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Impact of cut to crush delay and bio-chemical changes in sugarcane

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Abstract

In the present era of economic liberalization, sugar has become an important commodity for human consumption as well as trade. The global importance of this versatile sweetner could be judged by the fact that its consumption is expected to go much high upto 2020 AD as compared to the present level. Sugarcane plant, once detached from ground loses its machinery to synthesize sucrose. Thus a well ripened harvested crop may lose its sugar within a few days, which tends to increase further due to high ambient temperature. These loses tend to increase during processing, especially in those units where hygienic conditions are rather unsatisfactory. The post harvest sugar lose is one of the most vexing problems of sugar industry and has attracted widespread attention in the recent years. A study was carried out to examine the effect of staling h on three early and three mid-late maturing varieties in view of biochemical changes occurred in staled cane juice from 0 h - 96 h. The ambient temperature ranged around 40-42^{0}C. TSS, sucrose, purity coefficient, dextran, proteins, free amino acid contents, acid and neutral invertases were assayed. The sucrose content rapidly deteriorated after 24 h staling. The invert sugar and dextran content increased rapidly after 48 h. free amino acid contents increased rapidly up to 72 h. Free amino acid contents were higher in early group of varieties than mid-late group of varieties. Findings indicated that both the group of varieties fastly deteriorated sucrose content after 24 h. Higher dextran formation was observed after 48 h in most of the early varieties due to higher concentration of sucrose thereby TSS increased during staling periods. It may be inferred that the early maturing varieties should be crushed within 24 h and mid-late maturing varieties within 48 h after harvest under high ambient temperature. Mid-late maturing varieties showed lower values of dextran, free amino acid content, protein and invertase activities. Therefore, these varieties were found capable in retaining quality characters up to longer durations after harvest. It has also been observed that mid-late varieties having lower sucrose content than early varieties, deteriorated less during staling. In the cane juice of early maturing varieties, where sucrose and brix values were higher than mid-late, produced relatively higher invertase activities, dextran, free amino acid content and proteins in the late staling h possibly due to higher microbial infestation.

Keywords: Sugarcane varieties, staling hours, invertase(s), dextran, free amino acids, protein.
Abbreviations: H- hours; TSS- total soluble solids; TCA- tri chloro acetic acid; BSA- bovine serum albumin; AI- acid invertase; NI- neutral invertase.

Introduction

Sugarcane industry is considered one of the organized sectors. This sector is among the countries leading economic enterprises. Sugar is mainly extracted from sugarcane and sugar beet. Studies have indicated that nearly 20-30% of total sucrose synthesized by sugarcane plant is lost during various stages of raw material handling and sugar mill processing. The post harvest sugar loss is one of the most alarming problems of sugar industry and has attracted widespread attention in the recent years. The published reports indicating loss of recoverable sugar following cane harvest began to appear towards the end of the 19th century (Cross & Belile, 1914, 1915). According to these authors, Went & Geerligs from Java reported deterioration of sugarcane in 1894. Early workers emphasized the importance of time lag between harvesting and milling as well as storage environment in deterioration process. Browne & Blouin (1907) in java reported considerable drop in juice purity during storage of cane, however, no scientific explanation was advocated. Indian scientists (Magdum and Kadam, 1996; Solomon et al, 1997; 2007; Solomon 2000; Siddhant et al., 2008, Srivastava et al., 2006, 2009) reviewed the work on post harvest deterioration of sugarcane. Their work highlighted the importance of lose reduction technology in improving sugar productivity especially in India and Asian countries where this is a serious problem. The biological losses such as inversion of sucrose by plant & microbial invertases, organic acids & dextran formation by micro organisms are largely responsible for loss of recoverable sugar after harvest of cane and its subsequent processing in sugar mill. Soil born bacteria viz; *Leuconostoc sp.* (*L. mesenteroides or L. dextranicum*) are often associated with the deterioration of cane entering through the cut ends. This bacteria has the ability to synthesise alpha- glucon polysaccharides (dextran) from sucrose through an extracellular enzyme called dextransucrase. The formation of organic acids such as lactic acid, acetic acid and butyric acid produced by microorganisms leads to loss of sucrose and lowering of juice pH. For each gram of acid produced about 2.77 gm (*L. mesenteroides*) and 11.09 gm (*E.coli*) sucrose is degraded. In the present study an attempt has been made to correlate the rate of sucrose degradation in staled cane juice resulting the formation of certain biomolecules like dextran, free amino acids, protein and reducing sugars in early and mid-late maturing elite sugarcane varieties.

Table 1. Maximum and minimum temperatures and relative humidity in different dates of storage

Date of storage	Maximum Temp^{0}C	Minimum Temp^{0}C	Relative Humidity (%)	
			08:30AM	04:45PM
10.04.2010	40.9	22.5	14	50
11.04.2010	41.8	20.5	12	34
12.04.2010	42.4	20.0	16	31
13.04.2010	41.0	22.8	23	37
14.04.2010	40.4	21.6	11	29
Average	41.30	21.48	15.20	36.20

Table 2. Unit loss in sucrose at different staling hours

Early Maturing Group					
Varieties	O h	24 h	48 h	72 h	96 h
CoS 96268	00	1.44	0.58	1.46	0.75
CoSe 98231	00	1.00	0.60	1.94	0.45
CoS 95255	00	0.51	0.48	1.23	0.35
Mean	00	0.98	0.55	1.54	0.65
Mid-late Maturing Group					
Varieties	O h	24 h	48 h	72 h	96 h
CoSe 01424	00	0.37	1.20	1.91	1.68
CoSe 95422	00	0.53	0.73	1.92	1.79
CoS 767	00	1.02	0.24	1.68	0.49
Mean	00	0.64	0.72	1.84	1.32

Materials and methods

The experiment was conducted at sugarcane research institute (U.P. Council of Sugarcane Research) Shahjahanpur located in the subtropical cane growing belt of India (27.58^{0}N latitude, 79.54^0 longitude and 154.53 meter above the sea level).

Materials

Samples were collected from 2009-2010 spring planted crop in the month of April. Sugarcane varieties of early and mid-late maturity groups were used for the studies. Varieties CoS 95255, CoS 96268 and CoSe 98231 of early maturing group and CoS 767, CoSe 01424, and CoSe 95422 of mid-late maturing group, were taken to assess the post-harvest quality losses during the late milling period i.e. 10-14 April 2010 when the maximum ambient temperature was around 40.4 - 42.4^{0}C (Table 1).

Methods

Canes of uniform size were harvested of each variety, topped, detrashed and kept in open natural field condition in three replicates. Five canes from each variety were selected randomly and juice was extracted at the interval of 0, 24, 48, 72 and 96 h respectively in a clean power operated vertical crusher. The deterioration in cane quality was assessed by analyzing extracted juice as given below:

Juice analysis

TSS (Total soluble solids)

TSS was measured by using brix spindle (0-20 and 21-30) with the method given by Meade and Chen (1977).

Sucrose per cent in juice

Sucrose was analysed in cane juice by following the method as described by Meade and Chen (1977). The results are expressed in percent sucrose in juice.

Reducing sugar per cent in juice

Reducing sugars in cane juice were estimated by the method of Nelson (1944). The results are expressed as percent reducing sugar in juice.

Invertases

The activity of the Acid and Neutral invertase(s) were determined in the cane juice by the method described by Huber (1989). The results are expressed in micro mole sucrose hydrolysed/min./ml juice.

Protein estimation

Protein was precipitated with 10% (w/v) chilled TCA and the clear residue was dissolved in 1.0 ml of 0.1 N NaOH at 80^{0}C for 10 minutes. Protein was estimated in 0.5 ml aliquot, according to Lowry et al., (1951). The intensity of the blue colour was measured on UV-1800 spectrophotometer (Shimadzu, Japan) at 640nm using BSA as standard. The results expressed as µl/ml juice.

Free amino acid content

Free amino acid content was estimated by following the method given by Yemm and Cocking (1955). The colour intensity was measured on UV-1800 spectrophotometer (Shimadzu, Japan) at 580nm. Leucine was used as standard. The results expressed as µl/ml juice.

Result and discussion

TSS (Brix)

Increased TSS (brix) was observed in each variety with increasing staling h (fig 1). Generally, higher TSS was recorded in early maturing varieties as compared to the mid-late maturing varieties. Bhatia et al., (2009) reported gradual

Table 3 . Unit loss in purity coefficient at different staling hours

Early Maturing Group					
Varieties	O h	24 h	48 h	72 h	96 h
CoS 96268	00	6.82	6.48	8.08	4.31
CoSe 98231	00	7.26	10.46	9.64	1.43
CoS 95255	00	3.87	3.60	6.12	4.51
Mean	00	5.89	6.85	7.95	3.42
Mid-late Maturing Group					
Varieties	O h	24 h	48 h	72 h	96 h
CoSe 01424	00	9.48	5.78	8.61	7.58
CoSe 95422	00	7.61	4.82	9.56	13.21
CoS 767	00	7.54	1.17	8.89	3.01
Mean	00	8.21	3.92	9.02	7.93

Table 4. Acid and Neutral invertase (s) in juice of staled canes.(micro mol sucrose hydrolysed/min/ml juice)over the varieties

Early Maturing Group			Mid-late Maturing group		
Staling hours	Acid	Neutral	Staling hours	Acid	Neutral
0 h	205.11	249.75	0 h	117.96	204.40
24 h	257.37	366.04	24 h	218.72	329.20
48 h	273.24	380.99	48 h	241.95	349.31
72 h	306.95	424.32	72 h	263.93	371.93
96 h	317.96	426.98	96 h	294.93	405.32
Mean	272.13	369.62	Mean	227.43	332.03

Table 5. Acid and Neutral invertase (s) in juice of staled canes.(micro mol sucrose hydrolysed/min/ml juice) over the hours

Early Maturing Group			Mid-late Maturing Group		
Variety	Acid	Neutral	Variety	Acid	Neutral
CoS 96268	303.00	398.94	CoSe 01424	227.51	326.12
CoSe 98231	243.40	341.12	CoSe 95422	232.14	328.15
CoS 95255	270.00	368.78	CoS 767	249.58	341.82
Mean	272.13	369.61	Mean	236.41	332.03

Table 6. Bio-molecules in different sugarcane varieties (over the hours)

Early Group					
Variety	Sucrose %	Reducing Sugar %	Dextran ppm	Protein µl/ml	Free amino acid µl/ml
CoS 96268	16.44	1.60	103.39	294.77	0.33
CoSe 98231	16.32	1.65	106.75	251.63	0.22
CoS 95255	17.36	1.47	104.41	278.87	0.30
Mean	16.70	1.57	104.85	275.09	0.28
Mid-Late Group					
Variety	Sucrose %	Reducing Sugar %	Dextran ppm	Protein µl/ml	Free amino acid µl/ml
CoSe 01424	16.20	1.78	82.27	301.2	0.24
CoSe 95422	15.66	1.80	70.95	234.22	0.05
CoS 767	15.51	1.53	79.89	278.68	0.20
Mean	15.79	1.70	77.70	271.37	0.16

Table 7. pH of cane juice in different sugarcane varieties at different staling hours

Variety	O h	24 h	48 h	72 h	96 h
CoS 96268	5.22	5.06	4.95	4.75	4.50
CoSe 98231	5.23	4.97	4.84	4.80	4.76
CoS 95255	5.30	5.26	4.85	4.84	4.58
CoSe 01424	5.24	5.14	5.02	4.97	4.82
CoS 95422	5.21	5.02	4.87	4.84	4.75
CoS 767	5.22	5.05	4.90	4.89	4.72

increase in TSS after storage of canes for twelve days regardless of the genotype or environmental conditions.

pH

The results revealed that the pH value of juice decreased with the increasing storage hours in each variety ranging from 5.30 to 4.50 from 0 to 96 h (Table 7). Bhatia et al., (2009) have reported a gradual increase in titrable acidity with parallel decline in pH of juice was also observed in all the genotype during storage (Bhatia et al., 2009). However, this effect was more pronounced during late crushing period i.e. at high temperature as reported by Mao et el., 2006.

Sucrose

Sucrose percent in juice showed decreasing trend with increasing staling hours in each variety (Fig. 2). The maximum rate of decrease was noted between 48 h to 72 h. The early maturing varieties possessed higher sucrose per cent in juice in their stalk as compared to mid-late maturing varieties upto 96 h. Data on unit decrease (Table-2) of sucrose per cent on maturity group basis indicated that after24 h staling the early maturing varieties lost less sucrose than that of mid-late maturing group (Table-2). On the basis of overall display of the varieties on group level, it may be concluded that due to higher storage capacity of sucrose in the sink of early maturing varieties consisting less fibre content they have better potential in retaining sucrose percent in juice as compared to the mid-late maturing varieties which have less sucrose per cent in their stalk and higher fibre per cent (Fig 8). Previous authors (Siddhant et al., 2008; Srivastava et al., 2006, 2009; Solomon, 2009) have advocated the quality losses in staled cane juice.

Purity coefficient

The purity coefficient showed decreasing trend with increasing staling hours (fig 3). From 0-72 h, varieties of both the maturity groups showed almost similar trend in loosing purity coefficient (fig 3). Drop in purity coefficient after 24 h, observed higher in mid-late maturing varieties while after 48 h, it was noted in early maturing varieties thereafter at 72 and 96 h staling the mid-late maturing group showed more unit loss in purity coefficient as compared to early maturing group (Table 3). Variety CoS 95255 showed minimum drop at 24 (3.87) and 48 (3.60) h in purity coefficient. At 72 and 96 h of staling more or less each variety of both the maturity groups except CoSe 95422, CoSe 98231 and CoS 767 showed almost similar behavior in reducing purity coefficient of juice. The remarkable point was noted for varieties CoSe 95422, CoSe 98231 (13.21, 1.43) at 96 h and CoS 767, CoSe 98231 (10.46, 1.17) at 48 h lost in purity coefficient respectively as maximum and minimum (Table 3). The result pointed out that the purity lose in staled cane juice does not depend upon the maturity status of the variety. Uppal et al., (2006) have also observed decreasing trend of purity coefficient with increasing storage periods. Steel and Trost (2006) reported that the presence of bacteria reduced the sugar purity, which may be another reason for reduction of purity on staling.

Reducing sugar

The results revealed that the reducing sugar content increased with the increasing staling hours in all the varieties (Fig 4). The rate of increase was noted and found that the reducing sugar increased in varieties as per their genotypic chara-

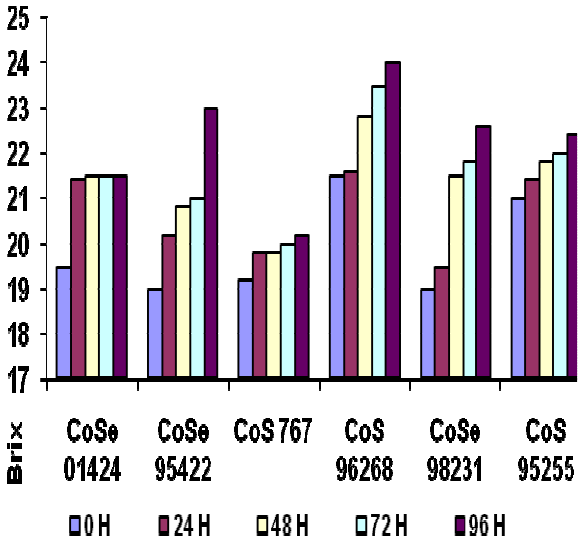

Fig 1. Post harvest changes in brix at different staling h.

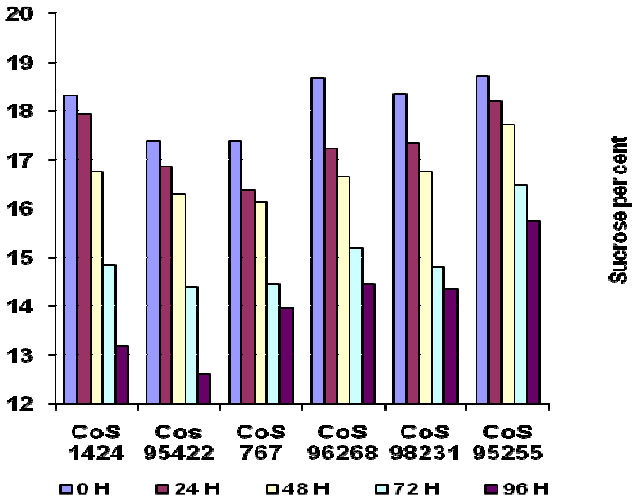

Fig 2. Post- harvest changes in Sucrose % in juice at different staling h.

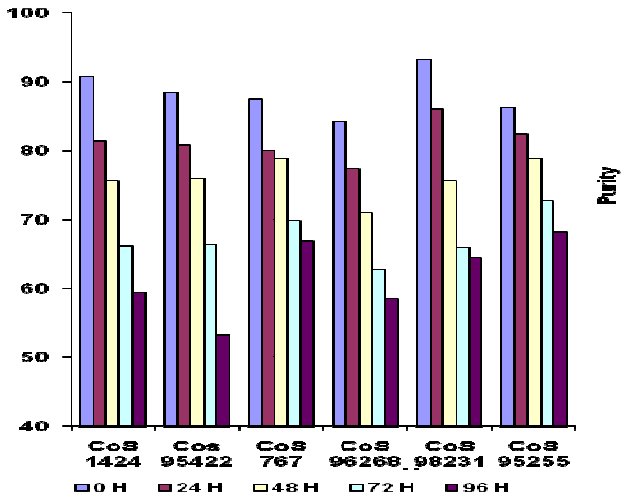

Fig 3. Post- harvest changes in purity coefficient at different staling h.

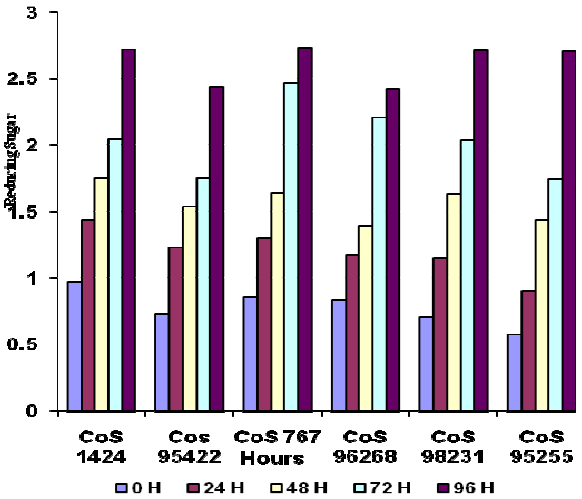

Fig 4. Post- harvest changes in reducing sugar at different staling h.

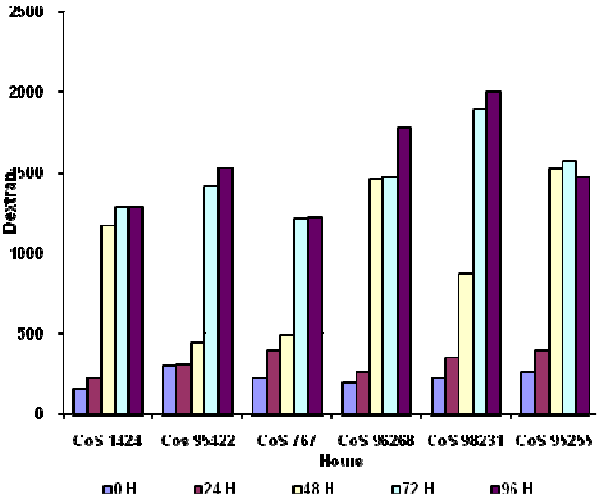

Fig 5. Post-harvest changes in Dextran at different staling h.

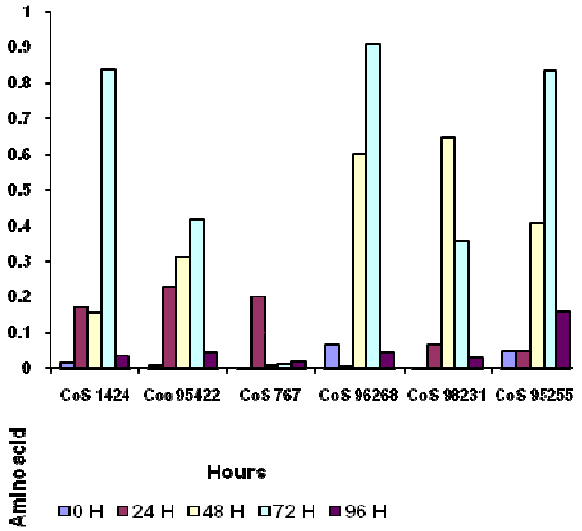

Fig 6. Post harvest changes in free amino acid at different staling h.

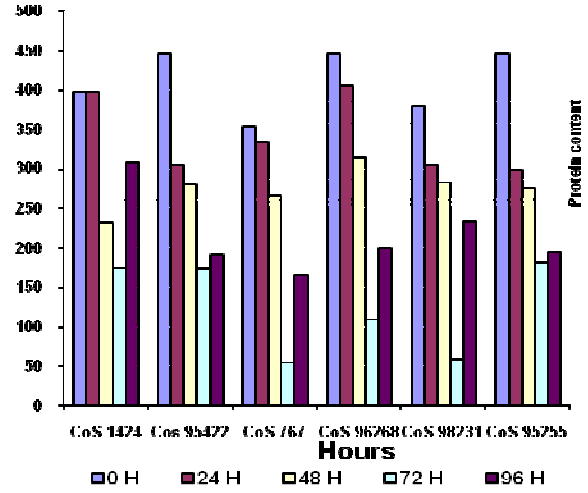

Fig 7. Post- harvest changes in protein content at different staling h.

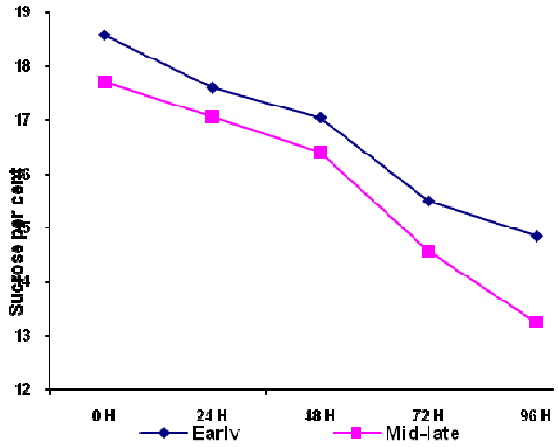

Fig 8. Mean data of sucrose % over the varieties of different maturity groups.

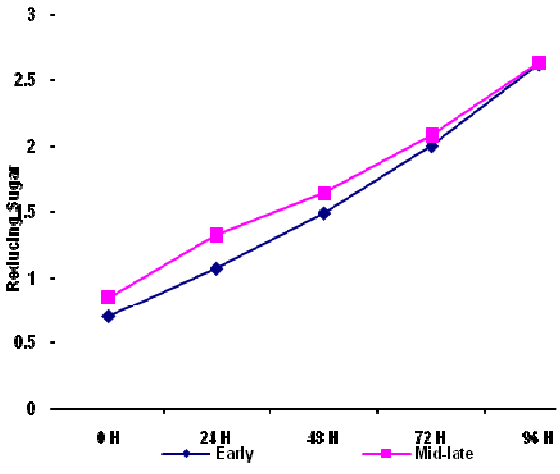

Fig 9. Mean data of reducing sugar over the varieties of different maturity groups.

cteristics (Fig 4). However on the basis of group level, the mean data of reducing sugar revealed that the mid-late maturing group consisted higher reducing sugar as compared to the early maturing group upto 72 h of staling but thereafter

at 96 h of staling both the group came akin to reducing sugar contents (Fig 9). It has been drawn out that the early maturing group lost the quality relatively less as compared to the mid-late maturing group (fig 9) showing lower reducing sugar content in juice. The decrease of sucrose concentration was not proportional to the increase of reducing sugar concentration. This might be due to the fact that reducing sugars are continuously utilized during the process of respiration and other metabolic activity. However, CoSe 95422 in mid-late group and CoS 95255 in early maturing group formed lower reducing sugar among their respective groups. The reducing sugars in juice are an important indicator of cane deterioration (Uppal and Sharma, 1997; Magdum et al.,1987; Ahmad and Khan, 1988; Gaur and Desai, 1988). Studies conducted by Solomon et al., (1997, 2007, 2008) have also reported higher levels of reducing sugars in juice on storage of the harvested cane.

Dextran

The dextran content of both the maturity groups differed with a very narrow range upto 24 h and thereafter showed wide range of difference till 96 h of staling (Fig 10). However, early maturing group produced more dextran as compared to the mid-late maturing group. At varietal level most of the varieties showed significantly higher dextran formation after 48 h of staling (Fig 5). Dextran is a glucose polymer, the glucose units are joined by a 1-6 link, with minor links 1-3 and 1-4, has a high molecular weight upto several millions. A healthy cane in field is generally free from any microbial infection. However, if crushing is delayed, the ambient temperature rises beyond 35^0C, 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 extra-cellularly secreted by *Leuconostoc* bacteria present in rhizophere. 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 and Robyt, 1995). Luzio and Mayer (1983) reported that dextransucrase catalysed 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).

Invertase(S)

In the month of April when the ambient temperature became so high, the acid invertase activity (AI) observed higher as the staling hours increased (Table 4). Higher acid invertase activity has been reported in immature internodes of sugarcane (Sachdeva et al., 2003). Enhancement in the acid invertase activity during storage of harvested cane was higher in all the genotypes under ambient environment from 24-96 h staling as compared to 0 h. Batta and Singh (1991) reported 7 folds increase in activity of acid invertase as compared to 4 folds increase in n eutral invertase after 12 days storage. In

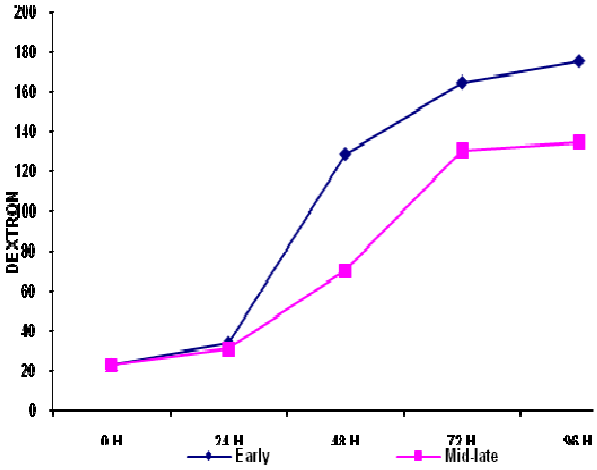

Fig 10. Mean data of dextrin over the varieties of different maturity groups.

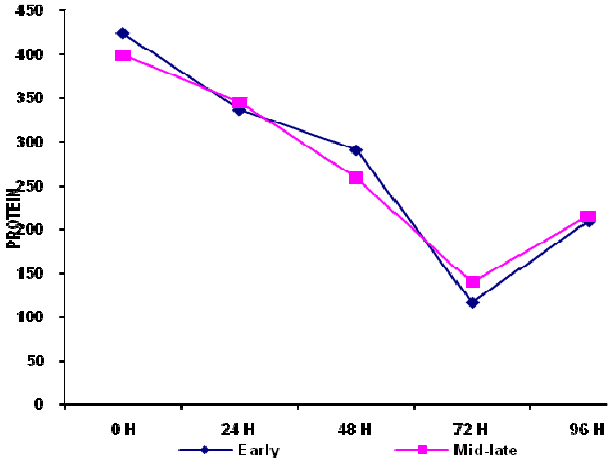

Fig 11. Mean data of protein over the varieties of different maturity groups.

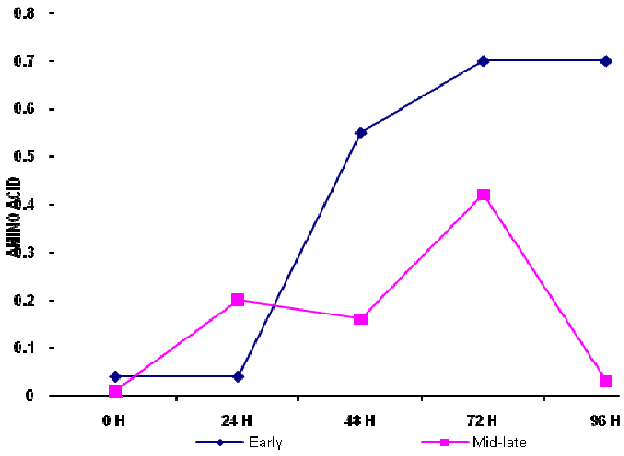

Fig 12. Mean data of free amino acid over the varieties of different maturity groups.

the present investigation the increase in acid invertase was almost 1.5-2.0 folds higher after 96 h of staling. Contradictory, Bhatia et al., (2009) has reported a decreasing trend in acid invertase and neutral invertase from November – March which did not support the present findings. It has also been noted that the acid invertase activity enhanced the dextran formation in both the maturity groups (Fig 10 & Table 5). Early experiments of Rizk and Normand (1968, 1969) demonstrated the presence of acid and neutral invertase in cane stalk and both the enzymes has the tendency to increase after harvest. Solomon et al., (1990) noticed increase in the activity of both, acid and neutral invertase after 72 h of storage of cane with a corresponding rise in the level of invertase sugar. Mao and Wang (2006) studied the sucrose metabolism in sugarcane stalks during storage and also recorded increase in invertase activity after harvest. Eggleston and Legendre (2003) advocated that the enhanced activity of acid invertase 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.

Free amino acids and protein contents

Free amino acid content showed almost increasing trend from 0-72 h staling (Fig 6). The protein content in both the maturity groups revealed a typical competition during the staling hours (Fig 11). However, it has been concluded that in fresh cane (0 h) early maturing group showed higher value. At 24 h staling, mid-late maturing group was higher while at 48 h, early maturing super seeded to mid-late and thereafter it went down against mid-late maturing group (72 h). At 96 h of staling both the groups came in close contact (Fig 4). Varieties Vs staling h showed that generally protein content observed higher at 24 and 48 h against 72 and 96 h staling (Fig 7). It has also observed that after 72 h, the protein contents increased in all the varieties. In general, at 72 h, the drop in protein contents was maximum in almost each variety. The protein content decreased gradually from 0-48 h and decreased rapidly at 72 h and thereafter increased at 96 h over 72 h (Fig 7). It might be due to the fact that in the month of April when ambient temperature becomes so high the dextran formation level became rapid and certain unknown bio-molecules might have formed which increased the protein content at 96 h of staling in both the maturity groups (Fig 11). Formation of dextran are the span of period which might have created toxicity for *Leuconostoc* bacterial colonies. Free amino acid content showed a competitive trend in between the maturity groups at 0 and 24 h of staling and thereafter early maturing group consisted higher free amino acid content at 48, 72 and 96 h of staling as compared to the mid-late maturing group (Fig 12). Mean data of early and mid-late maturing group over the staling hours indicated that sucrose percent in juice, dextran content, and free amino acid contents observed higher in early maturing group than that of mid-late maturing group. But reducing sugars in early maturing group found lower than mid-late maturing group (Table 6). It indicated that early maturing variety although produced less reducing sugar during staling but formed more dextran content in juice and thereby may be treated more prone for staling losses than that of mid-late maturing varieties. It may be argued that the microbes produced more dextran in early maturing group as compared to mid-late maturing group because the former group consisted higher sucrose percent in juice than the later group. The higher enzymatic activity of micro organisms in early maturing variety resulted higher free amino acid content in juice than the mid-late maturing group.

Conclusion

In the present study where six sugarcane varieties were studied, it was observed that at varietal level, CoS 767 showed lower TSS (total soluble solids), higher sucrose percent, low dextran content, better pH, lower invertase activity, lower protein content and lower free amino acids in cane juice after 96 h staling around 40-42^{0}C ambient temperature in the month of April. Besides this, CoSe 01424 and CoSe 95422 also showed relatively better results than others on above attributes. Thereby these varieties may be considered in retaining qualitative attributes for a longer durations under high ambient temperature after harvest (Table 6). In general, sucrose content fastly deteriorated after 24 h staling but simultaneously after 48 h to 72 h the invert sugar and dextran content increased. After 72 h, there was lower formation of dextran and increased level of total protein content alongwith free amino acid content. It was also noticed that protein content in early maturing group of varieties was slightly higher whereas free amino acid contents were much higher in mid-late varieties in comparison to early maturing group of varieties. It might be due to the higher protein contents and free amino acids in nodes of the cane stalk of mid-late maturing varieties. There may be formation of alcohol or toxic substances in staled cane juice that created toxicity for *Leuconostoc spp* bacteria that affected the dextran formation after 72 h of storage. Thus, it may be concluded that both the groups of varieties fastly deteriorated sucrose content after 24 h. After 48-72 h, higher dextran formation was observed in most of the early varieties due to higher concentration of sucrose in their juice than mid-late group of varieties that might be due to the higher rate of multiplication of bacterial colonies. There was no use of protein and free amino acids after the degradation of bacterial colonies. Therefore protein and free amino acids have been increased thereby resulted increasing trend of brix during staling periods. It may be inferred that early maturing varieties should be crushed within 24 h and mid-late maturing varieties should be crushed within 48 h. As the staling period increased, dextran formation increased as well, but after 72 h, the trend of dextran formation decreased, as the trend of dextran formation decreased the protein contents and free amino acid contents increased. At maturity group basis though the mid-late maturing group consisted relatively more reducing sugars in the early staling hours than that of early maturing group which was known to be undesirable quality character but on the other hand the former group produced less dextran than the later group. Dextran formation under late harvesting conditions has already been discussed in the text that this product deteriorates more quality characters of the cane juice thereby mid-late maturing group of varieties have better potential in avoiding more dextran formation than early maturing group of varieties. Conclusively the mid-late maturing variety may be considered suitable for post-harvest delay by showing less dextran formation during staling hours as compared to early maturing group. Studies have clearly indicated that post-harvest quality of each cane genotype is an important parameter and it is variable. Therefore, it is an ardent need to develop post-harvest quality profile of each sugarcane variety/genotype. Singh and Solomon (2003) also viewed accordingly.

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