RESEARCH ARTICLE

Chemical Preservatives-Based Storage Studies and Ethanol Production from Juice of Sweet Sorghum Cultivar, ICSV 93046

C. Ganesh Kumar · P. Srinivasa Rao ·
Soma Gupta · Jayalakshmi Malapaka ·
Ahmed Kamal

Abstract Sweet sorghum is an ideal feedstock for ethanol production, in view of the increased global demand for biofuels. Sweet sorghum juice is a perishable commodity and the stalk juice has a short shelf life (4–5 h) post-crushing due to its high fermentable sugar content and the rapid sugar degradation during storage is due to the metabolic activities of contaminating spoilage bacteria. Hence, the preservation of the juice is required for quality retention and to extend the storage shelf life of the juice. In the present study, the effect of chemical preservatives to extend the storage stability of sweet sorghum juice and its later bioconversion to ethanol was studied. Among the chemical preservatives evaluated, the juice samples spiked with sodium benzoate and sorbic acid delayed the increase in reducing sugars and thus prevented browning of juice during storage. Sodium benzoate and sorbic acid-spiked samples showed a decrease in the total sugar content from 13.03 to 10.7 % and 11.35 to 10.16 %, respectively, over a storage period of 96 h. Ethanol yield was in the range of 0.425–0.475 g g^{-1} and 0.405–0.445 g g^{-1} with optimal efficiency of 93 and 92 % for sodium benzoate and sorbic acid, respectively, while the control showed a reduction in yield from 0.36 to 0.26 g g^{-1} and efficiency by 57 %. Sodium benzoate (at 1,000 ppm concentration) was identified as suitable preservative to retain the quality and extend the storage shelf life of fresh sweet sorghum juice up to 2 days at 37 °C.

Keywords Sweet sorghum juice · Sodium benzoate · Sorbic acid · Preservatives · Storage · Ethanol

C. Ganesh Kumar and P. Srinivasa Rao these authors contributed equally.

C. Ganesh Kumar (✉) · S. Gupta · J. Malapaka · A. Kamal
Chemical Biology Laboratory, CSIR-Indian Institute of
Chemical Technology, Uppal Road, Hyderabad 500007, India
e-mail: cgkumar@iict.res.in; cgkumar5@gmail.com

P. Srinivasa Rao
International Crops Research Institute for the Semi-Arid Tropics,
Patancheru, Hyderabad 502324, India

Introduction

Sweet sorghum (*Sorghum bicolor* (L.) Moench) can be cultivated in tropical, sub-tropical, temperate, semi-arid regions as well as in marginal and poor quality soils due to its high carbon assimilation (50 g m^{-2} day^{-1}), shorter growing period, low water requirement (4,000 cubic m ha^{-1}), wider adaptability and special ability to accumulate high quantities of fermentable sugars in the stalks (Reddy et al. 2005), that favors ethanol production (Laopaiboon et al. 2009; Wu et al. 2010). This multipurpose biofuel feedstock offers grain for human consumption, fodder for livestock and ethanol for transportation purposes over other biofuel feedstocks such as sugarcane, corn grain, cassava, sugarbeet, jatropha, etc. (Srinivasa Rao et al. 2009). Sweet sorghum is best suited for ethanol production in view of its high reducing sugar content when compared to sugarcane (Huilgol and Misale 2004); with high water use efficiency, radiation use efficiency and is widely believed to be a climate change ready feedstock (Srinivasa Rao et al. 2011). Moreover, the sweet sorghum juice-derived ethanol (Sorganol) is environment-friendly, free of sulphur and aldehydes (Kundiyana 1996).

Sweet sorghum juice contains approximately 16–18 % fermentable sugars, amenable for direct fermentation by yeast to ethanol. However, the technical challenge of sweet sorghum value chain is that the stalk juice has a short shelf life (4–5 h) post-crushing due to its high fermentable sugar

content, and the rapid sugar degradation during storage is due to the metabolic activities of various contaminating spoilage bacteria (Srinivasa Rao et al. 2012). Thus, the preservation and storage of sweet sorghum juice is needed for its further utilization in ethanol production, as an alternate energy source that is renewable, sustainable, efficient, cost-effective, convenient and safe (Wyman and Goodman 1993; Chum and Overend 2001). The different preservation methods used in the food industry are the removal of water content, controlling temperature, freezing, drying, pH control, irradiation, vacuum packaging, modified atmosphere packaging, aseptic packaging, acidification, fermentation, heating (pasteurization and sterilization) and addition of chemical preservatives (Gould 1989, 2000). The existing propensities for juice preservation depends on the utilization of these methods that assures quality products with no added preservatives, high nutritional value, as well as safe from a microbiological perspective at the yeast fermentation step which is critical for the viability of the whole value chain (Srinivasa Rao et al. 2012).

In food industry, the most common preservatives used include benzoic acid, sodium benzoate, sorbic acid and potassium sorbate. In view of their established antimicrobial activity, these preservatives are frequently used in fruit pulps and juices for extending their storage shelf life (Sofos and Busta 1981; Manganelli and Casolari 1983; Lück 1990). In USA and Europe, sodium benzoate has Generally Recognized as Safe (GRAS) status for use in foods (Hussain et al. 2011). Sorbic acid and potassium sorbate are effective preservatives against a wide spectrum of food spoilage microorganisms acting at low pH that persist to be efficient at pH 6.5. Sorbic acid is tasteless, odorless and non-toxic preservative and finds use in a wide range of food products including cheese, yogurt, sour cream, margarine, mayonnaise, bread, cakes, beverages, fermented vegetables, fruit products, smoked and salted fish (Hussain et al. 2010). In the present study, the effect of six chemical preservatives, namely, benzoic acid, sodium benzoate, sorbic acid, citric acid, sodium citrate and ascorbic acid, were evaluated on the stability of sweet sorghum juice to enhance its storage shelf life and also their effect on ethanol fermentation by yeast.

Materials and Methods

Crop Cultivation and Management

The sweet sorghum cultivar, ICSV 93046, was cultivated during the post-rainy (*rabi*) season (October-February), 2010–2011 in vertisols of the experimental farm of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located in Patancheru, Andhra Pradesh,

India (altitude 545 m above mean sea level, latitude 17.53°N and longitude 78.27°E). This cultivar was sown in a plot size of 3 m wide and 4 m long, i.e. four rows of four meters long spaced at 75 cm $\times$ 15–20 cm. The planting was done on ridges with a plant stand of about 100,000 ha^{-1}. Sweet sorghum was initially planted dense but 15 days later after seedling emergence (DAS) thinned to one plant in each hill. Hand weeding was done followed by two inter-cultivations. Surface irrigation was applied in furrows to the crop to maintain proper growth. Standard agronomic package of practices (80-40-0 NPK ha^{-1}; 2/3rd N and total P as basal dose and 1/3rd at 25 DAS) and plant protection measures were adopted throughout the crop growth period in all the plots. After flowering, sorghum heads were covered with nylon bags for protection against bird damage on the developing grain. All the four rows were harvested at physiological maturity (when hilum turns black). The stalks were squeezed once for juice extraction on a three-roller cane press mill. The juice was sieved through a muslin cloth to remove the plant parts that come while extracting the juice. The juice was collected into sterile sample bottles and then transported under cold ice-jacketed conditions to the laboratory for further analysis. Data on juice yield (t ha^{-1}), pH and the stalk yield (t ha^{-1}) were collected following standard procedures for each plot. Approximate sugar yield (t ha^{-1}) was estimated as the product of Brix% and juice yield (t ha^{-1}) (Wortmann et al. 2010).

Effect of Different Preservatives

Different chemical preservatives used in the study were benzoic acid, sodium benzoate, sorbic acid, citric acid, sodium citrate and ascorbic acid. All these chemicals used were of analytical grade and procured from Sisco Research Laboratories (SRL), Mumbai, India. These chemical preservatives were initially spiked at a concentration of 1,000 ppm to 100 ml of each juice sample. Controls were run in parallel without addition of any preservative to the juice sample. These juice samples were stored at 37 °C for 4 days. Experiments were carried out in triplicates and sample aliquots were collected at 24 h periodic intervals for the analysis of different sugars (glucose, fructose and sucrose) and ethanol yield.

Inoculum Preparation

Saccharomyces cerevisiae strain ICTY 417 previously isolated and maintained in the in-house culture collection of CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad, India, identified as promising ethanol producer, was cultured in yeast extract-malt extract (YM) broth at 30 °C and agitated on a gyratory shaker at 150 rev min^{-1} for 18 h. The actively growing cells in the broth with an

absorbance of about 0.5 at 600 nm which corresponded to 10^6 CFU ml^{-1} served as inoculum for ethanol production.

Fermentation Studies

Sweet sorghum juice (600 ml) was dispensed in two 1,000 ml Erlenmeyer flasks and sodium benzoate and sorbic acid as preservatives were added individually at a concentration of 1,000 ppm per 100 ml sample and a third 1,000 ml Erlenmeyer flask containing 600 ml of juice sample with no preservatives served as control. All these three flasks were incubated at room temperature (37 °C). At zero hour period, 100 ml aliquots of the juice with and without preservatives was sampled from each of these flasks to which mineral salts (0.05 % MgSO$_4$ and 0.2 % (NH$_4$)$_2$SO$_4$) were added and autoclaved at 121 °C for 20 min. These flasks were cooled, inoculated with 1 ml of yeast culture (inoculum OD$_{600}$ 0.5) and incubated at 30 °C with agitation at 150 rpm. Further, at 24 h periodic intervals, 100 ml of the juice sample aliquots (control and with preservatives) was collected from the flasks incubated at room temperature and processed as described above. This sampling process was continued till 96 h. The fermented samples (10 ml) were withdrawn from the inoculated flasks at every 24 h interval for 2 days, centrifuged at 8,000 rpm for 10 min for cell separation and the cell-free supernatants were analyzed on gas chromatography (GC) to quantify the amount of ethanol produced. The reducing sugar content in the juice samples before and after fermentation was also analyzed by dinitrosalicylic acid (DNS) method (Miller 1959).

Analytical Methods

Sugar concentration in terms of Brix (%) was measured using a hand-held pocket refractometer (Model PAL, Atago Co. Ltd., Tokyo, Japan) (Kumar et al. 2010). The pH was recorded using a microprocessor-based pH meter (Model DPH506, Global Electronics, Hyderabad, India). Between two different sample readings, the refractometer and the pH meter were cleaned with distilled water and dried with a paper towel. The sweet sorghum juice was centrifuged at 10,000 rpm for 10 min and total soluble sugars (TSS) content in the supernatant was determined using phenol sulphuric acid method (Dubois et al. 1956), while the reducing sugar content was determined using 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). Sugar profiling to determine the contents of individual hexose sugars, i.e., glucose, fructose and sucrose, present in the supernatant were analyzed on a HPLC system (Shimadzu, Kyoto, Japan) equipped with a Luna 5 μm NH2 100R column (4.6 × 250 mm, 5 μm particle size, Phenomenex, Inc., USA) (Kumar et al. 2010).

In addition, the ethanol concentrations (P, g L^{-1}) in the samples were analyzed using the gas chromatograph (Model GC2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and interfaced with a Zebron ZB-624 column (Phenomenex Inc., USA) having dimensions of 30 m × 0.53 mm × 3.00 μm, and set at 60 °C. Ethanol (GR grade) was used for the construction of standard curve, while isopropanol (GR grade) was used as an internal standard. GC operation conditions: Oven temperature 60 °C; injecting temperature 250 °C using nitrogen as carrier gas and hydrogen as a flaming gas both at a flow rate of 41 mL/min with a column flow rate of 1.9 mL/min; flame ionization detector temperature was 280 °C; helium gas was used for cooling the column. Head pressure was 11.5 kPa with a 25:1 split ratio; sample volume used was 1 μL. All experiments were carried out in triplicates and the data values are represented as mean ± standard error (SE) and the SE values are shown as Y-error bars in all figures.

Kinetic studies were also carried out for the fermented samples. The ethanol concentration estimations were performed at periodic intervals of 4 h up to 60 h. The ethanol yield ($Y_{p/s}$) was calculated as the actual ethanol produced and expressed as g ethanol per g total sugar utilized (g g^{-1}). The ethanol productivity (Q_p, g L^{-1} h^{-1}) and the percentage of conversion efficiency or yield efficiency (E_y) were calculated using the following equations (Laopaiboon et al. 2007):

$$Q_P = P/t \text{ and } E_Y = (Y_{p/s} \times 100)/0.51,$$

where P is the actual ethanol concentration produced (g L^{-1}), t is the fermentation time (h) giving the highest ethanol concentration and 0.51 is the maximum theoretical ethanol yield of glucose consumption.

Results and Discussion

Sugar Analysis as a Function of Fermentation Time in Presence of Different Chemical Preservatives

Storage studies were carried out on fresh sweet sorghum juice samples spiked with different chemical preservatives like benzoic acid, sodium benzoate, sorbic acid, citric acid, sodium citrate and ascorbic acid. The results clearly indicated that the amount of total soluble sugars and the percentage of the individual hexose sugars like glucose, fructose and sucrose as a function of time decreased significantly in the juice samples spiked with citric acid (Fig. 1a), sodium citrate (Fig. 1b), ascorbic acid (Fig. 1c) and benzoic acid (Fig. 1d), indicating the deterioration of sugars as compared to the juice samples-spiked with sodium benzoate (Fig. 1e) and sorbic acid (Fig. 1f). It was also observed that the amount of reducing sugars increased, while the amount of non-reducing sugars decreased with an increase in the storage time as a result of breakdown of non-

reducing sugar (sucrose) to reducing sugars. The fructose and glucose content increased from 1.69 to 4.42 % 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. 1e. The sorbic acid-spiked samples showed an increase in fructose and glucose content from 1.47 to 3.3 % and 2.7 to 5.84 %, respectively, whereas sucrose content decreased from 7.18 to 1.02 % as evident from Fig. 1f. The total soluble sugar content decreased from 13.03 to 10.7 % and 11.35 to 10.16 % for sodium benzoate and sorbic acid-spiked samples, respectively. The data corroborates with an earlier study where the juice samples stored at room temperature (~ 25 °C), resulted in sharp

decrease in sucrose content (31 %) out of the total soluble sugar content after 5th day (Wu et al. 2010). The addition of sodium benzoate or sorbic acid at 1,000 ppm seemed to be more effective in quality retention and increase in the storage shelf life of sorghum juice from 5 h to 2 days at 37 °C. The deterioration of fresh sorghum juice (control) was observed with an obvious browning and rapid increase of viscosity (visual observation) which may be due to the fermentation by spoilage microflora within 12 h (Srinivasa Rao et al. 2012). Further, the juice deterioration was comparatively much lower in the sodium benzoate and sorbic acid-spiked juice samples suggesting their significant positive effect in restricting the microbial growth in the juice.
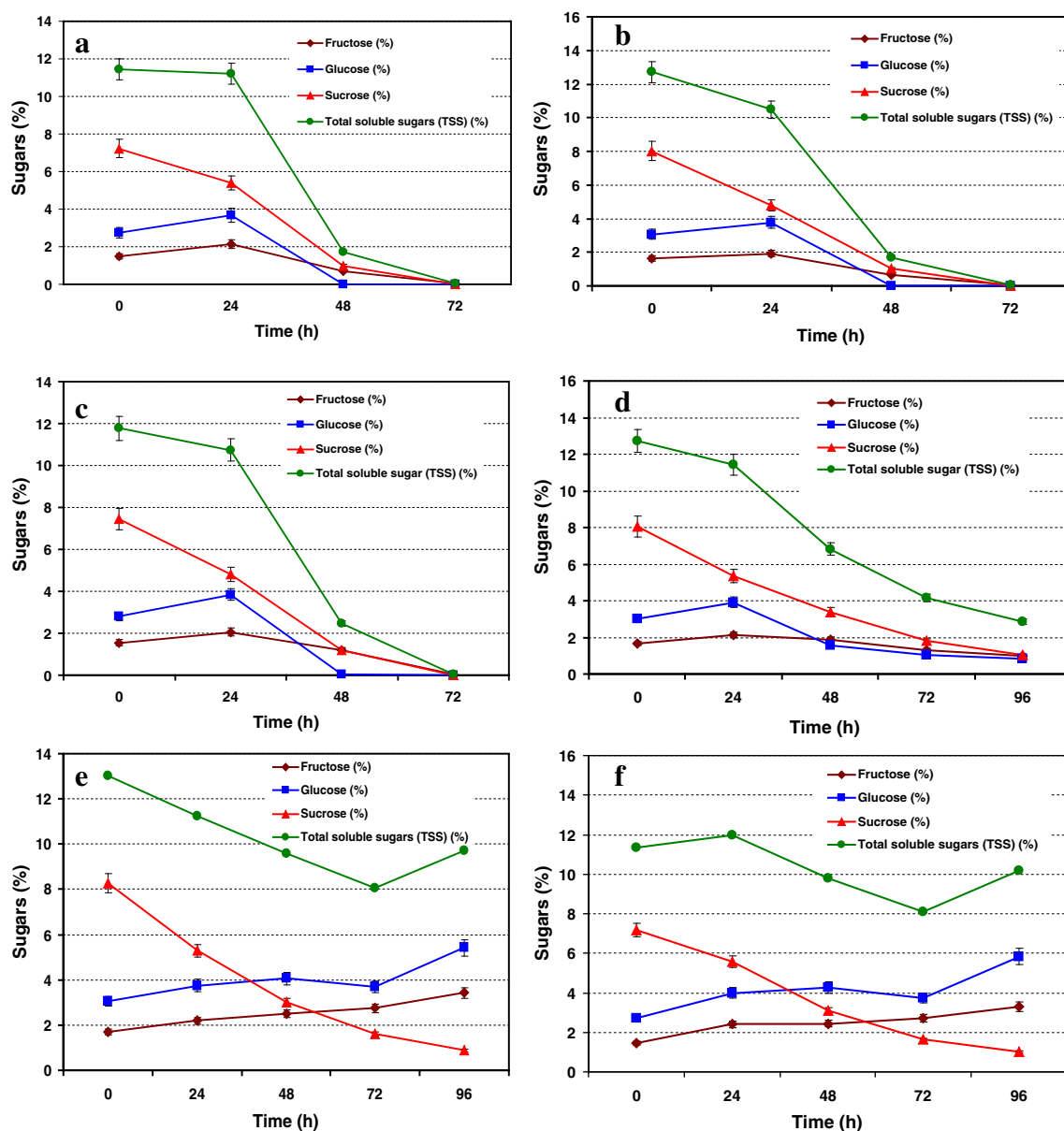

Fig. 1 Sugar analysis of sweet sorghum juice sample spiked with **a** citric acid, **b** sodium citrate, **c** ascorbic acid, **d** benzoic acid, **e** sodium benzoate, and **f** sorbic acid as a function of incubation period
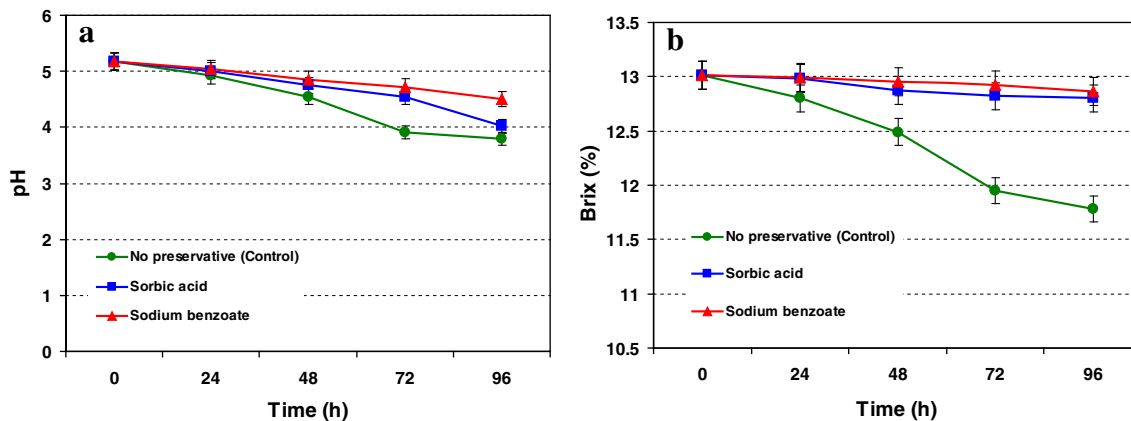
Fig. 2 Effect of different preservatives on **a** pH and **b** Brix% values of sweet sorghum juice as a function of incubation period
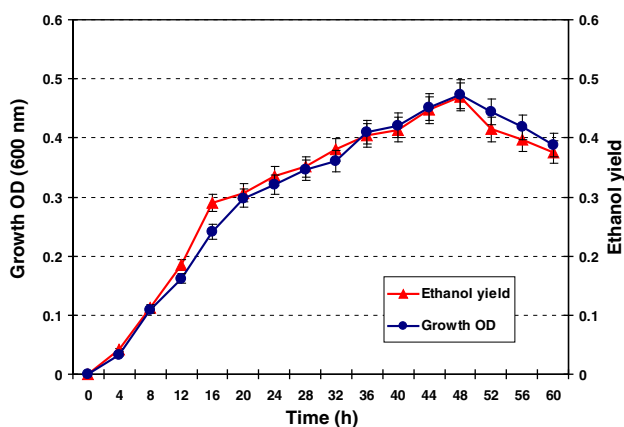

Fig. 3 Growth kinetics profile of *S. cerevisiae* strain ICTY 417 with reference to ethanol production

Considering these advantages, sodium benzoate and sorbic acid preservatives exhibited higher storage stability as compared to the other tested preservatives and these were only selected for further fermentation studies. It was earlier demonstrated that some yeast strains are resistant to these preservatives (Warth 1985), and this feature can be very well exploited in the context of yeast fermentation.

pH Changes as a Function of Fermentation Time

Based on the results shown in Fig. 2a, the pH changes observed in the sorghum juice were considerable and comparable. The pH value decreased with an increase in the fermentation time in the control from pH 5.18 to pH 3.8 as compared to the preservative-spiked samples which decreased from pH 5.18 to pH 4.5 for sodium benzoate and pH 5.18 to pH 4.02 for sorbic acid. In addition, the lowering of pH was somewhat more in the sorbic acid-spiked sample as compared to the benzoic acid-spiked sample. It was also noticed that the pH changes were not that significant and remained fairly constant at pH 4. This observation can be

explained by the fact that carbon dioxide released during yeast fermentation is dissolved in the fermented juice which is converted to carbonic acid producing carbonate ions and protons, and this maintained the pH of the juice at a relatively constant value of pH 4 (Shen et al. 2004). This lowering of pH value in the preservative-spiked samples may also contribute to the inhibition in growth of spoilage microbes and the extension in storage shelf life of the juice. The changes in pH value of the juice remained almost constant during the initial period of the fermentation after which it dropped and remained constant for the rest of the fermentation period. These results were comparable with some earlier fermentation studies carried out on sweet sorghum juice under different conditions (Khongsay et al. 2010; Ariyajarearnwong et al. 2011).

Brix Value Changes as a Function of Fermentation Time

A steady state changes in the Brix values were observed in the control samples as compared to the preservative-spiked samples (Fig. 2b). The Brix values reduced from an initial Brix value of 13 to 11.78 % in case of control as compared to the sorbic acid and sodium benzoate-spiked juice samples which reduced to 12.8 and 12.86 %, respectively, after 96 h incubation.

Ethanol Production as a Function of Fermentation Time

The ethanol production paralleled with the yeast growth and the fermentation of juice showed maximum concentration of ethanol (0.69 g g^{-1}) after 48 h with the onset of the stationary phase of growth, after which a decrease in the ethanol concentration was observed (Fig. 3). Since the optimal ethanol production was observed at 48 h, the later fermentation studies were carried out only for 48 h incubation.

Table 1 Kinetic parameters of batch ethanol production as a function of fermentation time by *S. cerevisiae* strain ICTY417 from sweet sorghum juice samples spiked with and without chemical preservatives like sorbic acid and sodium benzoate and stored for 96 h

Juice samples	Parameters (mean ± SE)			
	P (g L^{-1})	$Y_{p/s}$ (g g^{-1})	Q_p (g L^{-1} h^{-1})	$E_y^{\#}$ (%)
0 h storage period				
Control (24 h fermentation)	48.41 ± 0.074	0.40 ± 0.440	2.02 ± 0.251	77.98 ± 0.108
Control (48 h fermentation)	50.62 ± 0.140	0.40 ± 0.615	1.05 ± 0.591	79.09 ± 0.717
24 h storage period				
Control (24 h fermentation)	51.89 ± 0.374	0.33 ± 0.412	2.16 ± 0.576	64.50 ± 0.728
Sorbic acid (24 h fermentation)	52.23 ± 0.209	0.43 ± 0.266	2.17 ± 0.581	85.49 ± 0.810
Sodium benzoate (24 h fermentation)	52.14 ± 0.308	0.44 ± 0.488	2.17 ± 0.651	85.68 ± 0.923
Control (48 h fermentation)	52.97 ± 0.091	0.35 ± 0.487	1.10 ± 0.362	69.01 ± 0.705
Sorbic acid (48 h fermentation)	53.33 ± 0.194	0.44 ± 0.746	1.11 ± 0.680	89.45 ± 0.629
Sodium benzoate (48 h fermentation)	53.3 ± 0.931	0.44 ± 0.407	1.11 ± 0.692	90.45 ± 0.875
48 h storage period				
Control (24 h fermentation)	53.81 ± 0.340	0.31 ± 0.404	2.24 ± 0.357	60.19 ± 0.772
Sorbic acid (24 h fermentation)	54.27 ± 0.146	0.45 ± 0.561	2.26 ± 0.389	85.03 ± 0.795
Sodium benzoate (24 h fermentation)	55.06 ± 0.092	0.45 ± 0.808	2.29 ± 0.491	87.41 ± 0.673
Control (48 h fermentation)	56.97 ± 0.618	0.34 ± 0.297	1.18 ± 0.305	66.47 ± 0.037
Sorbic acid (48 h fermentation)	57.54 ± 0.610	0.44 ± 0.471	1.20 ± 0.584	92.74 ± 0.904
Sodium benzoate (48 h fermentation)	57.96 ± 0.161	0.47 ± 0.509	1.21 ± 0.714	93.13 ± 0.273
72 h storage period				
Control (24 h fermentation)	51.44 ± 0.971	0.29 ± 0.451	2.14 ± 0.728	57.05 ± 0.609
Sorbic acid (24 h fermentation)	51.55 ± 0.484	0.43 ± 0.847	2.15 ± 0.892	84.31 ± 0.491
Sodium benzoate (24 h fermentation)	52.53 ± 0.114	0.44 ± 0.230	2.19 ± 0.147	86.07 ± 0.408
Control (48 h fermentation)	52.60 ± 0.106	0.32 ± 0.099	1.09 ± 0.579	62.54 ± 0.627
Sorbic acid (48 h fermentation)	52.85 ± 0.470	0.44 ± 0.309	1.10 ± 0.672	88.66 ± 0.182
Sodium benzoate (48 h fermentation)	52.64 ± 0.493	0.44 ± 0.467	1.09 ± 0.877	90.47 ± 0.220
96 h storage period				
Control (24 h fermentation)	49.03 ± 0.368	0.26 ± 0.476	2.04 ± 0.119	50.98 ± 0.375
Sorbic acid (24 h fermentation)	49.10 ± 0.589	0.41 ± 0.374	2.04 ± 0.772	80.39 ± 0.191
Sodium benzoate (24 h fermentation)	49.08 ± 0.491	0.40 ± 0.701	2.04 ± 0.684	79.80 ± 0.275
Control (48 h fermentation)	49.54 ± 0.402	0.29 ± 0.905	1.03 ± 0.557	57.45 ± 0.621
Sorbic acid (48 h fermentation)	49.94 ± 0.835	0.42 ± 0.106	1.04 ± 0.721	88.76 ± 0.040
Sodium benzoate (48 h fermentation)	49.95 ± 0.676	0.42 ± 0.351	1.04 ± 0.117	90.96 ± 0.237

P actual ethanol concentration produced, $Y_{p/s}$ ethanol yield, Q_p ethanol productivity, $E_y^{\#}$ percentage of conversion efficiency or yield efficiency

The total soluble sugars and total soluble solids present at the beginning of the fermentation were 150 g L^{-1} and 13°Brix, respectively. The fermentation was carried out for sodium benzoate and sorbic acid-spiked juice samples and in juice sample without preservative, as a control. Ethanol yield (g g^{-1}) as a function of fermentation time was estimated for control and sodium benzoate-spiked samples (Fig. 1e) and for control and sorbic acid-spiked samples (Fig. 1f) at different time durations like 0, 24, 48, 72 and 96 h. It was observed that ethanol yield was highest after 48 h of fermentation in case of both sodium benzoate and sorbic acid-spiked samples. After 48 h, the ethanol production decreased which might be due to the ethanol feedback inhibition. A similar trend was observed in case of blank (un-spiked sample). Furthermore, the production followed a somewhat constant trend in both the preservative-spiked samples, while in case of control, the ethanol production was found to be lower as compared with the preservative-spiked samples. The decrease in the ethanol production of the control samples were the result of the rapid deterioration of the fermentable juice components by microorganisms.

Based on the kinetic parameters of batch ethanol production as a function of fermentation time (Table 1), it can be inferred that at 24 h of juice preservation, the sorbic acid-spiked sample recorded 21 % higher fermentation

efficiency at 24 h of fermentation period, while sodium benzoate-spiked sample exhibited 21.2 % increased fermentation efficiency over the control. However, when the fermentation period was extended to 48 h, the fermentation efficiency was 20.44 and 21.44 % higher for both the sorbic acid and sodium benzoate-added samples vis-a-vis control. Further, at 48 h of juice preservation, sorbic acid and benzoic acid recorded 25 and 27 % higher fermentation efficiency, respectively, at 24 h of fermentation period. The 48 h fermentation period showed higher fermentation efficiency of 26.27 and 26.66 % for sorbic acid and sodium benzoate, respectively, as compared to control. Similarly, an increasing trend of higher fermentation efficiency was observed even at fermentation period of 72 and 96 h for both sorbic acid and sodium benzoate as compared to the control.

Based on the results shown in Table 1, the ethanol yield remained in the range of 0.42–0.47 g g^{-1} in sodium benzoate-spiked samples which showed an optimal efficiency of 93 %, while in case of sorbic acid-spiked samples, the ethanol yield was in the range of 0.40–0.44 g g^{-1} which corresponds to an optimal efficiency of 92 %. In case of control, the ethanol yield was observed in the range of 0.36 g g^{-1} that reduced to 0.26 g g^{-1} and the optimal efficiency reduced to 57 %. The ethanol yields were consistent on fermentation in case of both the preservatives-spiked juice samples since the total soluble sugar content was maintained in the juice samples as compared to the control (un-spiked juice sample) which showed a reduction in the total soluble sugar content with an increase in the fermentation time. However, the sodium benzoate-spiked samples showed comparatively better results than sorbic acid-spiked samples. Since all the experiments were performed at room temperature, it is believed that the storage shelf life of sweet sorghum juice can be extended with the addition of sodium benzoate as preservative under room temperature conditions. Ethanol fermentation of greater than 90 % efficiency was observed in frozen, autoclaved and juice containing 25 % sugar samples, whereas less than the above was seen in normal juice fermentation (Imam and Capareda 2011). The fermentation efficiency of around 90 % was also observed in fermentations performed under very high gravity conditions (Nuanpeng et al. 2011). Therefore, the use of chemical preservatives employed in the present work were beneficial to enhance the storage shelf life of sweet sorghum juice with no special treatment required for the preservation of juice and the fermentation efficiency was also not compromised.

Conclusions

The results observed in the present study demonstrated that sodium benzoate and sorbic acid were suitable chemical preservatives among the tested preservatives and they preserved the fermentable sugars in the sweet sorghum stalk juice at room temperature, thus extending the storage shelf life of juice from 5 h (normal shelf life) to 48 h and also enabled the later fermentation of the juice to ethanol. On the other hand, the control juice sample with no added preservatives showed a significant reduction in the total soluble sugar content and resulted in a sharp decrease in the ethanol yield as well as the efficiency. The highest fermentation efficiency of 93 % was recorded at 48 h incubation. The chemical preservatives identified in this study may be helpful to the biofuel and other allied industries in the development of cost-effective strategies to preserve the fermentable sugars and retain the organoleptic and rheological properties of the sweet sorghum juice during processing, transportation, and storage under normal ambient conditions as compared to refrigerated conditions which is energy-intensive.

References

Ariyajarearnwong, P., L. Laopaiboon, P. Jaisil, and P. Laopaiboon. 2011. Repeated-batch ethanol fermentation from sweet sorghum juice by free cells of *Saccharomyces cerevisiae* NP 01. *African Journal of Biotechnology* 10: 13909–13918.

Chum, L.M., and R.P. Overend. 2001. Biomass and renewable fuels. *Fuel Bioprocessing Technology* 17: 187–195.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356.

Gould, G.W. 1989. *Mechanisms of action of food preservation procedures*. London: Elsevier Applied Science.

Gould, G.W. 2000. Preservation: Past, present and future. *British Medical Bulletin* 26: 84–96.

Huilgol, R.V., Ramakrishna, and G. Misale. 2004. A trial with sweet sorghum. In *Alternative Uses of Sorghum and Pearl Millet in Asia: Proceedings of the Expert Meeting, ICRISAT, Andhra Pradesh, India*, 1–4 *July* 2003, *CFC Technical paper No.* 34, 333–337. Patancheru: International Crops Research Institute for the Semi-Arid Tropics.

Hussain, I., A. Zeb, and M. Ayub. 2010. Quality attributes of apple and apricot blend juice preserved with potassium sorbate during storage at low temperature. *Internet Journal of Food Safety* 12: 80–86.

Hussain, I., A. Zeb, and M. Ayub. 2011. Evaluation of apple and apricot blend juice preserved with sodium benzoate at refrigeration temperature. *World Journal of Agricultural Sciences* 7: 136–142.

Imam, T., and S. Capareda. 2011. Fermentation kinetics and ethanol production from different sweet sorghum varieties. *International Journal of Agricultural and Biological Engineering* 4: 33–40.

Khongsay, N., L. Laopaiboon, and P. Laopaiboon. 2010. Growth and batch ethanol fermentation of *Saccharomyces cerevisiae* on sweet sorghum stem juice under normal and very high gravity conditions. *Biotechnology* 9: 9–16.

Kumar, C.G., A. Fatima, P. Srinivasa Rao, B.V.S. Reddy, A. Rathore, R. Nageswar Rao, S. Khalid, A.A. Kumar, and A. Kamal. 2010. Characterization of improved sweet sorghum genotypes for biochemical parameters, sugar yield and its attributes at different phenological stages. *Sugar Tech* 12: 322–328.

Kundiyana, D.K. 1996. Sorganol: In-field production of ethanol from sweet sorghum. M.Sc. Thesis. Stillwater, OK: Oklahoma State University.

Laopaiboon, L., P. Thanonkeo, P. Jaisil, and P. Laopaiboon. 2007. Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. *World Journal of Microbiology and Biotechnology* 23: 1497–1501.

Laopaiboon, L., S. Nuanpeng, P. Srinophakun, P. Klanrit, and P. Laopaiboon. 2009. Ethanol production from sweet sorghum juice using very high gravity technology: Effects of carbon and nitrogen supplementations. *Bioresource technology* 100: 4176–4182.

Lück, F. 1990. Food applications of sorbic acid and its salts. *Food Additives & Contaminants* 7: 711–715.

Manganelli, E., and A. Casolari. 1983. Sensitivity of yeasts to sorbic and benzoic acids and their salts. *Ind Conserve* 58: 23–25.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugars. *Analytical Chemistry* 31: 426–428.

Nuanpeng, S., L. Laopaiboon, P. Srinophakun, P. Klanrit, P. Jaisil, and P. Laopaiboon. 2011. Ethanol production from sweet sorghum juice under very high gravity conditions: Batch, repeated-batch and scale up fermentation. *Electronic Journal of Biotechnology*. doi: http://dx.doi.org/10.2225/vol14-issue1-fulltext-2 (accessed 14 May 2014).

Reddy, B.V.S., S. Ramesh, P. Sanjana Reddy, B. Ramaiah, P.M. Salimath, and R. Kachapur. 2005. Sweet sorghum-A potential alternative raw material for bioethanol and bio-energy. *International Sorghum and Millets Newsletter* 46: 79–86.

Shen, H.Y., S.D. Schrijver, N. Moonjai, K.J. Verstrepen, F. Delvaux, and F.R. Delvaux. 2004. Effects of CO_2 on the formation of flavor volatiles during fermentation with immobilized brewer's yeast. *Applied Microbiology and Biotechnology* 64: 636–643.

Sofos, J.N., and F.F. Busta. 1981. Antimicrobial activity of sorbate. *Journal of Food Protection* 44: 614–622.

Srinivasa Rao, P., S.S. Rao, N. Seetharama, A.V. Umakanth, P. Sanjana Reddy, B.V.S. Reddy, and C.L.L. Gowda. 2009. *Sweet sorghum for biofuel and strategies for its improvement. Information bulletin No.* 77. Patancheru: International Crops Research Institute for the Semi-Arid Tropics (ISBN978-92-9066-518-2).

Srinivasa Rao, P., J.V.N.S. Prasad, A.V. Umakanth, and B.V.S. Reddy. 2011. Sweet sorghum (*Sorghum bicolor* (L.) Moench)-A new generation water use efficient bioenergy crop. *Indian Journal of Dryland Agricultural Research and Development* 11: 65–79.

Srinivasa Rao, P., C.G. Kumar, M. Jayalakshmi, A. Kamal, and B.V.S. Reddy. 2012. Feasibility of sustaining sugars in sweet sorghum stalks during post-harvest stage by exploring cultivars and chemicals: A desk study. *Sugar Technology* 14: 21–25.

Warth, A.D. 1985. Resistance of yeast species to benzoic and sorbic acids and to sulfur dioxide. *Journal of Food Protection* 48: 564–569.

Wortmann, C.S., A.J. Liska, R.B. Ferguson, D.J. Lyon, R.N. Klein, and I. Dweikat. 2010. Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. *Agronomy Journal* 102: 319–326.

Wu, X., S. Staggenborg, J.L. Propheter, W.L. Rooney, J. Yu, and D. Wang. 2010. Features of sweet sorghum juice and their performance in ethanol fermentation. *Industrial Crops and Products* 31: 164–170.

Wyman, C.E., and B.J. Goodman. 1993. Biotechnology for production of fuels, chemicals, and materials from biomass. *Applied Biochemistry and Biotechnology* 39: 41–59.