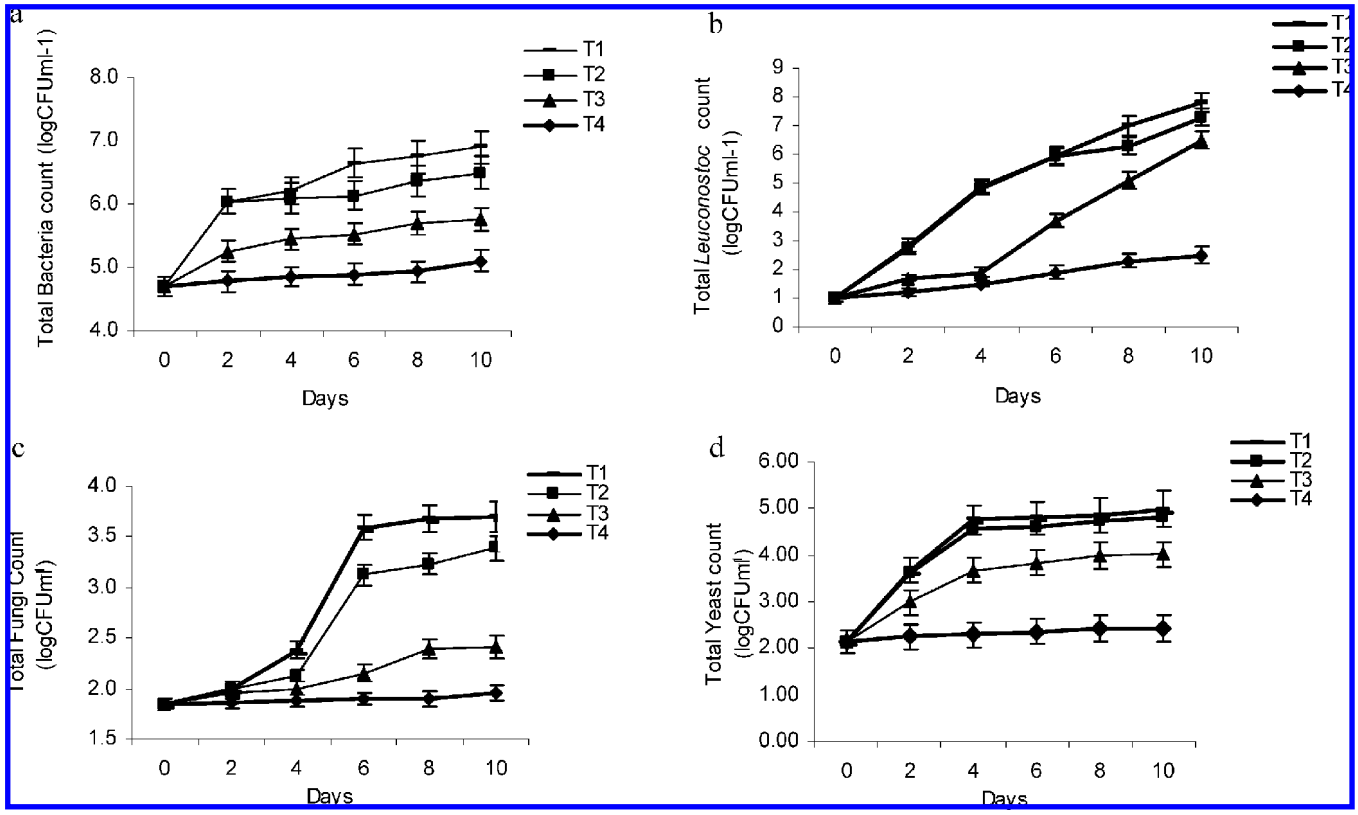

Figure 6. Changes in (**a**) total bacterial count, (**b**) total *Leuconostoc* count, (**c**) total fungal count, and (**d**) total yeast count in post-harvest sugar cane under storage at different days. Results are the mean of triplicate samples, and vertical bars represent ± S.E. T1—: control (untreated). T2■: H_2O spray. T3 ▲: (glutaraldehyde, 1000 ppm). T4 ◆: (glutaraldehyde + benzalkonium chloride, 1000 + 250 ppm).

between acid invertase activity and reducing sugars and a strong negative correlation between acid invertase and sucrose ($R^2 = -0.99$, **Table 1**) were observed. The accelerated metabolism and thus the advanced tissue senescence contributed to the increase in titrable acidity (51.04%), activities of acid invertase (67.15%), and reducing sugar content (manifold), thus being partially responsible for sucrose and weight losses in post-harvest sugar cane.

Total Phenols, Dextran, and Ethanol. Total phenolic content, dextran, and ethanol contents increased significantly (76.2, 99.0, and 100%, respectively) in sugar cane juice with storage days in the untreated canes (**Figure 4**). The strong positive correlation between the total bacterial count and the acid invertase activity ($R^2 = 0.96$) explain the reduction in sucrose content and the increase in reducing sugar content. (**Figure 7a** and **b**). The acid invertase activities led to sucrose hydrolysis resulting in the formation of reducing sugars, and the increase in titrable acidity accelerates the formation of other intermediary compounds.

A strong positive correlation among total *Leuconostoc* count, acid invertase activity, and respiration rates ($R^2 = 1.0$ and 0.97, respectively) was found to exist (**Table 1**). This explains that the respiration rate has a significant effect on the metabolism of sugars, initially forming reducing sugar and then diverting it to the formation of other intermediate compounds (*44*). Microbial counts and dextran levels increased rapidly in all of the samples of sugar cane during transportation (*2*).

Effects of Glutaraldehyde and Benzalkonium Chloride on Physio-Chemical Changes. No significant changes in weight loss, color, pH, and titrable acidity were found to occur with T_2, and the trends in their changes with storage days were similar to that observed in the untreated cane. However T_3 and T_4 showed significant decline in weight, color, and titrable acidity with days of storage (**Figure 2**). There was a significant decline of 59.80% in loss in weight with T_3 as compared to the untreated cane (T_1). The maximum decrease of 91.8% loss of weight was recorded with T_4. Similarly, it was observed that though the physio-chemical changes were reduced significantly with T_3, maximum reduction occurred with T_4 (**Figure 2**).

Ethylene Production and Respiration Rate. The results revealed that there was a slight reduction (2.1%) in ethylene production with T_2, and the increasing trend remained similar to that of T_1. However, there was significant reduction in ethylene production in canes treated with T_3 and T_4 (by 53.8 and 84.0%), as compared to the control. With regard to the respiration rate, it was found that CO_2 increased significantly (88.3%) in the untreated cane, and a strong positive correlation was observed with days of storage ($R^2 = 0.99$). However, with T_3 and T_4 significant reduction in respiration rates (47.16 and 86.3%, respectively) occurred.

Sucrose Losses, Reducing Sugars, Acid Invertase Activity, and Intermediate Compounds. The sucrose losses were observed to be reduced in all of the treatments. The losses in sucrose on the 10th day were 25.65, 9.35, and 7.14% with T_2, T_3, and T_4, respectively, as compared to 30% in T_1 (**Figure 3**). A reduction of 30.3% and 59.8% reducing sugar content was observed with T_2 and T_3, respectively, while a drastic reduction of 95.7% was found with T_4. Thus, there was an improvement by 76.9% in the sucrose content in cane treated with T_4 as compared to that in untreated canes. A strong negative correlation is observed between the sucrose content and reducing sugar content ($R^2 = -0.99$, **Table 1**). With T_3 and T_4, the acid invertase activity was reduced by 15 and 60%, respectively.

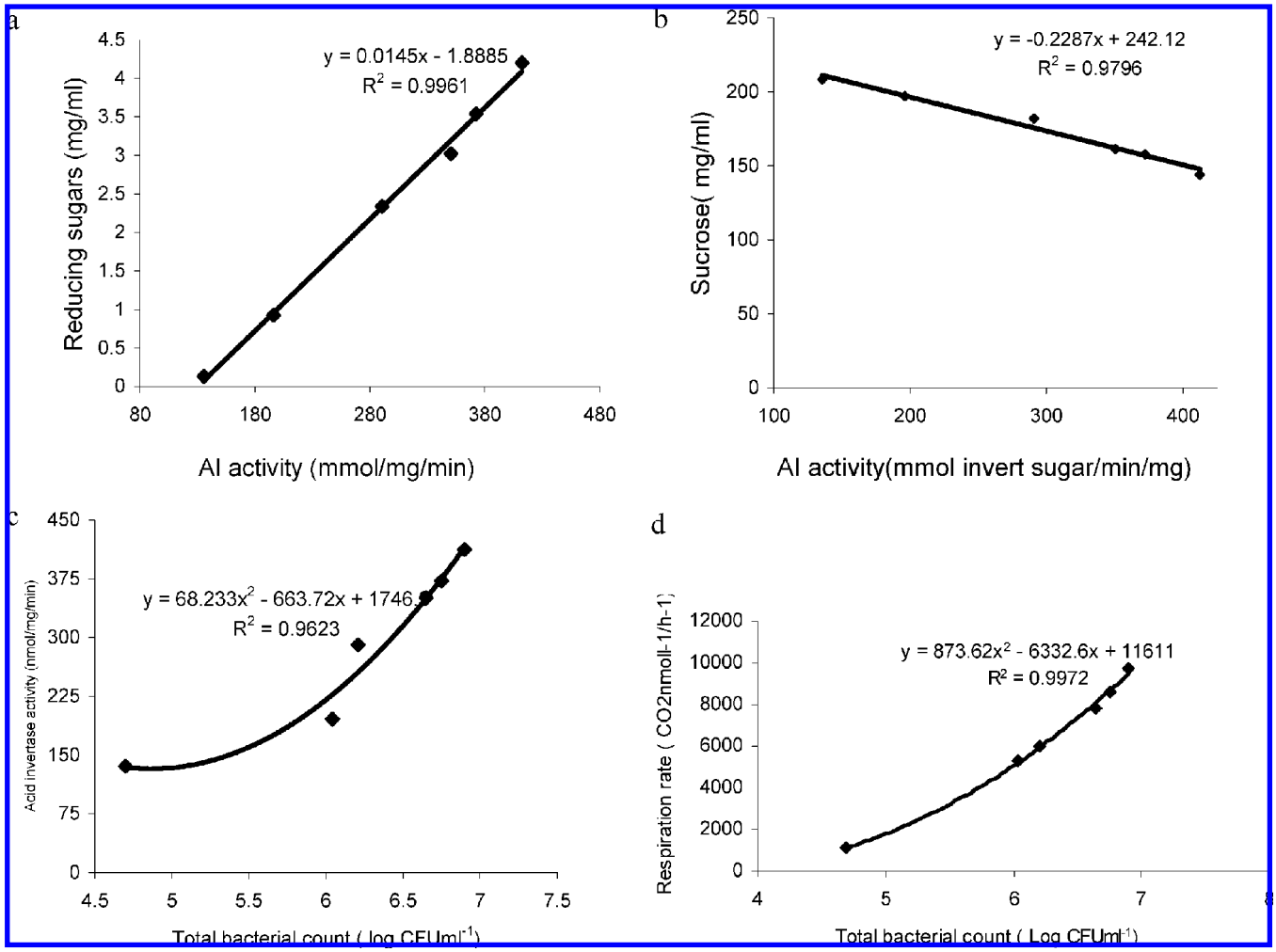

Figure 7. Effect of acid invertase activity on sucrose, reducing sugars (a, b) and total bacterial count on acid invertase activity and respiration rate (c, d) in post-harvest sugar cane under storage.

Table 1. Correlation Coefficients among the Different Parameters of Sugarcane Kept under Storage for 10 Days[a]

parameter	loss in wt	pH	TA	color	sucrose	RS	AI	TPC	C_2H_2	resp rate	TBC	TFC	TYC	TLC	ethanol	dextran
loss in wt	1															
pH	−0.87	1.0														
TA	0.88	−0.98	1.0													
color	0.96	−0.94	0.96	1												
sucrose	−0.97	0.96	−0.96	−0.98	1											
RS	0.99	−0.91	0.93	0.98	−0.99	1										
AI	1.0	−0.90	0.90	0.97	−0.99	1	1									
TPC	0.85	0.95	0.96	0.93	−0.91	0.89	0.87	1								
C_2H_2	0.96	−0.86	0.97	1	−0.98	0.98	0.97	0.94	1							
resp rate	0.96	−0.78	0.88	0.96	−0.95	0.96	0.96	0.84	0.96	1						
TBC	0.93	−0.96	0.80	0.91	−0.90	0.92	0.93	0.76	0.90	0.99	1					
TFC	0.93	−0.75	0.93	0.92	−0.97	0.94	0.94	0.81	0.93	0.89	0.84	1				
TYC	0.96	−0.75	0.77	0.89	−0.90	0.93	0.95	0.73	0.88	0.97	0.99	0.84	1			
TLC	1.0	−0.90	0.92	0.98	−0.98	1	1	0.88	0.98	0.97	0.94	0.93	0.95	1		
ethanol	0.83	−0.98	0.99	0.93	−0.92	0.88	0.85	0.93	0.94	0.82	0.73	0.90	0.68	0.86	1	
dextran	0.89	−0.97	0.98	0.97	−0.94	0.92	0.90	0.91	0.97	0.88	0.80	0.92	0.76	0.91	0.98	1

[a] TA, titrable acidity; RS, reducing sugars; AI, acid invertase activity; TPC, total phenol content; C_2H_2, ethylene production; TBC, total bacterial count; TFC, total fungal count; TYC, total yeast count; TLC, total *Leuconostoc* count; resp rate, respiration rate.

The treatments were effective in reducing the concentrations of the compounds. The maximum reduction of 76.0, 89.8, and 83% in total phenols, dextran, and ethanol contents, respectively, occurred with T_4 (**Figure 4**).

Effects of Glutaraldehyde and Benzalkonium Chloride on Microbial Counts. It was observed that with T_2 no reduction in the microbial counts occurred and that the counts were at par with T_1. However, with T_3, there was significant decline in the bacterial and the fungal counts (by 69.7 and 15.8%, respectively) while a reduction of 35.8 and 17.3% in the total *Leuconostoc* and yeast counts, respectively, occurred on the 10th day of analysis. The maximum reduction in the counts was with T_4 till the 10th day. The total bacterial and fungal counts were reduced by 67.92 and 51.3%, while a reduction of 26.08 and 51.2% in the total *Leuconostoc* and yeast counts, respectively, occurred with T_4 on the 10th day of storage. A stable trend,

however, was noticeable with T_4 in contrast to the increasing trends in the control.

As with T_3 and T_4, the microbial count and the acid invertase activity declined, and the sucrose breakdown and formation of reducing sugars were reduced significantly. In this sense, a physiological role of the disinfectants glutaraldehyde and BKC in decreasing the metabolism in post-harvest sugar cane could be proposed, although the intrinsic mechanism is not clear and deserves further research. The effect was found to be cumulative as antibacterial and antifungal, though the antibacterial effect was more pronounced.

The antibacterial and antifungal activities of glutaraldehyde and BKC could be attributed to their broad-spectrum activity against bacteria, fungi, and their spores. The mechanism of action involves the binding of glutaraldehyde to the outer layers of the bacterial cells, especially with unprotonated amines on the cell surface, and inhibited the action of the transport and enzyme system (*23, 45*). The mechanism of benzalkonium chloride's microbicidal action is thought to be due to the dissociation of cellular membrane bilayers, which compromises cellular permeability, controls and induces leakage of cellular contents, causing the loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging effects to the bacterial cell (*46–48*). The results reported here have thus demonstrated that the application of glutaraldehyde and benzalkonium chloride were effective in reducing the total bacterial, fungal, *Leuconostoc*, and yeast counts significantly till 10 days of storage. Apart from antimicrobial effects, they also exhibited physio-chemical roles during post-harvest storage by lowering the weight losses, acid invertase activity, breakdown of sucrose, titrable acidity, reducing sugars, dextran, ethanol, ethylene production, and respiration rates. The work thus indicated that the use of glutaraldehyde and benzalkonium chloride can be helpful in minimizing the post-harvest deterioration of sugar cane during its storage.

ACKNOWLEDGMENT

We thank the Director for his constant encouragement and guidance. We extend our thanks to the technical staff of the Division of Plant Physiology and Biochemistry and the Jaggery unit for their help in analysis during the experimentation. The help provided by Chatrapal, Yogendra Yadav, and Rajesh Kumar during the experimentation is duly acknowledged.

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Received for review May 5, 2008. Revised manuscript received June 19, 2008. Accepted June 20, 2008.

JF801394J