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Sugar Profile of Sugarcane Culm during Maturity

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The reducing and non reducing sugars in sugarcane juice and their changes in relation to maturity (9th to 13th month after planting) were analysed using HPLC in the popular sugarcane variety Co 86032. In CO 86032 sugarcane juice, no peaks of maltose, xylulose were detected while glucose, fructose and sucrose peaks were seen at 4.11, 3.76 and 4.79 retention times using individual sugar as standard. Maximum sucrose accumulation of 228.58 mg/g of dry weight occurred at 10th month after planting (MAP); after which the stored sucrose concentration reduced to 62.08% at 13 month after planting. With maturity, the activity of sucrose synthesizing enzymes, cell wall invertase, neutral invertase decreased while, the acid invertase activity was found to increase. Comparing the two reducing sugars in CO 86032 juice samples, fructose concentration was increasing till 10th month after planting and after which glucose concentration started increasing. At the time of maturity the cane juice tasted sweeter due to the high content of sucrose and fructose contents as fructose has relatively 1.73 times higher sweetness to sucrose.

Key words: Sugarcane, Sucrose, Glucose, Fructose, Co 86032 and HPLC

Sugar is used as the most plentiful sweetener. About 78 % of world sugar is produced from sugarcane (Saccharum officinarum) and is planted commercially in tropical and sub-tropical areas (Chandra et al., 2012). The primary use for sugarcane is to extract sucrose which can be utilized in a range of products. To recover maximum amount of sucrose, sugarcane needs to be harvested at appropriate time. Any delay in harvest, results in sudden decline in stored sucrose content and taste changes; thereby, reducing the quality of sugarcane juice. Hence, the present study was carried out with a view to identify the different sugars present in the sugarcane juice; their relative changes with maturity in relation to the sucrose metabolizing enzyme activities and to recommend the harvesting period of sugarcane.

Materials and Methods

Plant material

Sugarcane variety, Co 86032 was planted and maintained following the recommended agricultural practices. Mature stalks with approximately 18 internodes in each were randomly selected and harvested. The samples were collected during maturity phase from 9th to 13th MAP at monthly intervals. The samples were collected between 7 to 8 Am to minimize the diurnal variation in enzyme activity and sugar levels.

Sample preparation

The sugarcane juice was extracted mechanically using juice extractor from the matured culms of Co 86032 variety during maturity from 9th to 13th month after planting. The samples were boiled for 5 minutes to destroy activities of all enzymes and centrifuged at 13,000 rpm for 10 minutes. The supernatant served as the source for sugar analysis. The sugar standards namely, glucose, fructose, sucrose, xylulose and maltose at different concentrations (100, 250, 500, 750 and 1000 ppm) were prepared in HPLC grade water.

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HPLC determination of sugars

HPLC analysis was performed in Shimadzu LC20AT HPLC equipped with class VP software according to Agblevor et al. (2007). About 20 µl of the sample was chromatographed on amino column (30 cm x 3 mm i.d) using acetonitrile:water (76:24, v/v) as mobile phase at the flow rate of 1.5 ml/min. Detection was performed using Evaporative Light Scattering Detector (ELSD) adopting evaporation T – 70 °C, nebulisation T– 40 °C and gas pressure – 350 kPa. The sugar concentration (mg/g) was calculated using the formula given below:

$$\text{Sugar concentration (mg/g)} = \frac{\text{Area of the sample peak}}{\text{Area of the standard peak}} \times \frac{\text{Volume of sugarcane juice extracted}}{\text{Volume of sugarcane juice injected}} \times \frac{\text{Concentration of standard in mg}}{\text{Weight of the sample taken}}$$

Extraction and assay of enzymes

Sucrose Phosphate Synthase (SPS; EC 2.4.1.14) and Sucrose Synthase (SS; EC 2.4.1.13)

Sucrose synthesising enzymes *viz*., Sucrose Phosphate Synthase (SPS) and Sucrose Synthase (SS) were extracted from the stem tissues and assayed employing the modified method of Hubbard *et al.* (1989). SPS assay mix consisted of 10 mM of fructose-6-phosphate, 2.5 mM of UDPG, 0.05 mM of Magnesium chloride and 0.05 M of NaF in 0.05 M of Tris HCl buffer pH 7.5, while SS assay mix

contained 10 mM fructose instead of fructose-6-phosphate as substrate. 0.5 ml of enzyme extract was added to the assay mix and incubated at 37 °C for 30 minutes after which the reaction was stopped by adding 30 % KOH. Similarly a control was maintained without UDP-Glucose. The tubes were heated for 10 minutes to destroy fructose-6-Phosphate and cooled immediately. The sucrose produced by these reactions was assayed using the anthrone method of Van Handel (1968). SPS and SS enzyme activities were expressed in units, where 1 unit = 1µg of sucrose formed per minute per mg of protein.

Invertases (EC 3.2.1.26)

Soluble Acid Invertase (SAI) and Neutral Invertase (NI) activities were isolated by the method of Hatch and Glasziou (1963). Activity of AI was assayed at 37 °C in 0.1 M sodium citrate buffer (pH 5.2) and 0.2 M sucrose. The assay system was same for NI except that 0.1 M potassium phosphate buffer (pH 7.0) was used instead of 0.1 M sodium citrate buffer (pH 5.2). Cell wall invertase (CWI) was extracted by the method of Batta *et al.* (2008). CWI activity was assayed employing 0.5 M sucrose and 50 mM Sodium acetate buffer (pH 5.5) at 37 °C. The reaction was stopped after 20 minutes by heating at 90 °C and the amount of reducing sugars produced were determined using Nelson's arsenomolybdate reagent (Nelson, 1944). Invertase enzyme activities were expressed in units, where 1 unit = 1µg of glucose released per minute per mg of protein.

Determination of total soluble protein

Total soluble protein was measured according to Bradford (1976) using bovine serum albumin as standard.

Results and Discussion

Enzymatic changes during sucrose accumulation

Sucrose accumulation in sugarcane storage tissue usually occurs during the last phase of growth (maturation stage). Cane serves as the sink for synthesized and transported sugar from source (leaf tissue) and has a major role in sugar accumulation. In Co 86032, maximum sucrose accumulation of 228.58 mg/g of dry weight was seen at 10 MAP and after which the stored sucrose content started to decrease gradually at the rate of 15.39 %, 14.25 % and 47.72 % at 11th (193.4 mg/g of dry weight), 12th (165.83 mg/g of dry weight) and 13th MAP (86.68 mg/g of dry weight) respectively (Table 1; Fig. 1). Hence, the optimum time of harvest for Co 86032 variety was 10 MAP

Table 1. HPLC analysis of sugars in Co 86032 during maturity

Sugars	Sugar in mg/g of dry weight				
	9 MAP	10 MAP	11 MAP	12 MAP	13 MAP
Sucrose	214.43	228.58	193.40	165.83	86.68
Glucose	0.06	0.05	0.72	0.38	0.15
Fructose	0.22	0.11	0.48	0.24	0.08
Glucose + Fructose (Reducing Sugars)	0.28	0.16	1.20	0.62	0.23

MAP – Month after planting

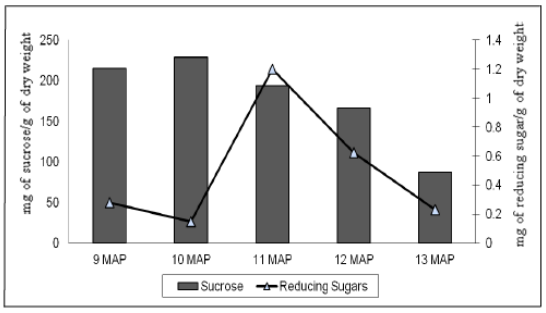


Fig.1. Amount of sucrose and reducing sugars in Co 86032 variety during maturity

for maximum sugar recovery in mills. This maximum sucrose accumulation in Co 86032, as early in 10 MAP makes Co 86032, a suitable variety for early harvest in southern states of India.

Sucrose synthesizing enzymes *viz*., Sucrose Phosphate Synthase (SPS) and Sucrose Synthase (SS) were found to decrease with maturity. SPS activity decreased from 52.06 units at 9 MAP to 5.86 Units at 13 MAP, while the SS activity in synthetic direction decreased from 7.01 to 1.20 units till 10 MAP, where maximum sucrose accumulation of 228.58 mg/g of dry weight occurred. Afterwards, its activity in cleavage direction started to increase (Fig. 2). SS is found to be active in cleavage and synthesis direction depending upon the substrate (sucrose) concentration (Schafer *et al*., 2004). A similar report of negative correlation between SS activity and stored sucrose content was reported by Verma *et al*.,2011.

Invertases are the major sucrose degrading enzymes. Acid Invertase (AI) activity in the matured sugarcane culm was almost equal (about 1.22 - 1.60 units) till 12 MAP after which its activity reduced to 0.24 units (Fig. 2). High AI activity at peak sucrose

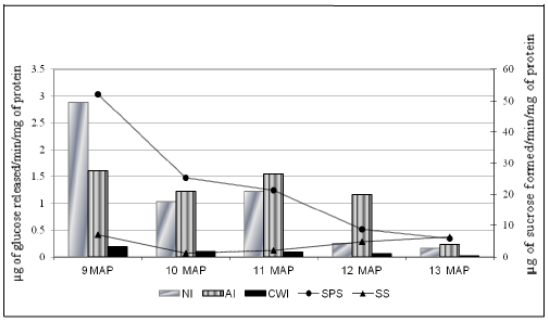


Fig.2. Sucrose accumulation and its related enzyme activities in Co 86032 variety during maturity

accumulation helps in the remobilization of stored sucrose from the vacuole and regulates the hexose levels in certain tissues (Singh and Kanwar, 1991); thereby, maintaining the life of plant at reduced or no photosynthate supply towards the crop end. The lower AI level in Co 86032 helps in storing higher sucrose making it a popular variety for sugar mills giving the maximum sucrose recovery. Higher AI activity and low sucrose content in the *Saccharum* group was reported by Pan *et al*., (2009).

Neutral invertase activity was high compared to AI at 9 MAP and later it was found to decrease constantly from 9 MAP (2.89 units) to 13 MAP (0.16 units). Since NI was involved in vascular transport of sucrose, NI activity was higher than AI during maturation phase and also in immature internodes (Rose and Botha, 2000). CWI activity was found to decrease constantly from 0.19 (at 9 MAP) to 0.02 units (at 13 MAP). CWI was reported to be involved in the apoplasic and symplasmic sucrose translocation between sources and sink tissues (Roitsch *et al.,* 1995). Hence, with the reduction in photosynthate supply from the sink tissue, the CWI activity also got reduced with maturity.

The decrease in stored sucrose content is due to the reduced Sucrose Phosphate Synthase (SPS) activity and increased acid and neutral invertase activities (Chandra *et al.,* 2012). The feedback inhibition of SPS activity by stored sucrose in early maturing variety as shown by Huber and Huber (1996) and higher activity of invertases (involved in the regulation of hexose levels) in the matured culms leads to the degradation of stored sucrose (Singh and Kanwar, 1991; Rose and Botha, 2000).

Sucrose and Reducing sugar contents

HPLC determination of different sugars in the juice of sugarcane variety Co 86032 showed no peak for maltose and xylulose; but glucose, fructose and sucrose peaks were seen at 4.11, 3.76 and 4.79 (Fig. 3a, b) retention times (RT) in comparision with

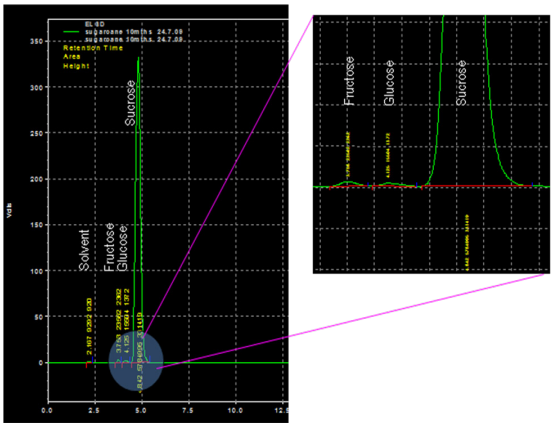


Fig. 3a. Enlarged HPLC analysis view of sugarcane juice from early maturing variety, Co 86032 at 10 MAP

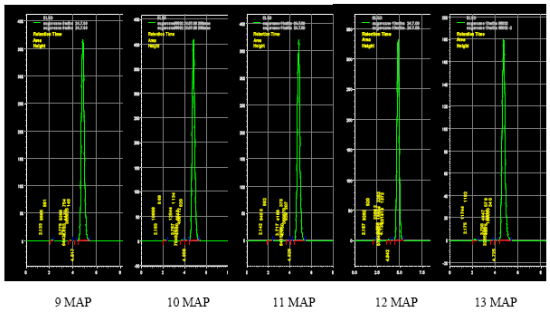
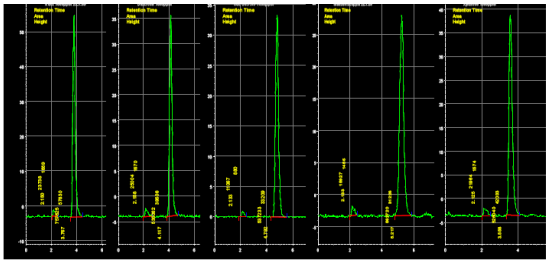


Fig. 3b. HPLC analysis of sugarcane juice from early maturing variety, Co 86032 during maturity

standard sugars (Fig. 4). Hence, sucrose, glucose and fructose were the only free sugars detected from sugarcane juice and among these sucrose



[Solvent peak was seen at 2.1 RT (Retention Time) in all the standards]

Fig. 4. HPLC analysis of sugar standards at 1000 ppm concentration

was found to be the predominant sugar (Batta *et al.,* 2008). Reducing sugar (mostly glucose and fructose) concentration was inverse in relation to sucrose content during maturity and a similar report was given by Batta and his co-workers in 2002. The amount of glucose and fructose in the Co 86032 juice samples was measured. Among the reducing sugars, fructose concentration was high compared to glucose till 10th MAP (Table 1), after which the glucose concentration increased over fructose content (fructose - 0.48, 0.24, 0.08 mg/g of dry weight, glucose – 0.72, 0.38, 0.18 mg/g of dry weight in 11th, 12th and 13th MAP respectively; Table 1; Fig. 5).

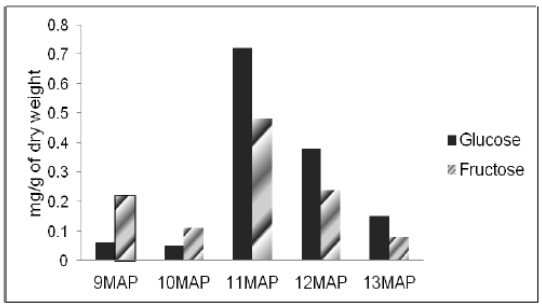


Fig. 5. Amount of glucose and fructose in Co86032 variety during maturity

Fructose, popularly known as 'fruit or honey sugar' has relatively higher in sweetness and is 1.73 times as sweet as sucrose. Hence, at the time of maturity the cane juice tasted sweeter due to the higher sucrose and fructose content. Af the time of maturity (10 MAP) sucrose and the relative concentration of fructose in the reducing sugar pool decreased, and glucose level increased. This leads to the poor taste of sugarcane juice with delayed harvest or over ripened culms. To develop cane varieties with superior juice quality, the concentration of sucrose and fructose need to be increased. Many approaches have been made to prevent the degradation of stored sucrose concentration by down regulation of acid and neutral invertase activities (Botha *et al*., 2001; Rossouw *et al*.,2007; Rossouw *et al*., 2010) and ectopic expression of a high value sugar, isomaltulose, which is resistant to invertase action (Wu and Birch, 2007). To increase the fructan

concentration, 1-SST (sucrose: sucrose 1- fructosyltransferase, EC 2.4.1.99) and 1-FFT (fructan: fructan 1-fructosyltransferase, EC 2.4.1.100) were targeted in potato and sugarbeet (Hellwege *et al*.,1997; Sévenier *et al*., 1998; Hellwege *et al*., 2000).

The results of the present study clearly shows that the early sugarcane varieties attain their maximum sucrose accumulation as early as 10 MAP, after which the stored sucrose get degraded by various enzymes involved in sucrose catabolism. Hence, any delay in cutting order may lead to considerable sucrose loss in the field, leading to low sugar recovery in mills, in turn low income to farmers. Hence, to increase the sugarcane juice quality, degradation of stored sucrose should be prevented. The concentration of fructan, the high value sugars need to be increased. With the advent of molecular biology tools new sugarcane varieties with superior juice quality for maximum sugar recovery in mills, can be developed in future.

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