

Chapter XIII: Assessing sweet sorghum juice and syrup quality and fermentation efficiency

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I. Introduction

Sweet sorghum is a C₄ crop with high photosynthetic efficiency with a unique ability of high carbon assimilation (50 g m⁻² day⁻¹) and accumulates high concentrations of easily fermentable sugars (glucose, fructose and sucrose) in the stalks. Hence, it is widely believed that it is an alternate energy source that is renewable, sustainable, efficient, cost-effective, convenient and safe to use. Sucrose is the major sugar in sweet sorghum juice which constitutes up to 85% of the total sugars (Woods 2000). The sugar yields ranged between 1.6 to 13.2 Mg ha⁻¹, with significant variations observed between years and regions (Jackson et al. 1980; Reddy et al. 2007; Zhao et al. 2009). The juice sugar content is dependent on the crop stage, because fructose is more abundant at the early development stage, whereas sucrose tends to be dominant after heading (Sipos et al. 2009). The sweet sorghum juice sugar content ranged from 10 to 25 Brix% at maturity (Reddy et al. 2007; Ritter et al. 2004). Research at the International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT) showed that sweet sorghum juice yield ranges between 16.8 to 27.2 m³ ha⁻¹ (Reddy et al. 2007) and accrues about 23% additional returns vis-à-vis grain sorghum (Rao et al. 2009).

II. Postharvest losses

Postharvest deterioration of sweet sorghum stalk, both qualitatively and quantitatively is a problem limiting the sustainability of the sweet sorghum value chain. If the time lag between harvesting to milling of the sorghum stalk is between 2 to 4 days, then it leads to huge losses in the recoverable sugars due to deterioration and souring of the harvested stalk. Weather conditions such as high temperatures and humidity also have a great impact on the stalk deterioration in tropics. In sweet sorghum, it has been observed that quality losses in stalk is primarily due to chemical (acid) and enzymatic

inversion where the sucrose could be hydrolyzed to the respective reducing sugars (glucose and fructose) by the acid invertase enzyme (acid inversion of sucrose) which is secreted by few yeast species like *Saccharomyces* (Rao et al. 2012). As the stalk deteriorates the stalk deterioration products such as invert sugars, polysaccharides (eg, dextran, levan) and microbial contaminants (eg, ethanol and lactic acid formation) increase, all of which has a negative effect on processing. The primary disadvantage of the sweet sorghum value chain is the short shelf life of the juice due to its high sugar content which favors contamination by the spoilage microbes. Thus, the preservation and storage of sweet sorghum juice is needed for its further utilization in ethanol production (Wyman and Goodman 1993; Rao et al. 2012). Therefore, this chapter discusses the critical areas of sustainability of sweet sorghum value chain such as genetic variability of sugar yield vis-a-vis phenology, juice and syrup preservation, fermentation efficiency etc. The chemical analysis of the juice and syrup were determined using standard methods and procedures (Dubois et al. 1956, Miller 1959; Kumar et al. 2010).

III. Dynamics of sugar yield vis-a-vis phenology

The analysis of variance (ANOVA) revealed that the mean sum of squares of juice yield, Brix%, sugar yield, sucrose, glucose and fructose contents, and pH were significantly ($P \leq 0.05$) different at all the three different phenological stages, ie, dough, physiological maturity and post-physiological maturity (Table 1) across 19 improved cultivars indicating quantitative and qualitative changes in sugar yield and allied traits vis-a-vis crop phenology. The genotypes evaluated also exhibited highly significant ($P \leq 0.01$) differences for sugar related traits. However, there is significant genotype x stage interaction for juice yield, Brix% and glucose content, at $P \leq 0.05$ level, while highly significant genotype x stage interaction was observed for sugar yield, sucrose and fructose levels besides pH ($P \leq 0.01$). This data suggests that there is high degree of variability among the genotypes for the sugar yield and its components and offers opportunity to harness high sugar yield owing to genotypic differences, stage-wise differences and also from the significant interaction of genotype with phenological stage for sucrose content.

Table 1. Analysis of variance (ANOVA) for metric traits and biochemical parameters at three phenological stages.

Source	DF ⁺	MS ⁺⁺ for juice yield (t ha ⁻¹)	MS for Brix%	MS for sugar yield (t ha ⁻¹)	MS for sucrose (%)	MS for glucose (%)	MS for fructose (%)	MS for pH
Stage	2	546.45**	108.28**	13.24**	159.79**	8.99**	2.49**	0.95**
Replication	6	17.51*	1.12	0.15	0.87	0.28	0.12	0.29**
Genotype	18	25.22**	25.14**	1.08**	5.46**	0.52**	0.19	0.55**
Genotype x Stage	36	6.38*	3.97*	0.39**	4.27**	0.33*	0.28**	0.14**
LSD		3.34	2.59	0.36	0.52	0.15	0.13	0.09

⁺DF: Degrees of freedom; ⁺⁺MS: Mean squares

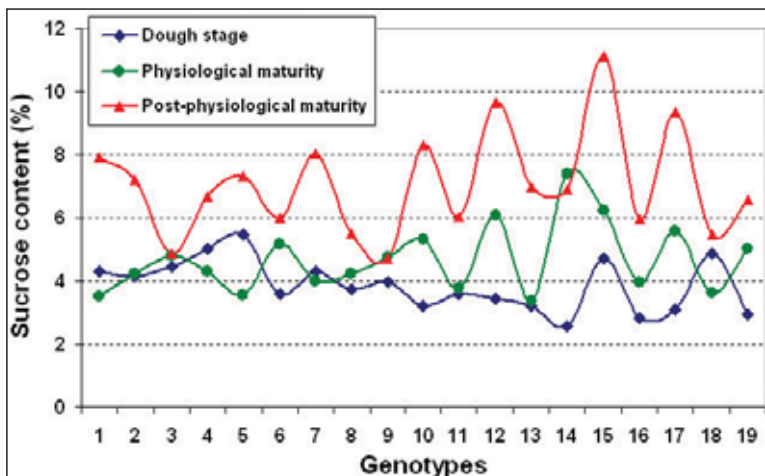
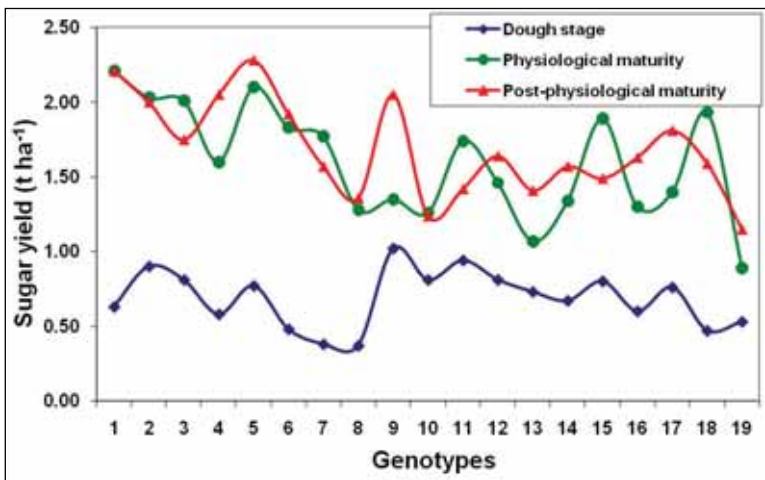
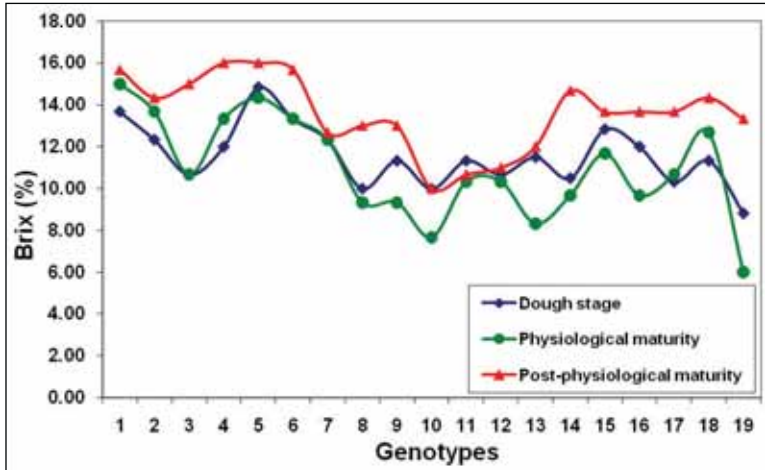
The juice yield at dough stage was highest and its variation among the 19 genotypes ranged between 3.03 (SP 4511-2) and 9.03 t ha⁻¹ (ICSA 38 x ICSV 700); while the Brix (%), in the juice varied between 8.83 (JK Recova) and 14.83 (SP 4495) (Fig. 1a); sugar yield ranged between 0.37 (ICSA 84 x E 36-1) and 1.02 (ICSA 38 x ICSV 700) (Fig. 1b). The sucrose content (%), a major disaccharide in sweet sorghum juice that contributes to the bulk of non-reducing sugars, ranged between 2.58 (ICSA 702 x SSV 74) and 5.48% (SP 4495) at dough stage (Fig. 1c); The glucose content (%), a major monosaccharide in sweet sorghum juice which has a significant bearing on the ethanol yield, showed variation in a narrow range of 1.12 (ICSA 702 x SSV 74) and 2.94 (CSH 22SS) at dough stage (Fig. 1d). Another prominent monosaccharide in the juice, fructose (Fig. 1e) ranged between 1.05 (ICSA 702 x SSV 74) and 2.39% (CSH 22SS), while the pH was in a range of 4.97 (ICSA 38 x ICSV 700) and 5.6 (ICSA 475 x SSV 74) (data not shown).

The juice yield at physiological maturity among the 19 genotypes ranged between 12.08 (SS 2016) to 18.41 t ha⁻¹ (SP 4487-3) with a mean of 14.64 t ha⁻¹; while the Brix (%) varied between 6.0 (JK Recova) and 15.0 (SP 4495) (Fig. 1a); sugar yield ranged between 0.89 (JK Recova) and 1.99 (ICSA 38 x ICSV 700) (Fig. 1b). The sucrose content (%) varied between 3.34 (ICSA 475 x NTJ 2) and 6.07 (ICSA 474 x SSV 74) at physiological maturity (Fig. 1c); The glucose content (%) showed variation in a narrow range of 0.83 (SP 4511-2) and 1.73 (JK Recova) with a mean of 1.53 showing a sharp decline of over

36.1% compared to that of dough stage (Fig. 1d). Fructose (Fig. 1e) ranged between 1.05 (ICSA 702 x SSV 74) and 2.39 % (CSH 22SS) with a mean of 1.59% showing a moderate increase of 16.1%, while the pH was in a range of 4.22 JK Recova) and 5.73 (SP 4511-3).

At post-physiological stage, the Brix (%) varied between 10.67 (ICSA 675 x ICSV 700) and 15.67 (SP 4511-3 and SP 4511-2) with a mean of 13.60% (Fig. 1a); sugar yield ranged between 1.15 (JK Recova) and 2.28 t ha⁻¹ (SP 4495) with a mean of 1.69 tha⁻¹ showing an increase of 146% over that of dough stage and 5.5% over that of physiological maturity (Fig. 1b). The sucrose content (%) varied between 4.73 (ICSA 38 x ICSV 700) and 11.15% (ICSA 475 x SSV 74) at post-physiological maturity (Fig. 1c) while the glucose content (%) showed variation in a narrow range of 1.07 (ICSA 475 x SSV 74) and 2.26 (ICSV 93046) (Fig. 1d). Another monosaccharide in sweet sorghum juice, fructose (Fig. 1e), ranged between 0.95 (JK Recova) and 1.67% (ICSA 675 x ICSV 700) while the pH was in a range of 4.97 (ICSA 38 x ICSV 700) and 5.6 (ICSA 475 x SSV 74) (data not shown).

The overall mean of total soluble solids i.e., Brix% was marginally high at dough stage, 11.57% vis-a-vis 10.96% at physiological maturity, but majority of the genotypes recorded the highest Brix% at post-physiological maturity as vindicated by the highest mean Brix% value of 13.6 owing to rapid accumulation of sucrose from dough stage (3.86%) to physiological maturity (4.67%) and also to post-physiological maturity (7.08%). It is reported in the literature that sucrose begins to accumulate after heading and shows maximum accumulation after the soft dough (McBee and Miller 1982) because the developing panicle represents a less competitive sink than elongating internodes (Lingle 1987). It was observed that there was about a two-fold increase of sucrose component in all the genotypes at post-physiological maturity ranging from 4.74% (ICSA 38 x ICSV 700) to 11.15 % (ICSA 475 x ICSA 74). A perusal of experimental data revealed that the reducing sugars, i.e., glucose and fructose, did not increase significantly ($P \leq 0.05$) from dough stage to either physiological or post-physiological maturity in the 19 improved sweet sorghum varieties and hybrids. The mean glucose levels fluctuated between 1.35% at physiological maturity, 1.9% at post-physiological maturity, but peaking at dough stage (2.12%). However, the fructose level is highest at physiological maturity, 1.6% followed by dough stage 1.37% and post-physiological maturity, 1.18%. A bird's eye view of the overall data supports the observation that the relative percentages of each sugar present in the juice were approximately 70%, 20%



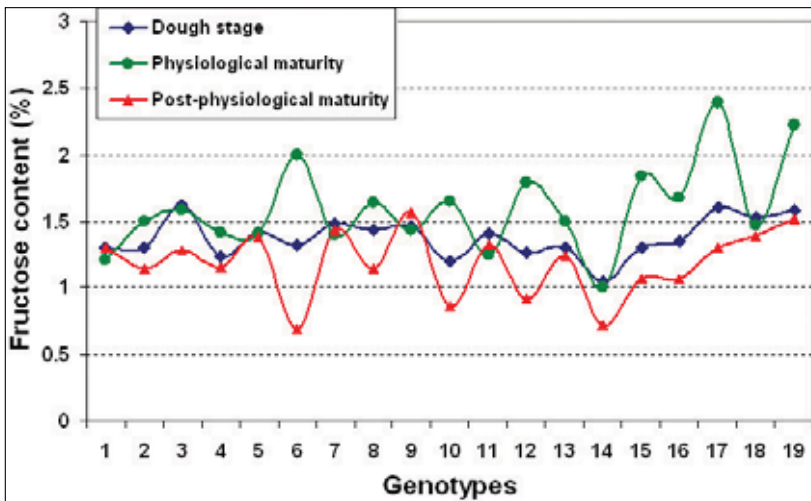
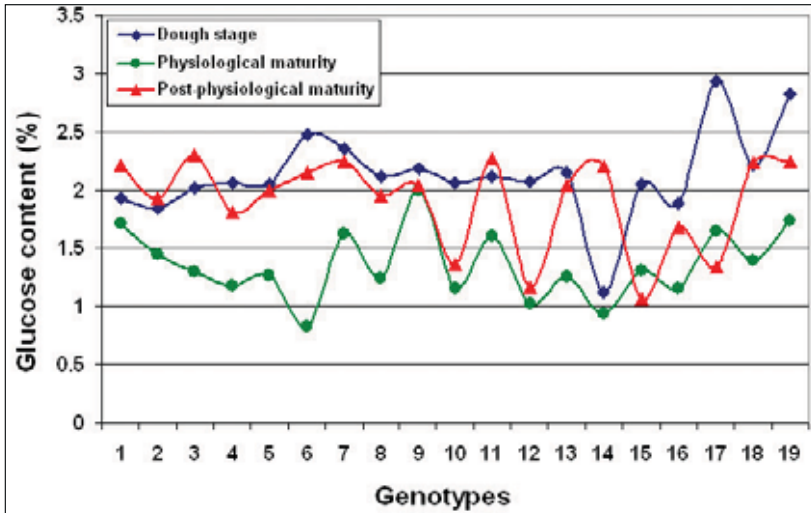


Fig. 1. Performance of sweet sorghum genotypes for (a) Brix (%) (b) sugar yield ($t\ ha^{-1}$) (c) sucrose content (%), (d) fructose content (%) and (e) glucose content (%) in three phenological stages (dough stage, physiological maturity and post-physiological maturity).

and 10% for sucrose, glucose and fructose, respectively. The incremental rise in sugar content during the physiological maturity stage has been attributed to decrease in the activity of amylases due to the aging processes and increase in temperatures during the maturation of the crop (Ikegaya et al. 1994; Channappagoudar et al. 2007). These observations shed light on the extent of variability for different sugars at three phenological stages and provide new window of opportunity in hybrids like ICSA 475 x SSV 74, ICSA 38 x ICSV 700 and varieties such as SP 4495 and SP 4511-3.

IV. Standardizing the storage conditions

Fresh sweet sorghum juice samples of two different cultivars, ICSV 93046 and CSH 22SS, stored at 4 and 15°C did not show any sugar losses, while marginal sugar losses were observed in juice samples stored at room temperature even after 24 hours of storage for both un-stripped and stripped juice samples, in case of both cultivars, ICSV 93046 and CSH 22SS (Table 2). It is concluded that temperature of 15-18°C would be ideal for storage of fresh sweet sorghum juice after crushing.

Table 2. Storage conditions of fresh sweet sorghum juice of two different cultivars, ICSV 93046 and CSH 22SS, at different time and temperature intervals.

S. No.	Cultivar	Temp. (°C)	Brix (%)	pH	Glucose (%)	Fructose (%)	Sucrose (%)
After 4 h							
1	ICSV 93046 (Unstripped)	4	14	5.71	2.36	2.49	5.47
		15	14	5.72	2.39	2.52	5.52
		RT	13.8	5.70	2.37	2.49	5.47
2	ICSV 93046 (UnStripped)		13	5.62	2.57	2.69	5.76
		15	13	5.72	2.62	2.71	5.77
		RT	12.9	5.61	2.58	2.67	5.74
3	CSH 22SS (Unstripped)	4	12	5.48	2.21	2.24	4.61
		15	12	5.63	2.24	2.26	4.62
		RT	11.8	5.46	2.23	2.27	4.59
4	CSH 22SS (Unstripped)	4	11.5	5.37	2.34	2.36	4.82
		15	11.5	5.49	2.36	2.37	4.83
		RT	11.4	5.35	2.32	2.36	4.81

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Table 2. Storage conditions of fresh sweet sorghum juice of two different cultivars, ICSV 93046 and CSH 22SS, at different time and temperature intervals.

S. No.	Cultivar	Temp. (°C)	Brix (%)	pH	Glucose (%)	Fructose (%)	Sucrose (%)
After 8 h							
5	ICSV 93046 (Unstripped)	4	14	5.72	2.34	2.46	5.46
		15	14	5.73	2.38	2.51	5.52
		RT	13.6	5.70	2.27	2.38	5.39
6	ICSV 93046 (Unstripped)	4	13	5.64	2.54	2.67	5.74
		15	13	5.65	2.59	2.70	5.76
		RT	12.8	5.60	2.46	2.57	5.67
7	CSH 22SS (Unstripped)	4	12	5.49	2.19	2.22	4.60
		15	12	5.50	2.23	2.24	4.61
		RT	11.7	5.46	2.17	2.19	4.47
8	CSH 22SS (Unstripped)	4	11.5	5.37	2.32	2.33	4.81
		15	11.5	5.40	2.34	2.36	4.82
		RT	11.3	5.33	2.24	2.25	4.73
After 24 h							
9	ICSV 93046 (Unstripped)	4	14	5.74	2.32	2.43	5.44
		15	14	5.74	2.38	2.51	5.47
		RT	13.5	5.7	2.19	2.24	5.27
10	ICSV 93046 (Stripped)	4	13	5.65	2.52	2.64	5.72
		15	13	5.67	2.57	2.69	5.74
		RT	12.8	5.6	2.37	2.46	5.54
11	CSH 22SS (Unstripped)	4	12	5.49	2.17	2.21	4.57
		15	12	5.52	2.21	2.23	4.58
		RT	11.7	5.41	2.06	2.08	4.31
12	CSH 22SS (Unstripped)	4	11.5	5.38	2.31	2.31	4.79
		15	11.5	5.41	2.32	2.34	4.81
		RT	11.1	5.3	2.18	2.17	4.64

Studies on syrup quality at different Brix% levels: Syrup samples of different Brix% values were collected from decentralized crushing unit (DCU) located at Ibrahimabad, Medak, Andhra Pradesh, India for storage studies: 4 samples with 40, 50, 60 and 70% Brix and 3 samples with 50, 60 and 65% Brix. Based on these results, it was observed that the syrup of different

Brix% values could be stored for one year; however, a slight deterioration was observed in total soluble sugars (%) and reducing sugars (%) values on storage of these samples. The chromatograms of syrup of 2008K are shown in Fig. 2. The chemical analysis of different syrup samples of Kharif (K) seasons for the years 2008, 2009, 2010 and 2011 were analyzed and shown in Table 3.

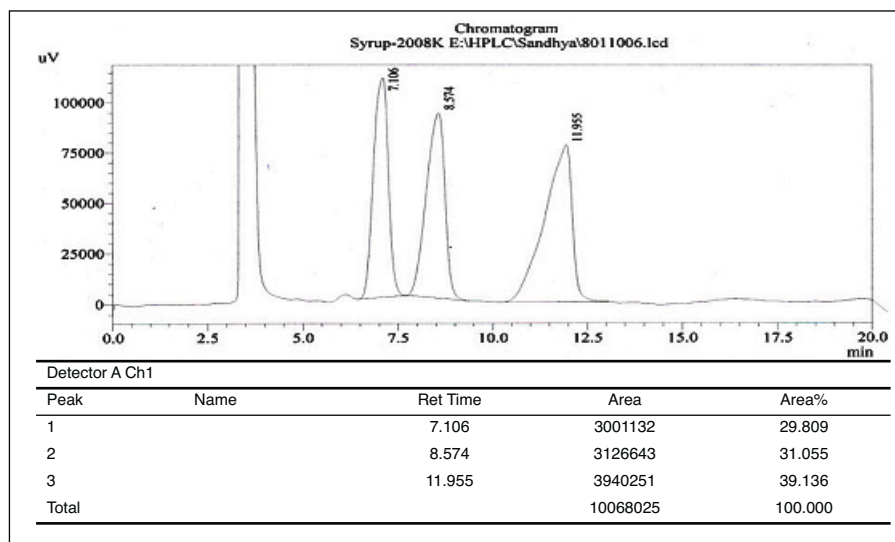


Fig. 2. Chromatogram of syrup of 2008K (1 year stored).

Table 3. Chemical analysis of syrup samples of kharif crop seasons for the years 2008, 2009, 2010 and 2011.

S. No.	Parameters	Analysis Method	2008K			2009K (fresh)	2010K (fresh)	2011K (fresh)
			(Fresh)	(1 year stored)	(2 years stored)			
1	Brix	Brixmeter	85	80.2	80	10	67	75
2	Calorific value	CA ^a	3730	2830	2940	2360	2772	ND
3	TSS (% wt)	UV ^b	75.3	73.2	94.98	42.8	95.12	78.98
4	Total reducing sugars (% wt)	UV	31.3	29.7	32.0	20.4	32.4	ND
5	Ash (% wt)	CA	3.6	3.6	3.05	0.12	2.64	2.84
6	Riboflavin	HPLC ^c	5791	2642	11	1243	10	84
7	Vitamin C (% wt)	IC ^d /HPLC	23	0.7	85	34	33	34

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Table 3. Chemical analysis of syrup samples of kharif crop seasons for the years 2008, 2009, 2010 and 2011.

S. No.	Parameters	Analysis Method	2008K			2009K (fresh)	2010K (fresh)	2011K (fresh)
			(Fresh)	(1 year stored)	(2 years stored)			
8	Nicotinic acid	HPLC	4	4	9	2	2	16
9	Benzoic acid (ppm)	HPLC	38	7	25	2	6	27
10	Iron (ppm)	AAS ^e	76.4	69.6	65.2	45.9	75.1	224.1
11	Calcium (ppm)	AAS	2455	2100	1909	400	770	759
12	Sodium (ppm)	AAS	1945	8400	1515	1300	662	442.9
13	Potassium (ppm)	AAS	11603	17500	9763.5	9300	9870.5	8100
14	Phosphorus (% wt)	CA	0.1	0.005	ND ^g	0.024	ND	ND
15	Sulphur (% wt)	CA	0.0	0.89	ND	0.68	ND	ND
16	Glucose (% wt)	HPLC	16.2	20.62	17.07	18.89	17.50	19.8
17	Fructose (% wt)	HPLC	5.6	17.79	14.93	14.87	14.92	15.3
18	Sucrose (% wt)	HPLC	45.0	20.63	23.62	10.25	16.28	23.4
19	Maltose (% wt)	HPLC	Nil	Nil	Nil	Nil	Nil	Nil
20	Other sugars (% wt) ⁱ	HPLC	Nil	Nil	Nil	Nil	Nil	Nil
20	Free acids (% wt)	Volumetric	0.5	0.52	ND	0.56	ND	ND
21	pH	pH meter	5.6	5.49	5.1	5.47	5.0	5.22

^aCA – Chemical analysis

^bUV – UV Spectroscopy

^cHPLC – High Performance Liquid Chromatography

^dIC – Ion Chromatography

^eAAS – Atomic absorption spectroscopy

^fSugars analyzed: Xylose, Ribose, Galactose, Mannose, Arabinose

^gND – Not determined

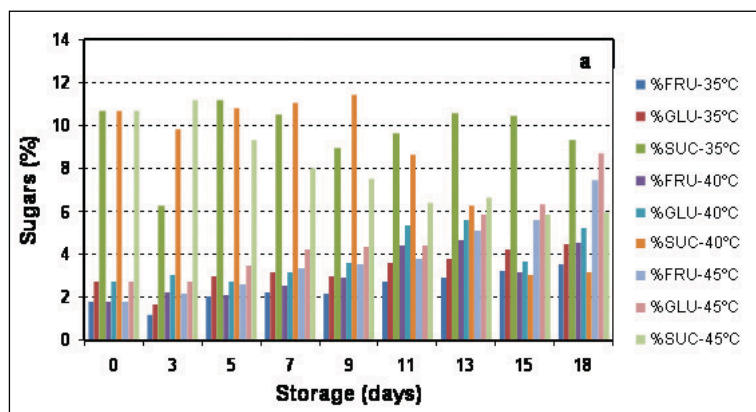
K- Kharif

2. Effect of pasteurization treatment on the shelf life of juice

The percentages of the individual sugars like glucose, fructose and sucrose as a function of time did not reveal much variations in the sugar levels on storage for 10 days (Fig. 3 a-c). The experimental data suggests that pasteurization at 80°C for 10 min and storage of juice at a temperature of 35°C was recommended as a good treatment method for enhancing the storage shelf life of the juice.

3. Effect of chemical preservatives on the shelf life of juice

Storage studies were carried out on sweet sorghum juice samples spiked with different chemical preservatives like benzoic acid, sodium benzoate, sorbic acid, citric acid, sodium citrate and ascorbic acid at 1000 parts per million (ppm). The results on the analysis of the amount of total soluble sugars and the percentages of the individual sugars like glucose, fructose and sucrose as a function of time decreased significantly in the juice samples spiked with citric acid (Fig. 4a), sodium citrate (Fig. 4b), ascorbic acid (Fig. 4c) and benzoic acid (Fig. 4d), as compared to the juice samples spiked with sodium benzoate (Fig. 4e) and sorbic acid (Fig. 4f). It was also observed that the amount of reducing sugars increased, while the amount of non-reducing sugars decreased with increase in the storage time. The fructose and glucose content increased from 1.69% to 3.42% and 3.07% to 5.41%, respectively, while sucrose content decreased from 8.27% to 0.87% in sodium benzoate-spiked samples as depicted in (Fig. 4e). The sorbic acid-spiked samples showed an increase in fructose and glucose content from 1.47% to 3.3% and



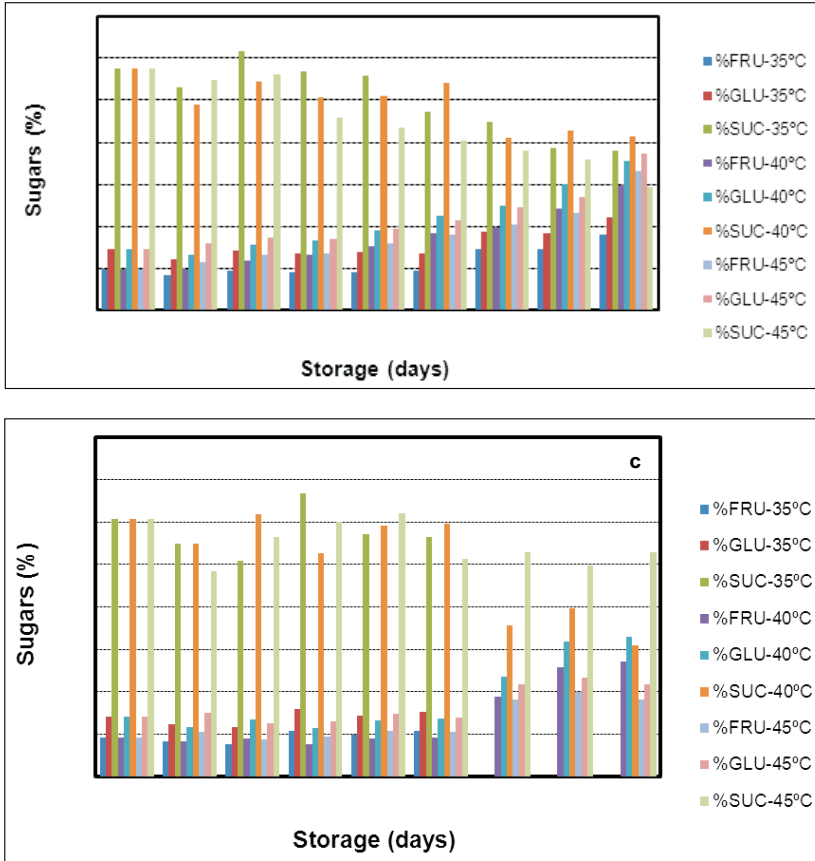


Fig. 3. Effect of pasteurization: (a) 70°C for 15 min, (b) 80°C for 10 min, and (c) 90°C for 5 min, as a function of time on the storage shelf life of sweet sorghum juice.

2.7% to 5.84%, respectively, whereas sucrose content decreased from 7.18 to 1.02% as evident from Fig. 4f. The total soluble sugar content decreased from 13.03% to 9.7% and 11.35% to 10.16% for sodium benzoate and sorbic acid-spiked samples, respectively. Based on these results, sodium benzoate and sorbic acid were identified as most suitable preservatives to enhance the storage shelf life of the sweet sorghum juice for 72 h.

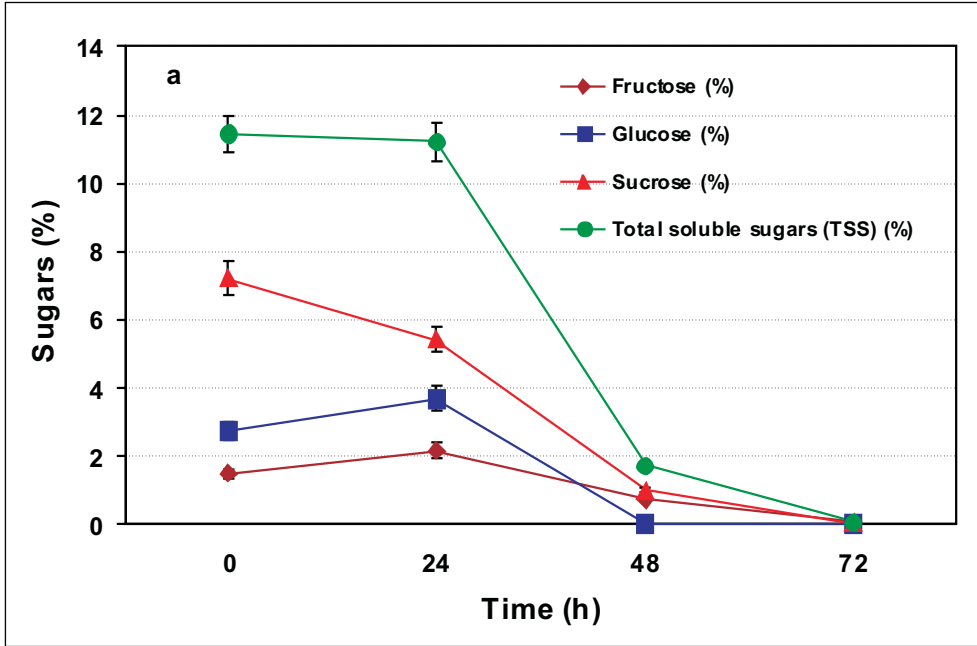


Fig. 4a.

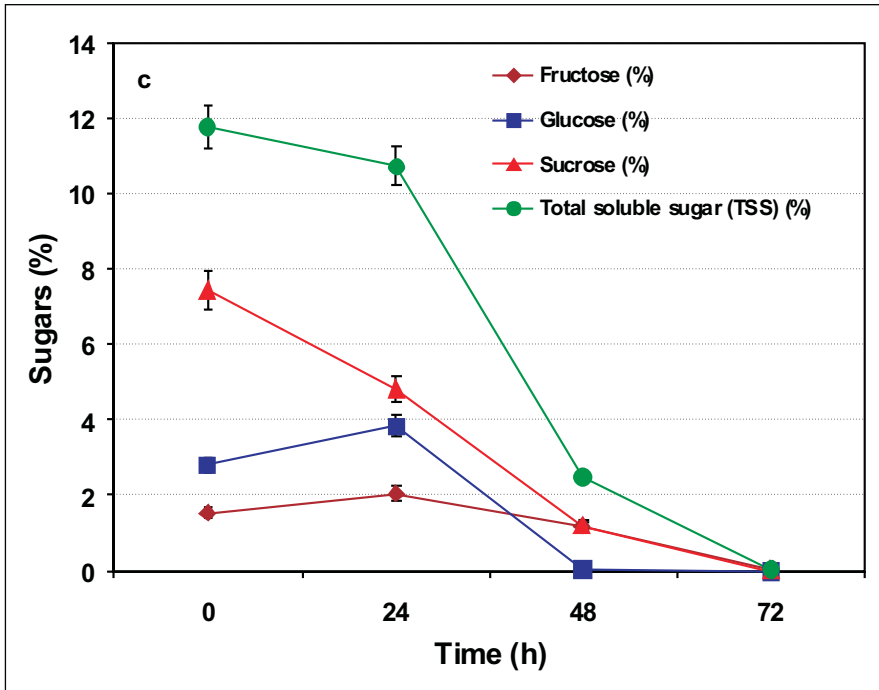


Fig. 4b.

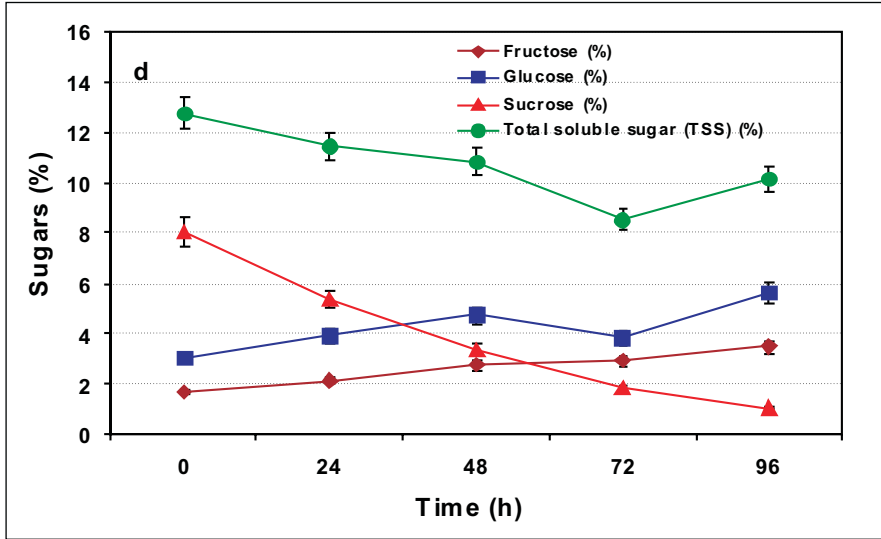


Fig. 4c.

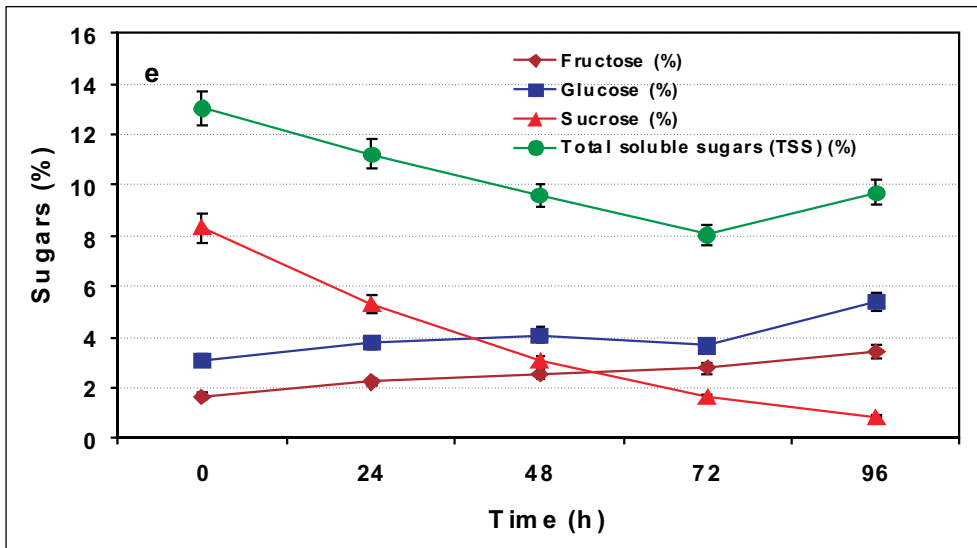


Fig. 4d.

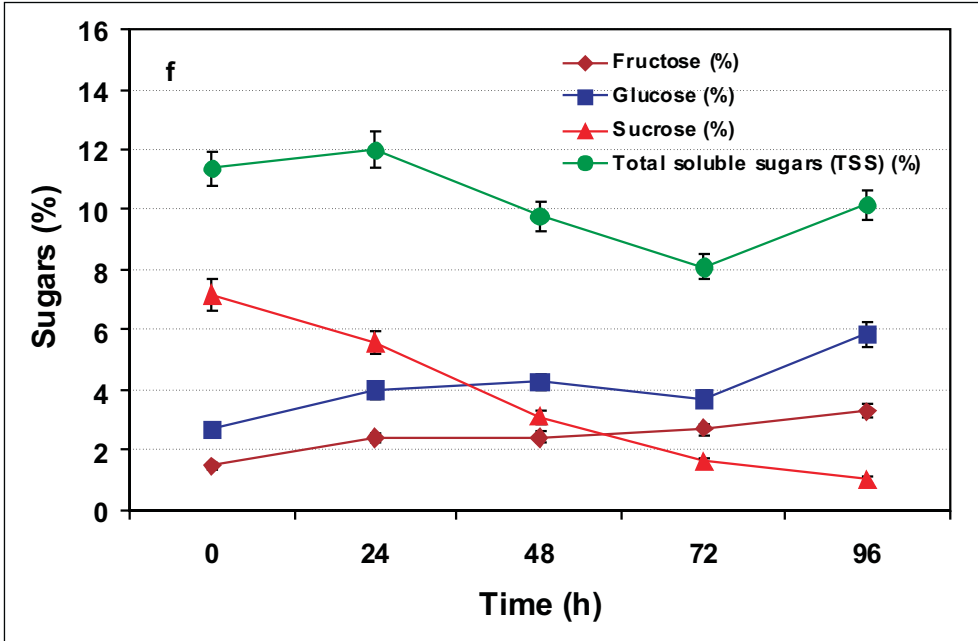


Fig. 4e.

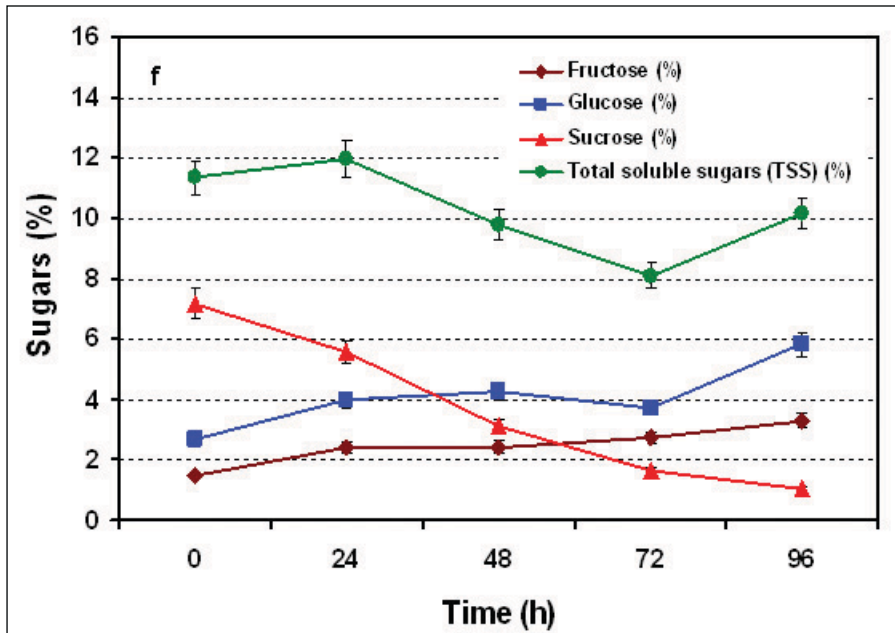


Fig. 4f.

Fig. 4. Sugar analysis as a function of time of juice sample spiked with (a) citric acid, (b) sodium citrate, (c) ascorbic acid, (d) benzoic acid, (e) sodium benzoate, and (f) sorbic acid.

V. Isolation of new yeasts for increased fermentation efficiency

Saccharomyces cerevisiae, the conventional baker's yeast is a Generally Regarded As Safe (GRAS) microorganism that is more tolerant to ethanol than other microorganisms and thus is commonly employed in industrial wine making, brewing and baking processes for the production of ethanol and CO₂ from fermentable sugars like glucose. It is reported that the possible ethanol yield can be 600-650 gallons/acre if all the fermentable sugars in sweet sorghum are converted to ethanol (Imam and Capareda 2010). Different types of yeasts were isolated from the surfaces of different spoiled fruits like mango, apple, grapes etc. (for epiphytic yeasts), toddy juice and sweet sorghum juice. Further, the short-listed isolates were subjected to secondary screening in shake-flasks through submerged fermentation. These isolates were subjected to secondary screening in the basal medium to determine the fermentation efficiency and ethanol yields. Based on this secondary screening, 15 yeast strains were shortlisted as good isolates based on the ethanol yield and fermentation efficiency. The results suggested that two strains (ICTY 417 and ICTY 685) exhibited maximum fermentation efficiency of 93% and 88% with ethanol yield of 0.47 and 0.45 g g⁻¹, respectively, after a fermentation period of 48 h.

The yeast strain, ICTY 417 was further used to ferment sweet sorghum juice of two cultivars (CSH 22SS and ICSV 93046). These studies suggested that undiluted juice (15% Brix) supplemented with mineral salts showed better ethanol yields after a fermentation period of 48 h as compared to the undiluted juice without mineral salts supplementation, diluted juice (1:1) supplemented with mineral salts and diluted juice (1:1) without mineral salts supplementation.

1. Effect of chemical preservatives on ethanol yield and fermentation efficiency

Since sodium benzoate and sorbic acid exhibited more stability as compared to the other tested preservatives further studies were carried out to evaluate their effect in the fermentation process. The fermentation efficiency of the yeast strain, ICTY 414 was further evaluated in presence of two preservatives (sodium benzoate and sorbic acid) on the storage of sweet sorghum juice. The storage studies indicated that there was not much difference in the efficiency

and yield of the fermentation till 96 h as compared to the samples without the addition of preservatives. The ethanol yield remained in the range of 0.425-0.475 g g⁻¹ in sodium benzoate-spiked samples (Fig. 5), which showed an optimal efficiency of 93%, while in case of sorbic acid-spiked samples (Fig. 6) the ethanol yield was in the range of 0.405-0.445 g g⁻¹ which corresponded to an optimal efficiency of 92%. In case of control (without preservatives), the ethanol yield was 0.36 g g⁻¹ which declined to 0.26 g g⁻¹ after 96 h of storage and the optimal efficiency reduced to 57%. The initial sugar levels determined in fresh juice was 150 mg ml⁻¹ and the left over sugars in the juice after fermentation ranged between 28-33 mg ml⁻¹. Overall the sodium benzoate-spiked samples showed comparatively better results than sorbic acid-spiked samples. Therefore, it is recommended that the addition of sodium benzoate as chemical preservative is necessary to prevent the spoilage by microbial contamination and thus extend the shelf life of the sweet sorghum juice under ambient temperature conditions.

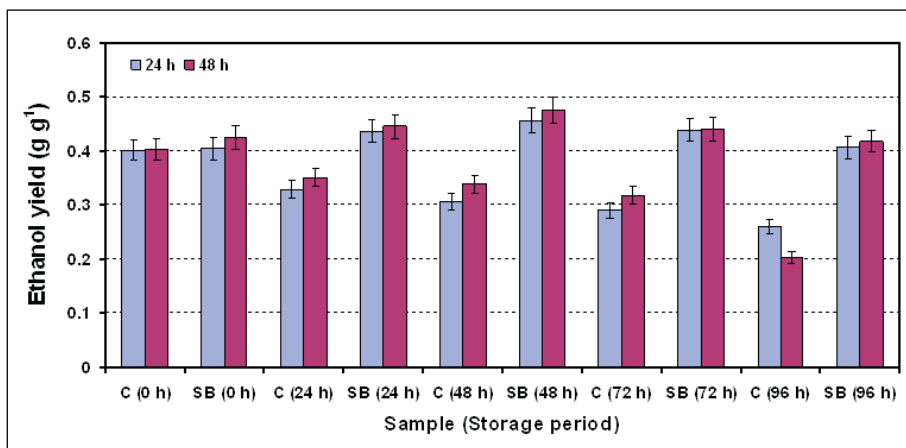


Fig. 5. Ethanol yield as a function of fermentation time with and without sodium benzoate-spiked juice samples.

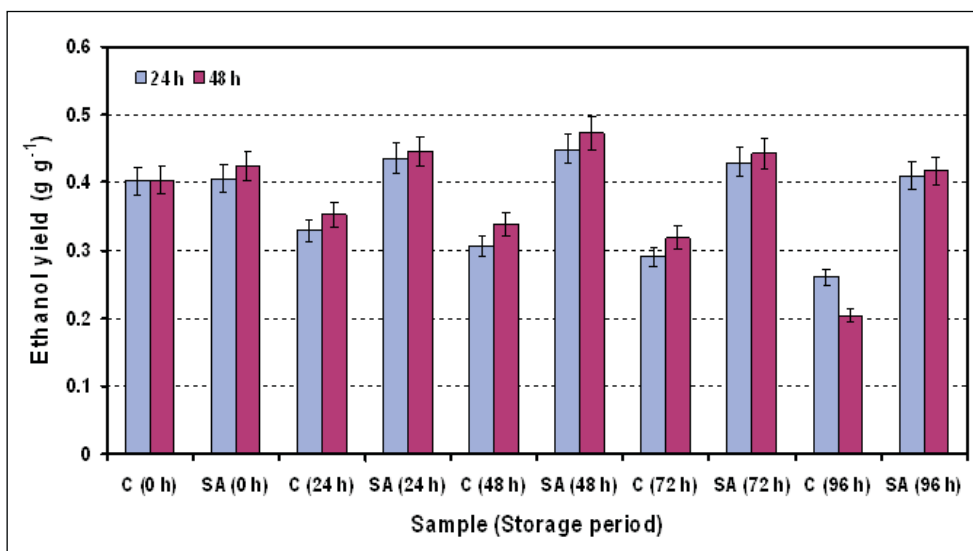


Fig. 6. Ethanol yield as a function of fermentation time with and without sorbic acid-spiked juice samples.

VI. Conclusions

In sweet sorghum, there is a significant genotype x stage interaction for juice yield, Brix%, sugar yield, glucose content, sucrose content and glucose content that can be exploited favorably by a centralized sweet sorghum ethanol distillery. A temperature of 15-18°C would be ideal for storage of fresh sweet sorghum juice. The sweet sorghum syrup with > 65% Brix is better for storage under ambient conditions without deterioration in quality. Two yeast strains (ICTY 417 and ICTY 685) exhibited maximum fermentation efficiency of 93% and 88% with ethanol yield of 0.47 and 0.45 g g⁻¹, respectively, after a fermentation period of 48 h. Based on the experimental results, sodium benzoate and sorbic acid at 1000 ppm were identified as most suitable preservatives to enhance the storage shelf life of the sweet sorghum juice for 72 h.

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