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RESEARCH ARTICLE

Post-harvest deterioration of sugarcane and its relationship with the activities of invertase and dextransucrase during late-crushing season in sub-tropics

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Abstract Sugar losses after harvest is one of the major problems of sugar processing units in many countries especially where milling is extended during a period of high ambient temperature. A study undertaken on the magnitude of post-harvest sucrose losses and its relationship with two enzymes viz. invertase and dextransucrase during late milling season showed significant reduction in CCS and increase in enzyme activities. The loss in CCS was nearly 60 percent in untreated cane compared to 27 percent in QUAT based chemical treatment after 240 hours of storage. There was marked increase in acid invertase and dextransucrase activities with the passage of time, however QUAT based formulation treatment recorded appreciable reduction in the enzyme activities. The study has indicated that it is possible to minimize post harvest sugar losses during late-season by application of QUAT based formulation.

Keywords Commercial cane sugar,acid invertase, dextransucrase, late milling season.

Introduction

Sugarcane harvesting-processing is a seasonal activity however, in the years of surplus cane production, extension of milling season during summer months (Temp 38-42^{0}C) become unavoidable. This leads to enormous loss in sucrose and subsequently results in low sugar recovery. A study revealed around 13.0 kg sugar loss per ton cane milled due to harvest-to-milling delays. Recent studies have indicated that over 1.0 unit pol (percent cane) is lost due to cut to crush delays (Solomon *et al*. 2007). The present study was directed at studying the expression of two important enzymes viz. acid invertase and dextransucrase, primarily responsible for the loss of sucrose in harvested cane. After harvest of cane the endogenous invertases get activated due to loss of moisture and lack of any physiological and biochemical control mechanism. Studies have shown gradual increase in invertase(s) activity and consequently a decline in commercial cane sugar in the harvested stored or stale cane (Solomon *et al*., 1990). The problem is further compounded by *Leuconostoc* bacteria which converts sucrose to a complex polysaccharide (dextran) through an extracellular dextransucrase, leaving fructose as a secondary product.(Legendre *et al*.,1985). The polysaccharide dextran directly and negatively affects the efficiency of factory processing as they interfere with the crystallization(Morel du Boil,1995) and pull down sugar recovery. This study was aimed at investigating the behavior of invertase and dextransucrase in relation to sucrose content and other quality parameters in harvested stored cane during late-milling period. Effect of applying a quaternary ammonium compound based formulation, developed at IISR,Lucknow (Solomon *et al.,* 2007) on the quality of harvested stored cane was also investigated.

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Material and Methods

The experiment was conducted at the Indian Institute of Sugarcane Research,Lucknow located in the sub-tropical cane growing belt of India (26° 56'N; 80° 52'E: 111m amsl). A mid-late maturing commercial sugarcane variety CoSe 92423 was used to asses the post-harvest quality losses during the late-milling period i.e. last week of April when the ambient temperature was around 39-40^{0}C. The canes of uniform size were harvested topped, detrashed and kept in separate bundles in small heaps under open field conditions in three replicates. First heap was kept in open and used as control (T-1), second heap was sprinkled with water and covered with a thick layers of trash (T-2) and third heap was mist sprayed with an aqueous solution of quaternary ammonium compound based bactericidal formulation and covered with a thick layer of trash (T-3).

Ten canes from each heap were selected randomly from the heaps and juice was extracted at the interval of 0,48,96,144,192 and 240 hours, respectively in a clean power operated vertical crusher. The deterioration in cane quality was assessed by observing following parameters:

The Total Soluble Solids (TSS) and pol% in juice were recorded by Foss NIR (Near Infra Red Reflectance) cane analyzer. Commercial Cane Sugar(CCS) in juice were calculated by using equation CCS%= 1.022(pol% juice) – 0.292(brix) (Bakshi Ram *et al*.2001). Reducing sugars in juice were estimated by the spectrophotometeric method of Nelson(1944). Acid invertase activity in the primary expressed juice was assayed by the method described by Rosario and Santisoparsi(2003). Proteins in the dialyzed juice were estimated by the method of Lowry *et al* (1951). Dextransucrase activity (transferase) was assayed by the method of Kobyachi and Matsuda(1974). Dextran in juice was estimated by Haze method.

Results and Discussion

Commercial Cane Sugar (CCS) is the major quality factor which is considered while studying the deterioration. The present study (Fig.1) shows significant decline in CCS in harvested cane(T-1) kept over a period of 240 hours. The CCS value of freshly harvested cane was 10.88, which declined by 2.27 and 6.59 units after 48 and 240 hours of harvest, respectively. This accounted for 60.56% loss in stored sucrose. In harvested cane treated with water and covered with trash (T-2) decline in CCS after 48 hours was 1.67 unit, whereas after 240 hours of storage CCS loss was 4.86 units ie: 44.6%. In the third heap of harvested cane, which was treated with chemical formulation and covered with trash (T-3), the decline in CCS after 48 hours was 0.20 unit, whereas after 240 hours the loss was 2.96 units i.e. about 27% loss of sucrose present in fresh cane. It can be seen that the loss of sucrose in untreated as well as treated cane increased during late milling season. However, a substantial amount of recoverable sugar could be saved by chemical treatment. Earlier studies conducted in north India (Solomon *et al.,*1997,2003, 2006, 2007) Sharma and Sunita (1994) have shown that loss in CCS per day after harvest varied 0.5-1.5 units depending upon the time of milling. In sub-tropical India, field losses in CCS were found to be 0.35, 1.0 and 1.32 unit per day during early, mid and late-crushing periods respectively.

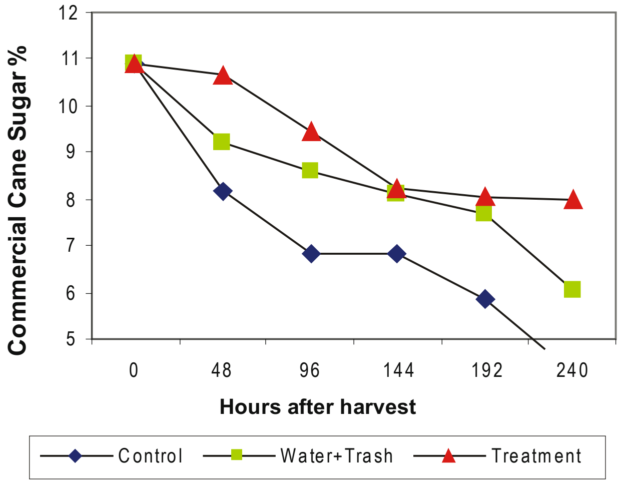

Fig.1. Commercial Cane Sugar in harvested cane

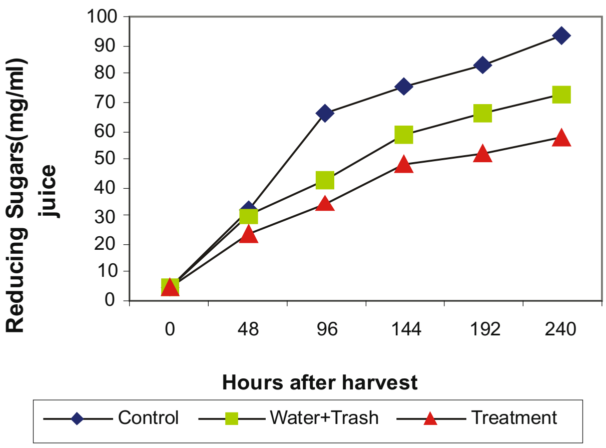

Fig.2. Reducing sugars during storage in juice

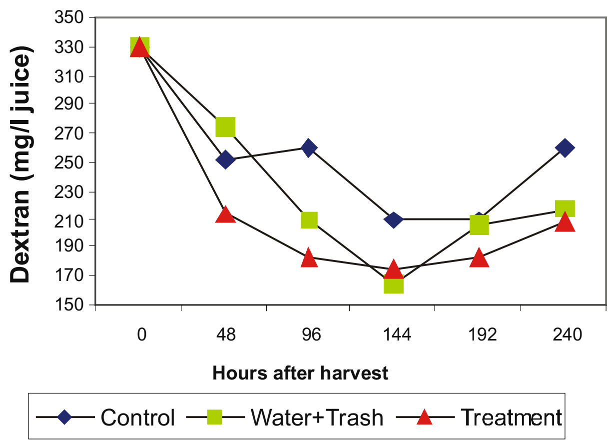

Fig.3. Dextran content in harvested cane

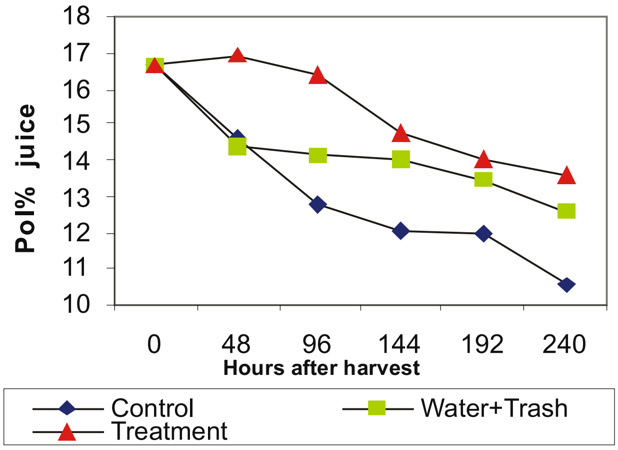

Fig.4. Change in pol% juice in harvested cane

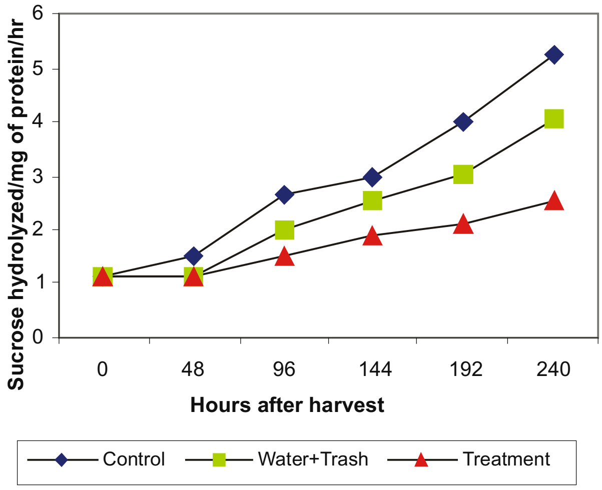

Fig.5. Change in Acid Invertase activity after harvest

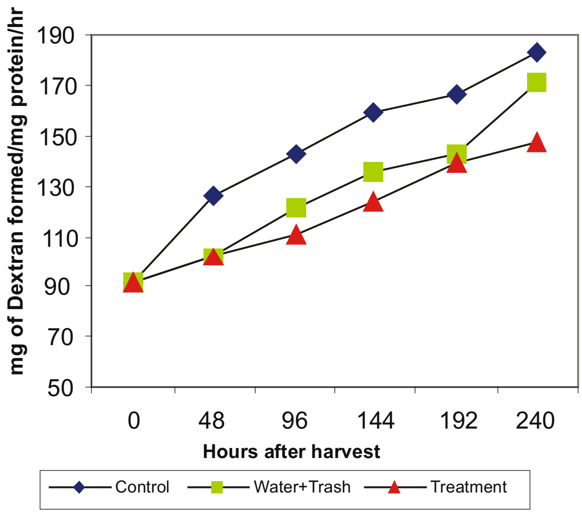

Fig.6. Activity of Dextransucrase in harvested stored cane

The level of reducing sugars at the time of harvest was 5.06 mg/ml juice which increased to 6 and 19 folds after 48 and 240 hours of staling, respectively in untreated (T-1) cane. In canes treated with water and trash (T-2), reducing sugars were 5.9, 14.35 folds higher after 48 and 240 hours of staling compard to their original level in cane (Fig.2). In chemically treated cane (T-3), the loss was 4.6 and 11.4 folds after 48 and 240 hours of staling, respectively. The reducing sugars in juice are an important indicator of cane deterioration (Uppal *et al.,* 1997; Magdum *et al.* 1987; Ahmad and Khan,1988 ; Gaur and Desai,1988). Studies conducted by Solomon *et al* (1997,2006, 2007) have also reported higher levels of reducing sugars in juice on storage of harvested cane.

A rapid increase in acid invertase activity during late milling season was noticed in harvested stored cane. This increase in invertase activity was 1.38, and 4.75 folds after 48 and 240 hours of staling in untreated (T-1) cane compared to its initial status in the freshly harvested cane (Fig.5). In cane treated with water and covered with trash (T-2) this increase was 1.04 and 3.69 folds after 48 and 240 hours, respectively. However, in chemically treated cane (T-3) rise in enzyme activity was 1.02 and 2.32 folds after 48 and 240 hours of staling, indicating the inhibitory effects of chemicals on deterioration. It is evident that the activity of acid invertase was reduced to half in cane treated with chemical compared to untreated control after ten days of storage. The higher acid invertase activity favored sucrose inversion which is responsible for loss of sucrose in the harvested stored cane. It has been noticed that soon after harvest of cane, endogenous invertases get activated due to loss of moisture and lack of any physiological and biochemical control mechanism (Solomon *et al* 1990).The invertases are of two types based upon pH performance(acid invertase,pH4.8 and neutral invertases pH 7.0). These two invertases are present in mature and immature tissues(Glasziou and Hatch,1963).The acid invertase are involved in sucrose inversion and decline in cane quality. A sharp increase in acid invertases leads to increase in reducing sugars and consequently there is drop in Commercial Cane Sugar(Solomon *et al.*, 1997). Eggleston and Legendre(2003) advocated that the enhanced activity of acid invertases could be due to mobilization of cell invertase, possible synthesis of cut induced invertase and decreased activities of sucrose synthesizing enzymes induced by pH change.

The transferase activity (dextran synthesized) in untreated cane (T-1) indicated an increase of 35.33 units and 91.06 units after 48 and 240 hours of staling compared to its initial status. In canes treated with water and covered with trash (T-2) 10.88 and 79.78 units increase after 48 and 240 hours of harvest was noticed , whereas in canes treated with chemical and covered with trash (T-3) the enzyme activity increased by 9.78 and 56.4 units after 48 and 240 hours, respectively (Fig.6). It is interested to note that the activity of dextransucrase is greatly affected by chemical treatment which is probably due to antimicrobial activity of QUAT, inhibiting the growth of dextran producing bacteria. A healthy cane in field is generally free from any microbial infection however, if crushing is delayed, then a water soluble polymer of glucose(dextran) is synthesized from the stored sucrose. The dextran content in stale cane juice increased sharply with increase in dextransucrase activity, an enzyme extracellularly secreted by *Leuconostoc* bacteria present in rhizosphere. The cut ends of the harvested cane facilitates invasion of microbes, particularly *Leuconostoc* bacteria inside cane, which converts stored sucrose into dextran through dextransucrase enzyme(Kin,1995;Haldane;1994).The action of dextran sucrose leads to formation of α(1-6) bonded lines dextran chains and releases fructose in the medium.

The post harvest quality deterioration is also characterized by decrease in glucose/fructose ratio, dextransucrase an enzyme secreted extracellularly by *Leuconostac* bacteria, hydrolyse glucose from sucrose molecule to form dextran, leaving fructose as a secondary product.(Legendre *et al.*,1985) Dextransucrase catalyzes the transfer of a D-glucosyl group from sucrose to a growing chain of the polysaccharide dextran. Luzio & Mayer(1983) reported that dextransucrase catalyzed the hydrolysis of the substrate sucrose and that a glucosylated enzyme had three competing activities, hydrolysis, D-glucosyl transfer,and polymerization. This enzyme, secreted mostly by *Leuconostoc* bacteria, not only catalyses dextran synthesis from sucrose, but in the presence of other carbohydrates such as glucose, fructose also transfer glucose from the sucrose molecule to form oligosaccharides such as leucrose and palatinose(Robyt, 1995; Robyt and Eklund,1982) and therefore, is a potential criteria for cane deterioration.(Eggleston and Legendre,2003).These oligosaccharides directly and negatively affect the efficiency of factory processing as they interfere with crystallization(Morel du Boil,1995) and pull down sugar recovery. Our studies have shown that loss of sucrose from the harvested stored cane during high ambient temperature is catalyzed by enzyme invertase and dextransucrase. Their joint action is probably responsible for a rapid loss in recoverable sugar under sub-tropical conditions. The study also support our earlier observations (Solomon *et al.* 2007)) that QUAT based chemical formulation developed in our laboratory could minimize post-harvest sucrose losses, even at high ambient temperature. The anti-inversion and anti-bacterial (QUAT) compounds present in the formulations perhaps reduce sucrose inversion and dextran formation(Fig.3 and 4) in the harvested stored cane.

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