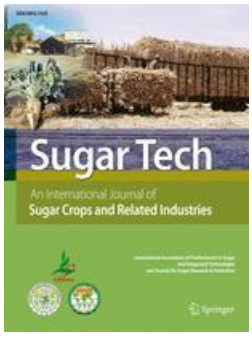


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# Enhancing the Shelf Life of Sweet Sorghum [Sorghum bicolor (L.) Moench] Juice Through Pasteurization While Sustaining Fermentation Efficiency

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**Enhancing the shelf life of sweet sorghum [*Sorghum bicolor* Moench] juice through pasteurization while sustaining fermentation efficiency**

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*Running title*: Preservation of sweet sorghum juice

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**Abstract**

The influence of pasteurization on storage stability of sweet sorghum juice and subsequent bioconversion to ethanol was studied. Juice samples were pasteurized at three different temperatures, i.e., 70 °C for 10 min, 80 °C for 5 min and 90 °C for 2 min and were further stored at three different temperatures of 35, 40 and 45 °C. The storage shelf life of the sorghum juice was observed to be extended for 21 days without compromising the ethanol conversion efficiency. Consistent fermentation efficiencies were observed for the juice samples pasteurized at 70 °C followed by storage at 45 °C, pasteurized at 80 °C followed by storage at 40 °C and pasteurized at 90 °C followed by storage at 35 °C and these samples showed an ethanol yield in the range of 0.473 to 0.477 g $g^{-1}$, 0.461 to 0.47 g $g^{-1}$ and 0.466-0.473 g $g^{-1}$, respectively. Hence, the juice samples pasteurized at 90 °C and stored at 35 °C was deemed as the superior preservation condition as it was close to

ambient temperature and increased the shelf life of sweet sorghum juice. The highest fermentation efficiency of 93% was observed after 48 h of fermentation.

**Keywords**: Sweet sorghum juice; pasteurization; shelf life; ethanol; storage

## Introduction

Biomass as a primary renewable energy resource for biofuels generation has gained immense importance in the last few decades and utilization of ethanol from biomass is predicted to increase from 1.0 mboe/d in 2010 to 3.4 mboe/d in 2035 (Anonymous 2012), which includes organic and animal wastes, wastewater, energy crops, agricultural and industrial residues (Antonopoulou et al. 2008). Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a C4 plant possessing high photosynthetic activity and drought tolerance that can be cultivated in all temperatures including tropical and temperate climatic areas requiring minimal quantity of water and fertilizer unlike other crops (Rao et al. 2009, 2011). It has been deemed as a potential feedstock for biofuels production since it has approximately equal quantities of soluble (glucose and sucrose) and insoluble (cellulose and hemicellulose) carbohydrates (Yu et al. 2012). These features make the production of biofuels such as ethanol from this feedstock juice advantageous (Anderson 2005).

Sweet sorghum juice has a short shelf life and prone to microbial spoilage due to the presence of sugars (Krishnakumar and Devadas 2006). Sweet sorghum juice like sugarcane juice gets affected by chemical (acid) and enzymatic inversion (Singh et al. 2006) due to the presence of both neutral invertase (NI) and acid invertase (AI). These enzymes cause sucrose inversion, the reason being their high correlation with sucrose and reducing sugar content during plant growth (Siswoyoa et al. 2007). The existing propensities for juice preservation depends on the utilization of the methods that assures qualitative products, high nutritional value and safe from a microbiological perspective at the downstream step of yeast fermentation which is critical for the viability of the whole value chain (Gould 2000; Ranken et al. 2005; 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 (Gould 2000; 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 (Ranken et al. 2005).The potential methods employed in the food preservation can thus be divided into physical, physicochemical, microbial-derived and miscellaneous, among which, the most important ones are high temperature, low temperature, water activity, acidity, redox potential (Eh), competitive microorganism (e.g. lactic acid bacteria) and preservatives (e.g. nitrite, sorbate, sulphite) (Leistner and Gorris 1995).

Pasteurization is one of the effective and widely practised preservation method employed in the food industry since heating at higher temperature kills a major fraction of microbes in foods stored in both room and refrigerated temperatures (Karmakar et al. 2010). The method of food preservation using pasteurization has been adapted for many fruits juices such as Nagpur mandarin (*Citrus reticulata* Blanco) (Pareek et al. 2011), aonla juice (Bhattacherjee et al. 2011), kinnow juice blends (Bhardwaj and Mukherjee 2011) as well as widely used in sugarcane juice preservation (Chauhan et al. 2002; Karmakar et al. 2010; Sankhla et al. 2012). The aim of the present study is to evaluate the effect of pasteurization on the stability of sweet sorghum juice so as to enhance its shelf life and also to study their effect on ethanol fermentation by yeast.

## Materials and methods

### Crop Cultivation and Management

The sweet sorghum cultivar, ICSV 93046, was grown during the post-rainy (*rabi*) season (October-February), 2010–11 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 × 4 m, i.e. four rows of four meters long spaced at 75 cm × 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 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 $ha^{-1}$; $2/3^{rd}$ N and total P as basal dose and $1/3^{rd}$ at 25 DAS) and plant protection measures were adopted throughout the crop growth period in all the plots. At 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 to extract the juice on a three-roller cane press mill. The juice was sieved through a muslin cloth to remove the plant parts that may 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 stalk yield (t $ha^{-1}$) were collected following standard procedures for each plot (Rao et al. 2010). The sugar yield (t $ha^{-1}$) was estimated as the product of Brix % and juice yield (t $ha^{-1}$) (Wortmann et al. 2010).

Microorganism and Inoculum Preparation

*Saccharomyces cerevisiae* strain ICTY 417 previously isolated and maintained in the in-house culture collection of CSIR-Indian Institute of Chemical Technology, Hyderabad, India was cultured in yeast extract-malt extract (YM) medium at 30ºC 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 $10^6$ cells $ml^{-1}$ was used as inoculum for ethanol production.

## Heat Treatment for Pasteurization

Sweet sorghum juice (1 liter each) was taken in 18 Erlenmeyer flasks that were divided into three sets consisting of 6 flasks in each set. Each set of juice were pasteurized at three different temperatures, i.e., 70, 80 and 90 °C for 10, 5 and 2 min, respectively. One flask from each set was stored under 35, 40 and 45 °C. Additional unpasteurized sweet sorghum juice samples were taken as blank, which were also maintained at all above mentioned three temperatures. Experiments were carried out in triplicates and the analysis for different sugars (glucose, fructose and sucrose) and ethanol yield was carried out at 24 h periodic intervals for 72 h.

## Fermentation Studies

Hundred milliliter aliquots of each of the pasteurized and blank sweet sorghum juice samples was used for the zero hour study to which mineral salts [0.05% $MgSO_4$ and 0.2% $(NH_4)_2SO_4$] were added and autoclaved at 121 ºC for 20 min. The flasks were then cooled and inoculated with 1 ml of the fresh grown yeast inoculum ($OD_{600}$ 0.5), incubated at 30 ºC with agitation at 150 rev $min^{-1}$. Further, at periodic intervals of 48 h till $13^{th}$ day after which the interval was doubled to 96 h for the last two sets of samples ($17^{th}$ and $21^{st}$ day), 100 ml of the juice samples from each of the three sets of pasteurized temperatures along with the blank and stored at three different temperatures was taken and processed as described above. This sampling process was continued till 72 h at every 24 h interval. The fermented samples were taken from the inoculated flasks after every 24 h,

centrifuged at 8000 rpm for 10 min for cell separation and the cell-free supernatants were subjected to gas chromatography (GC) analysis to determine the amount of ethanol produced. The reducing sugar content present 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) (Zoecklien et al. 1995). 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 rev $min^{-1}$ for 10 min and total soluble sugars (TSS) content in the supernatant was determined using the phenol sulphuric acid method (Dubois *et al*. 1956), while the reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). Sugar profiling to determine the contents of individual hexose sugars, like glucose, fructose and sucrose, present in the extracted sweet sorghum juice were analyzed on a HPLC system (Kumar et al. 2010).

In addition, ethanol concentrations (P, g $l^{-1}$) in the samples were analyzed using the gas chromatograph (Model GC2014, Shimadzu, 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 ºC. Ethanol (GR) and isopropanol (GR) were used for the standard curve and as an internal standard,

respectively. Operation conditions: Oven temperature was 60 ºC; injecting temperature was 250 ºC using nitrogen as carrier gas and hydrogen as a flaming gas both at a flow rate of 41 ml $min^{-1}$ with a column flow rate of 1.9 ml $min^{-1}$; flame ionization detector temperature was 280 ºC; helium gas was used for cooling the column. Head pressure was 11.5 kPa with a 25:1 split ratio; sample volume was 1 µl. All experiments were carried out in triplicates and the data values are represented as mean ± standard error (S.E.) and the S.E. 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):

$Q_P = P/t$ 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 Pasteurization Carried Out and Stored at Different Temperatures

The pasteurization studies were carried out on fresh sweet sorghum juice samples at different temperature conditions. The fresh juice samples were first pasteurized at three different temperatures, that is, 70 °C, 80 °C and 90 °C which was further incubated at three different temperatures *viz*., 35 °C, 40 °C and 45 °C for 21 days. The results suggest that the amount of total soluble sugars and the percentage of hexose sugars like glucose, fructose and sucrose as a function of time did not show significant changes over the period of time. 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 (Fig. 1 and Fig. 2). From the figures, it can be inferred that the fructose and glucose content increased, while the sucrose content decreased in the case of the samples pasteurized at 70 °C and stored at 35 °C, 40 °C, and 45 °C. Similarly, the fructose and glucose content increased in case of samples pasteurized at 80 °C and stored at 35 °C, 40 °C and 45 °C. The sucrose content also decreased for the samples pasteurized at 80 °C and stored at 35 °C, 40 °C and 45 °C. However, there was no much significant changes observed in case of total soluble sugar content for the samples pasteurized at 80 °C and stored at 35 °C, 40 °C and 45 °C. However, in case of samples pasteurized at 80°C and stored at 35 °C, the fructose and glucose contents increased from 1.85% to 4.84% and 2.83% to 4.46%; at 40 °C, it increased from 1.85% to 5.41% and 2.83% to 6.61% and at 45 °C storage temperature, it increased from 1.85% to 4.31 and 2.83% to 4.99%, respectively. In case of samples pasteurized at 90 °C and stored at 35 °C, 40 °C and 45 °C, the sucrose content decreased from 12.15% to 9.91%, 12.15% to 10.18% and 12.15% to 9.62%, respectively. The changes observed in total soluble sugar content for samples pasteurized at 90 °C and

stored at 35 °C, 40 °C and 45 °C were from 16.85% to 20.92%, 16.85% to 18.2% and 16.85% to 15.43%, respectively. The observed data was comparable with an earlier study where the juice samples stored at room temperature (≈25 °C), resulted in a sharp decline in the sucrose content of the total soluble sugar content to 31% after the $5^{th}$ day (Wu et al. 2010). The effect of pasteurization thus, increased the storage shelf life of the fresh sweet sorghum juice from 5 h to three weeks. The fresh sorghum juice (control sample) deterioration was observed with an obvious browning and rapid increase in the viscosity (visual observation) which may be due to the fermentation by spoilage microflora within 12 h. Later, all these pasteurized samples were subjected to fermentation to check their fermentation ability.

Changes in pH and Brix% Values as a Function of Pasteurization Time

The results depicted in Fig. 3, showed that the pH changes observed during the fermentation process of the sorghum juice were not that significant and comparable. The pH values in case of all the fermentation experiments of the samples pasteurized at 70 °C, 80 °C and 90 °C and stored at 35 °C, 40 °C and 45 °C decreased from pH 5 and remained fairly constant around pH 4. This negligible change in the pH is plausibly due to the release of carbon dioxide, which in turn was converted to carbonic acid and produced carbonate ions and protons, and thus the pH of the fermented juice maintained at a relatively constant value (Shen et al. 2004). This decrease in pH also aided in the prevention of the growth of spoilage microbes resulting in enhancement of the storage shelf life of the sorghum juice. The minimal changes in the pH values of the sweet

sorghum juice for the entire period of the 21 days of experiments showed stability in the ethanol production. 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). Further, the changes in the brix values in the pasteurized samples were found to be comparatively in a steady state as evident from Fig.4. The Brix values showed slight fluctuation as it reduced slightly and then again increased slightly. The almost near consistency in the brix value of the sweet sorghum juice samples evidently showed the maintenance of total soluble sugars which in turn will influence the consistency in the fermentation of the pasteurized juice samples.

Ethanol Production as a Function of Fermentation Time

The ethanol production paralleled with the growth of the yeast in the submerged culture medium. However, the fermentation of fresh 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 reduction in the ethanol concentration was observed (Fig. 6). Since the optimal ethanol production was observed at 48 h, the later fermentation studies were carried out for 48 h. The decrease in the ethanol production after 48 h indicates the end of stationary phase of the organism. The supplementation of the sweet sorghum juice with ammonium sulphate as substrate contributed to the yield and productivity of the ethanol production (Laopaiboon et al. 2007).

The total soluble sugars and total soluble solids present at the beginning of the fermentation were 170 g $l^{-1}$ and 13.03 Brix%, respectively. The fermentation was carried

out for all the samples pasteurized at 70 °C, 80 °C and 90 °C further stored at three different temperatures of 35 °C, 40 °C and 45 °C. The ethanol yield (g $g^{-1}$) as a function of fermentation time was estimated for the pasteurized samples (Fig. 5) at different time durations like 0, 24, 48, 72 and 96 h. The ethanol yield was found to be highest after 48 h of fermentation in case of all the three pasteurized samples. After 48 h, the ethanol production decreased which might be due to the ethanol feedback inhibition. The blank (unpasteurized sample) showed similar tendency. The ethanol production in the pasteurized samples in all conditions showed a relatively constant trend whereas, the ethanol production in control was found to be comparatively lower than that of the pasteurized samples. The kinetic parameter studies of the control (unpasteurized sample) showed an initial ethanol production efficiency value of 88% with the yield of 0.453 g $g^{-1}$ on the $1^{st}$ day sample at 48 h of fermentation. The second day sample showed lower ethanol production efficiency and yield, that is 75% and 0.332 g $g^{-1}$ at 48 h of fermentation. The production of ethanol further reduced with each consecutive day and was found to be minimal by $5^{th}$ day that showed a fermentation efficiency of just as low as 40% and yield of 0.102 g $g^{-1}$. The reduced ethanol production exhibited by the control is as a result of rapid degradation of the fermentable components of the juice by microorganisms.

The kinetic parameters of ethanol production for the pasteurized samples as a function of fermentation time is shown in tables 1, 2 and 3 for samples pasteurized at 70 °C, 80 °C and 90 °C, respectively. The fermentation efficiency for the samples stored at 70 °C was in the range of 89% to 93%. The juice sample pasteurized at 80 °C, showed a variation in efficiency values ranging from 88% to 92% and the samples pasteurized at

90°C, the variation was in the range of 88% to 93%. The ethanol yield for the samples pasteurized at 70 °C and stored at 35 °C, 40 °C and 45 °C was in the range of 0.473-0.462 g $g^{-1}$, 0.473 to 0.454 g $g^{-1}$ and 0.473 to 0.477 g $g^{-1}$, respectively. The samples stored at 35 °C, 40 °C and 45 °C for juice samples pasteurized at 80 °C showed the range of 0.47 to 0.456 g $g^{-1}$, 0.47 to 0.461 g $g^{-1}$ and 0.47 to 0.459 g $g^{-1}$, respectively, for ethanol yield. The ethanol yield for samples pasteurized at 90 °C and stored at 35 °C, 40 °C and 45 °C was in the range of 0.473-0.466 g $g^{-1}$, 0.473 to 0.458 g $g^{-1}$ and 0.473 to 0.461 g $g^{-1}$, respectively. On fermentation, the ethanol yields were almost consistent in all the three pasteurized temperatures and kept at a different storage conditions. However, the efficiency was more consistent and subsequent ethanol yield was observed in case of samples pasteurized at 70 °C and stored at 45 °C, 40 °C for samples pasteurized at 80 °C and 35 °C for samples pasteurized at 90 °C. Therefore, we can say that the samples pasteurised at 90 °C and stored at 35 °C which is approximately close to room temperature is the best preservation condition for storage and increasing the shelf life of the sweet sorghum juice. Some of the earlier studies indicated that the ethanol fermentation efficiency of >90% was observed in frozen, autoclaved and juice containing 25% sugar samples, whereas less than the above was observed in normal juice fermentation (Imam and Capareda 2011). The fermentation efficiency of around 90% was also reported in the fermentations carried out under very high gravity conditions (Nuanpeng et al. 2011). Therefore, the conditions employed in the present work is beneficial for the enhancement of the shelf life of sweet sorghum juice and pasteurization was suggested as an efficient preservation method of the sweet sorghum juice samples and the fermentation efficiency was also maintained.

## Conclusions

The results observed in the present study identified a suitable pasteurization temperature that was capable of preserving the fermentable sugars in sweet sorghum stalk juice and maintained the sugar profiles reasonably well at near room temperature, i.e. pasteurization at 90 ºC followed by storage at 35 ºC. The storage shelf life of the juice was extended up to 21 days and also enabled efficient bioconversion of the juice to ethanol. On the other hand, the control juice sample which was not preserved well showed a significant reduction in the total soluble sugar content and thus resulted in a sharp decrease in the ethanol yield due to reduced fermentation efficiency. The highest fermentation efficiency of 93% was recorded after 48 h of fermentation. Hence, the pasteurization method identified in the present study can be a cost-effective strategy to preserve fermentable sugars and retain the processing properties of the sweet sorghum juice during processing, transportation, and storage under normal conditions as compared to refrigerated conditions.

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**References**

Anderson I.C. 2005. Ethanol from sweet sorghum. http://www.energy.istate.edu/renewable/biomass/csanerobicz.html

Anonymous (IEA). 1998. World Energy Outlook, 1998, Edition, Available from: <www.iea.org>.

Antonopoulou G., H.N. Gavala, I.V. Skiadas, K. Angelopoulos, and G. Lyberatos. 2008. Biofuels generation from sweet sorghum: Fermentative hydrogen production and anaerobic digestion of the remaining biomass. *Bioresource Technology* 99: 110–119.

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.

Bhardwaj, R.L., and S. Mukherjee. 2011. Effects of fruit juice blending ratios on kinnow juice preservation at ambient storage condition. *African Journal of Food Science* 5: 281–286.

Bhattacherjee, A.K., D.K. Tandon, A. Dikshit, and S. Kumar. 2011. Effect of pasteurization temperature on quality of aonla juice during storage. *Journal of Food Science and Technology* 48: 269–273.

Chauhan, O.P., D. Singh, S.M. Tyagi, and D.K. Balyan. 2002. Studies on preservation of sugarcane juice. *International Journal of Food Properties* 5: 217–229.

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

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

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.

Karmakar R., A.K. Ghosh, and H. Gangopadhyay. 2010. Study on the nutritional and microbiological changes of sugarcane juice and determination of optimum conditions during pasteurization. *Asian Journal of Food and Agro-Industry* 3: 453–461.

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.

Krishnakumar, T., and C.T. Devadas. 2006. Microbiological changes during storage of sugarcane juice in different packaging materials. *Beverage and Food World* 33: 82–83.

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. *SugarTech* 12: 322–328.

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.

Leistner, L., and M. Gorris. 1995. Food preservation by hurdle technology. *Trends in Food Science and Technology* 6: 4–9.

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* http://dx.doi.org/10.2225/vol14-issue1-fulltext-2 [accessed 14 August 2012]

Pareek S., R. Paliwal, and S. Mukherjee. 2011. Effect of juice extraction methods and processing temperature-time on juice quality of Nagpur mandarin (*Citrus reticulate* Blanco) during storage. *Journal of Food Science and Technology* 48: 197–203.

Ranken, M.D., R.C. Kill, and C.G.J. Baker. 2005. Food Industries Manual. 24th Edition, Springer-Verlag, Weinheim, Germany.

Rao, P.S., 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, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. 80 Pp. ISBN 978-92-9066-518-2.

Rao, P.S., 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 Agriculture* 26: 65–71.

Rao, P.S., 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. *SugarTech* 14: 21–25.

Sankhla, S., A. Chaturvedi, A. Kuna, and K. Dhanlakshmi. 2012. Preservation of sugarcane juice using hurdle technology. *SugarTech* 14: 26–39.

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.

Singh, I., S. Solomon, A.K. Shrivastava, R.K. Singh, and J. Singh. 2006. Post–harvest quality deterioration of cane juice: physiobiochemical indicators. *SugarTech* 8: 128–131.

Siswoyoa, T.A., Ika Oktavianawatia, D.U. Murdiyantob, and B. Sugihartoa. 2007. Changes of sucrose content and invertase activity during sugarcane stem storage. *Indonesian Journal of Agricultural Science* 8: 75–81.

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.

Yu, J., T. Zhang, J. Zhong, X. Zhang, and T. Tan. 2012. Biorefinery of sweet sorghum stem. *Biotechnology Advances* 30: 811–816.

Zoecklien, B.W., K.C. Fugelsang, B.H. Gump, and F.S. Nury. 1995. *Wine Analysis and Production*. Chapman and Hall, New York.

**Table 1** Kinetic parameters of batch ethanol production as a function of fermentation time by *Saccharomyces cerevisiae* strain ICTY417 using sweet sorghum juice samples (pasteurization at 70°C for 48 h fermentation)

| Juice samples (Storage period / Storage temperature) | Parameters (mean ± S.E.) | | | |
|---|---|---|---|---|
| | $P^{\dagger}$ ( g $L^{-1}$) | $Y_{p/s}^{\ddagger}$ (g $g^{-1}$) | $Q_p^{\$}$ (g $L^{-1}$ $h^{-1}$) | $E_y^{\#}$ (%) |
| 0 h storage period for 70°C | 65.47 ± 0.074 | 0.473 ± 0.440 | 1.343 ± 0.251 | 93.58 ± 0.108 |
| 3rd Day (35°C) | 63.44 ± 0.374 | 0.453 ± 0.412 | 1.322 ± 0.576 | 89.76 ± 0.728 |
| 3rd Day (40°C) | 61.92 ± 0.209 | 0.462 ± 0.266 | 1.29 ± 0.581 | 90.52 ±0.810 |
| 3rd Day (45°C) | 60.7 ± 0.308 | 0.466 ± 0.488 | 1.264 ± 0.651 | 91.32 ± 0.923 |
| 5th Day (35°C) | 62.94 ± 0.091 | 0.454 ±0.487 | 1.311 ± 0.362 | 90.82 ± 0.705 |
| 5th Day (40°C) | 60.25 ± 0.194 | 0.449 ± 0.746 | 1.255 ± 0.680 | 89.45 ± 0.629 |
| 5th Day (45°C) | 61.76 ± 0.931 | 0.463 ± 0.407 | 1.287 ± 0.692 | 92.45 ± 0.875 |
| 7th Day (35°C) | 63.22 ± 0.340 | 0.464 ± 0.404 | 1.317 ± 0.357 | 91.67 ± 0.772 |
| 7th Day (40°C) | 61.25 ± 0.146 | 0.452 ± 0.561 | 1.276 ± 0.389 | 90.67 ± 0.795 |
| 7th Day (45°C) | 61.82 ± 0.092 | 0.468 ± 0.808 | 1.288 ± 0.491 | 92.06 ± 0.673 |
| 9th Day (35°C) | 63.49 ± 0.618 | 0.454 ± 0.297 | 1.323 ± 0.305 | 90.89 ± 0.037 |
| 9th Day (40°C) | 60.7 ± 0.610 | 0.459 ± 0.471 | 1.265 ± 0.584 | 92.74 ± 0.904 |

| | | | | |
|---|---|---|---|---|
| 9th Day (45°C) | 61.38 ± 0.161 | 0.464 ± 0.509 | 1.277 ± 0.714 | 93.13 ± 0.273 |
| 11th Day (35°C) | 62.68 ± 0.971 | 0.458 ± 0.451 | 1.306 ± 0.728 | 89.76 ± 0.609 |
| 11th Day (40°C) | 60.7 ± 0.484 | 0.453 ± 0.847 | 1.265 ± 0.892 | 89.81 ± 0.491 |
| 11th Day (45°C) | 60.89 ± 0.114 | 0.458 ± 0.230 | 1.269 ± 0.147 | 89.72 ± 0.408 |
| 13th Day (35°C) | 63.22 ± 0.106 | 0.448 ± 0.099 | 1.317 ± 0.579 | 91.82 ± 0.627 |
| 13th Day (40°C) | 60.81 ± 0.470 | 0.46 ± 0.309 | 1.267 ± 0.672 | 90.21 ± 0.182 |
| 13th Day (45°C) | 60.41 ± 0.493 | 0.468 ± 0.467 | 1.259 ± 0.877 | 92.47 ± 0.220 |
| 17th Day (35°C) | 63.76 ± 0.368 | 0.461 ± 0.476 | 1.328 ± 0.119 | 90.71 ± 0.375 |
| 17th Day (40°C) | 61.79 ± 0.589 | 0.454 ± 0.374 | 1.287 ± 0.772 | 89.03 ± 0.191 |
| 17th Day (45°C) | 62.14 ± 0.491 | 0.468 ± 0.701 | 1.295 ± 0.684 | 91.33 ± 0.275 |
| 21st Day (35°C) | 64.3 ± 0.402 | 0.462 ± 0.905 | 1.339 ± 0.557 | 89.48 ± 0.621 |
| 21st Day (40°C) | 60.7 ± 0.835 | 0.454 ± 0.106 | 1.265 ± 0.721 | 90.05 ±0.040 |
| 21st Day (45°C) | 61.71 ± 0.676 | 0.477 ± 0.351 | 1.285 ± 0.117 | 90.96 ± 0.237 |

1 $^{\dagger}$P, actual ethanol concentration produced; $^{\ddagger}Y_{p/s}$, ethanol yield; $^{\$}Q_p$, ethanol productivity; $^{\#}E_y$, percentage of conversion efficiency or
2 yield efficiency

**Table 2** Kinetic parameters of batch ethanol production as a function of fermentation time by *Saccharomyces cerevisiae* strain ICTY417 from sweet sorghum juice samples (pasteurization at 80°C for 48 h fermentation)

| Juice samples (Storage period / Storage temperature) | Parameters (mean ± S.E.) | | | |
|---|---|---|---|---|
| | $P^{\dagger}$ ( g $L^{-1}$) | $Y_{p/s}^{\ddagger}$ (g $g^{-1}$) | $Q_p^{\$}$ (g $L^{-1}$ $h^{-1}$) | $E_y^{\#}$ (%) |
| 0 h storage period for 80°C | 63.98 ± 0.209 | 0.471 ± 0.581 | 1.332 ± 0.692 | 93.03 ± 0.875 |
| 3rd Day (35°C) | 63.98 ± 0.140 | 0.462 ± 0.615 | 1.332 ± 0.581 | 90.11 ± 0.717 |
| 3rd Day (40°C) | 62.46 ± 0.676 | 0.469 ± 0.412 | 1.301 ± 0.181 | 92.63 ± 0.609 |
| 3rd Day (45°C) | 60.48 ± 0.351 | 0.458 ± 0.925 | 1.26 ± 0.752 | 89.78 ± 0.273 |
| 5th Day (35°C) | 62.94 ± 0.117 | 0.455 ± 0.557 | 1.311 ± 0.365 | 90.81 ± 0.118 |
| 5th Day (40°C) | 59.17 ± 0.237 | 0.463 ± 0.611 | 1.232 ± 0.221 | 92.23 ± 0.857 |
| 5th Day (45°C) | 61.76 ± 0.040 | 0.457 ± 0.265 | 1.287 ± 0.172 | 89.64 ± 0.671 |
| 7th Day (35°C) | 64.3 ± 0.721 | 0.456 ± 0.674 | 1.339 ± 0.626 | 91.56 ± 0.578 |
| 7th Day (40°C) | 61.25 ± 0.106 | 0.462 ± 0.711 | 1.276 ± 0.438 | 91.33 ± 0.145 |
| 7th Day (45°C) | 61.82 ± 0.835 | 0.461 ± 0.481 | 1.287 ± 0.491 | 90.39 ± 0.892 |
| 9th Day (35°C) | 63.49 ± 0.728 | 0.461 ± 0.106 | 1.326 ± 0.037 | 89.49 ± 0.374 |
| 9th Day (40°C) | 62.33 ± 0.714 | 0.467 ± 0.454 | 1.299 ± 0.673 | 91.32 ± 0.412 |

| | | | | |
|---|---|---|---|---|
| 9th Day (45°C) | 61.38 ± 0.374 | 0.464 ± 0.971 | 1.278 ± 0.808 | 90.03 ± 0.581 |
| 11th Day (35°C) | 64.3 ± 0.476 | 0.466 ± 0.161 | 1.339 ± 0.491 | 90.77 ± 0.651 |
| 11th Day (40°C) | 62.87 ± 0.467 | 0.463 ± 0.471 | 1.309 ± 0.772 | 93.30 ± 0.362 |
| 11th Day (45°C) | 60.89 ± 0.309 | 0.454 ± 0.584 | 1.268 ± 0.357 | 90.05 ± 0.209 |
| 13th Day (35°C) | 63.76 ± 0.098 | 0.462 ± 0.904 | 1.328 ± 0.424 | 91.92 ± 0.576 |
| 13th Day (40°C) | 62.44 ± 0.231 | 0.465 ± 0.305 | 1.301 ± 0.610 | 92.57 ± 0.680 |
| 13th Day (45°C) | 63.12 ± 0.837 | 0.465 ± 0.297 | 1.315 ± 0.340 | 91.23 ± 0.629 |
| 17th Day (35°C) | 63.76 ± 0.451 | 0.459 ± 0.618 | 1.328 ± 0.875 | 89.70 ± 0.705 |
| 17th Day (40°C) | 61.79 ± 0.519 | 0.463 ± 0.092 | 1.287 ± 0.692 | 90.03 ± 0.923 |
| 17th Day (45°C) | 62.14 ± 0.569 | 0.461 ± 0.795 | 1.294 ± 0.407 | 90.32 ±0.810 |
| 21st Day (35°C) | 64.3 ± 0.453 | 0.456 ± 0.389 | 1.339 ± 0.728 | 88.79 ± 0.266 |
| 21st Day (40°C) | 60.7 ± 0.471 | 0.461 ± 0.561 | 1.265 ± 0.308 | 93.39 ± 0.488 |
| 21st Day (45°C) | 61.71 ± 0.104 | 0.459 ± 0.146 | 1.285 ±0.487 | 89.93 ± 0.746 |

1 $^{\dagger}$P, actual ethanol concentration produced; $^{\ddagger}Y_{p/s}$, ethanol yield; $^{\$}Q_p$, ethanol productivity; $^{\#}E_y$, percentage of conversion efficiency or
2 yield efficiency

**Table 3** Kinetic parameters of batch ethanol production as a function of fermentation time by *Saccharomyces cerevisiae* strain ICTY417 using sweet sorghum juice samples (pasteurization at 90°C for 48 h fermentation)

| Juice samples (Storage period / Storage temperature) | Parameters (mean ± S.E.) | | | |
|---|---|---|---|---|
| | $P^{\dagger}$ ( g $L^{-1}$) | $Y_{p/s}^{\ddagger}$ (g $g^{-1}$) | $Q_p^{\$}$ (g $L^{-1}$ $h^{-1}$) | $E_y^{\#}$ (%) |
| 0 h storage period for 90°C | 63.39 ± 0.140 | 0.475 ± 0.615 | 1.320 ± 0.591 | 93.01 ± 0.717 |
| 3rd Day (35°C) | 63.98 ± 0.629 | 0.469 ± 0.576 | 1.333 ± 0.362 | 92.11 ± 0.488 |
| 3rd Day (40°C) | 61.92 ± 0.618 | 0.465 ± 0.923 | 1.29 ± 0.581 | 90.52 ± 0.808 |
| 3rd Day (45°C) | 61.24 ±0.810 | 0.465 ± 0.728 | 1.276 ± 0.471 | 90.23 ± 0.209 |
| 5th Day (35°C) | 64.02 ± 0.692 | 0.468 ± 0.357 | 1.333 ± 0.407 | 91.70 ± 0.308 |
| 5th Day (40°C) | 60.79 ± 0.680 | 0.46 ± 0.404 | 1.267 ± 0.389 | 90.24 ± 0.037 |
| 5th Day (45°C) | 61.76 ± 0.746 | 0.464 ± 0.904 | 1.287 ± 0.875 | 90.98 ± 0.194 |
| 7th Day (35°C) | 64.3 ± 0.091 | 0.469 ± 0.305 | 1.339 ± 0.487 | 92.83 ± 0.795 |
| 7th Day (40°C) | 61.25 ± 0.651 | 0.460 ± 0.673 | 1.276 ± 0.772 | 89.99 ± 0.705 |
| 7th Day (45°C) | 60.73 ± 0.374 | 0.463 ± 0.266 | 1.265 ± 0.412 | 90.83 ± 0.795 |
| 9th Day (35°C) | 60.24 ± 0.676 | 0.446 ± 0.106 | 1.255 ±0.040 | 91.44 ± 0.375 |
| 9th Day (40°C) | 60.7 ± 0.402 | 0.459 ± 0.905 | 1.264 ± 0.191 | 89.62 ± 0.772 |

| | | | | |
|---|---|---|---|---|
| 9th Day (45°C) | 61.38 ± 0.491 | 0.461 ± 0.557 | 1.278 ± 0.368 | 90.72 ± 0.476 |
| 11th Day (35°C) | 62.68 ± 0.275 | 0.465 ± 0.684 | 1.306 ± 0.119 | 92.76 ± 0.374 |
| 11th Day (40°C) | 60.7 ± 0.237 | 0.458 ± 0.351 | 1.264 ± 0.835 | 88.81 ± 0.701 |
| 11th Day (45°C) | 60.89 ± 0.161 | 0.460 ± 0.467 | 1.268 ± 0.117 | 89.39 ± 0.621 |
| 13th Day (35°C) | 63.22 ± 0.309 | 0.463 ± 0.493 | 1.317 ± 0.147 | 93.03 ± 0.721 |
| 13th Day (40°C) | 60.81 ± 0.470 | 0.461 ± 0.297 | 1.266 ± 0.484 | 89.89 ± 0.220 |
| 13th Day (45°C) | 61.43 ± 0.491 | 0.458 ± 0.610 | 1.275 ± 0.451 | 91.08 ± 0.877 |
| 17th Day (35°C) | 60.41 ± 0.609 | 0.460 ± 0.728 | 1.258 ± 0.584 | 91.33 ± 0.099 |
| 17th Day (40°C) | 63.76 ± 0.892 | 0.458 ± 0.273 | 1.328 ± 0.092 | 88.37 ± 0.106 |
| 17th Day (45°C) | 62.11 ± 0.971 | 0.459 ± 0.509 | 1.348 ± 0.714 | 90.56 ± 0.579 |
| 21st Day (35°C) | 61.79 ± 0.114 | 0.466 ± 0.847 | 1.287 ± 0.627 | 92.31 ± 0.672 |
| 21st Day (40°C) | 62.14 ± 0.230 | 0.458 ± 0.561 | 1.294 ± 0.491 | 90.24 ± 0.182 |
| 21st Day (45°C) | 62.01 ± 0.931 | 0.461 ± 0.340 | 1.301 ± 0.146 | 90.13 ± 0.408 |

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

**Figure legends**

**Fig. 1** Sugar analysis of sweet sorghum juice samples pasteurized at (a) 70°C, (b) 80°C and (c) 90°C.

**Fig. 2** TSS and reducing sugar analysis of sweet sorghum juice samples pasteurized at (a) 70°C, (b) 80°C and (c) 90°C.

**Fig. 3** pH analysis of sweet sorghum juice samples pasteurized at (a) 70°C, (b) 80°C and (c) 90°C.

**Fig. 4** Brix analysis of sweet sorghum juice samples pasteurized at (a) 70°C, (b) 80°C and (c) 90°C.

**Fig. 5** Fermentation analysis of sweet sorghum juice pasteurized at (a) 70°C, (b) 80°C and (c) 90°C.

Figure 1

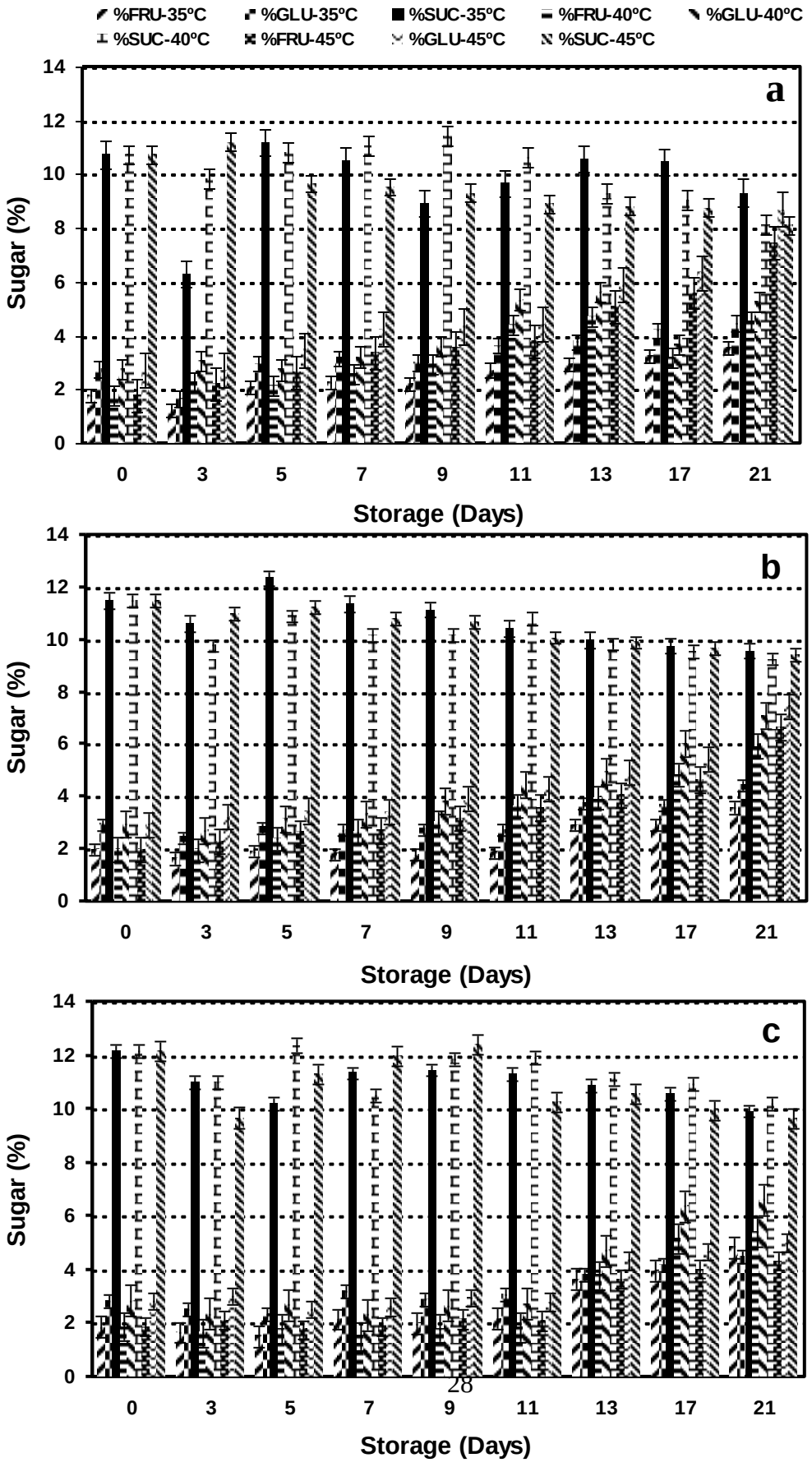
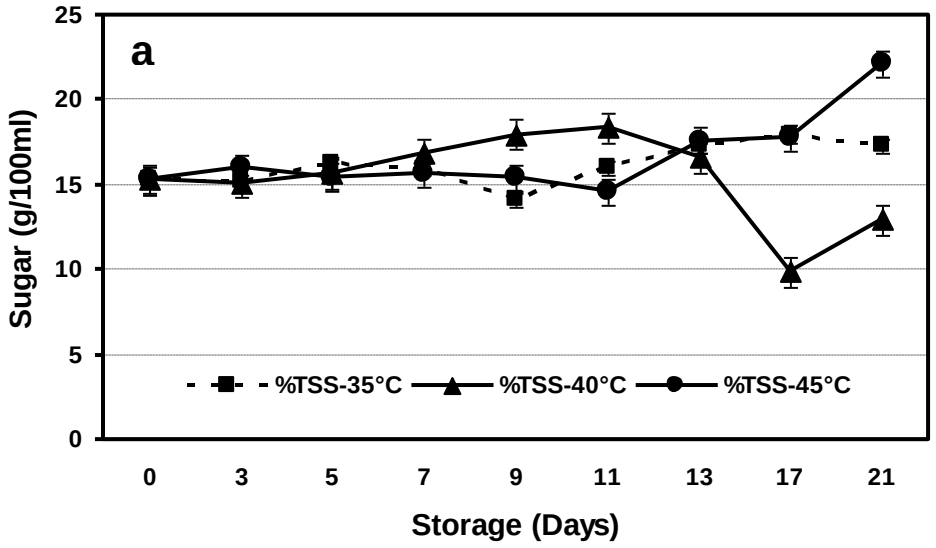
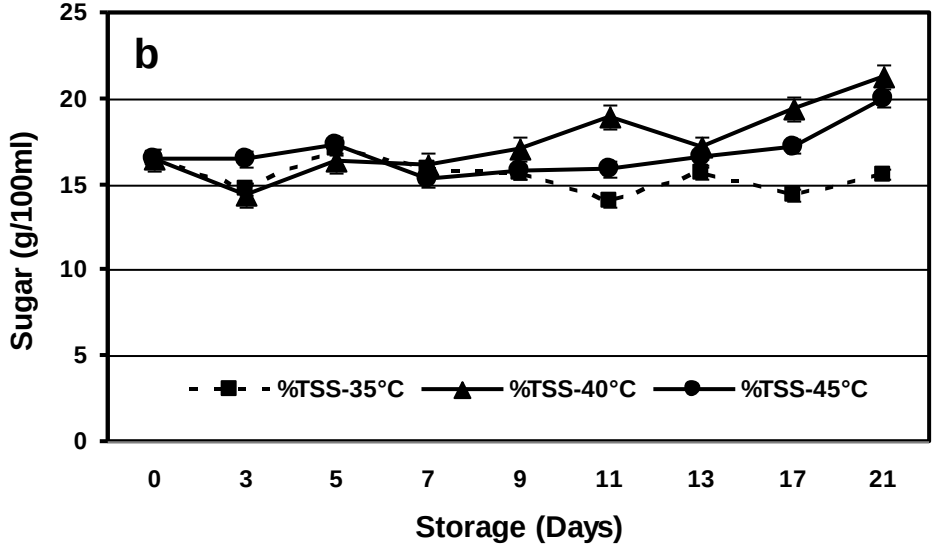

%FRU-35ºC
%GLU-35ºC
%SUC-35ºC
%FRU-40ºC
%GLU-40ºC
%SUC-40ºC
%FRU-45ºC
%GLU-45ºC
%SUC-45ºC
a
b
c
Sugar (%)
Storage (Days)
0
2
4
6
8
10
12
14
0
3
5
7
9
11
13
17
21

Figure 2


a
Sugar (g/100ml)
25
20
15
10
5
0
%TSS-35°C
%TSS-40°C
%TSS-45°C
0
3
5
7
9
11
13
17
21
Storage (Days)


b
Sugar (g/100ml)
25
20
15
10
5
0
%TSS-35°C
%TSS-40°C
%TSS-45°C
0
3
5
7
9
11
13
17
21
Storage (Days)

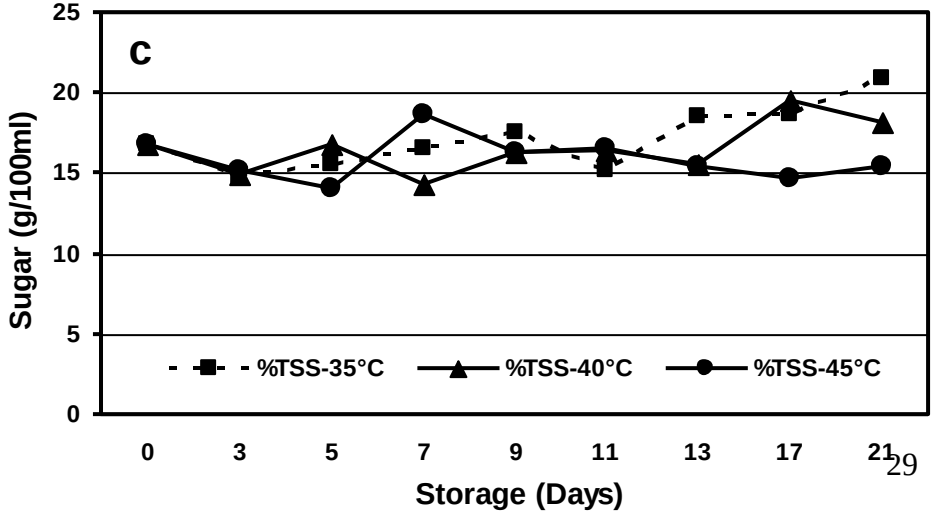

c
Sugar (g/100ml)
25
20
15
10
5
0
%TSS-35°C
%TSS-40°C
%TSS-45°C
0
3
5
7
9
11
13
17
21
Storage (Days)

Figure 3

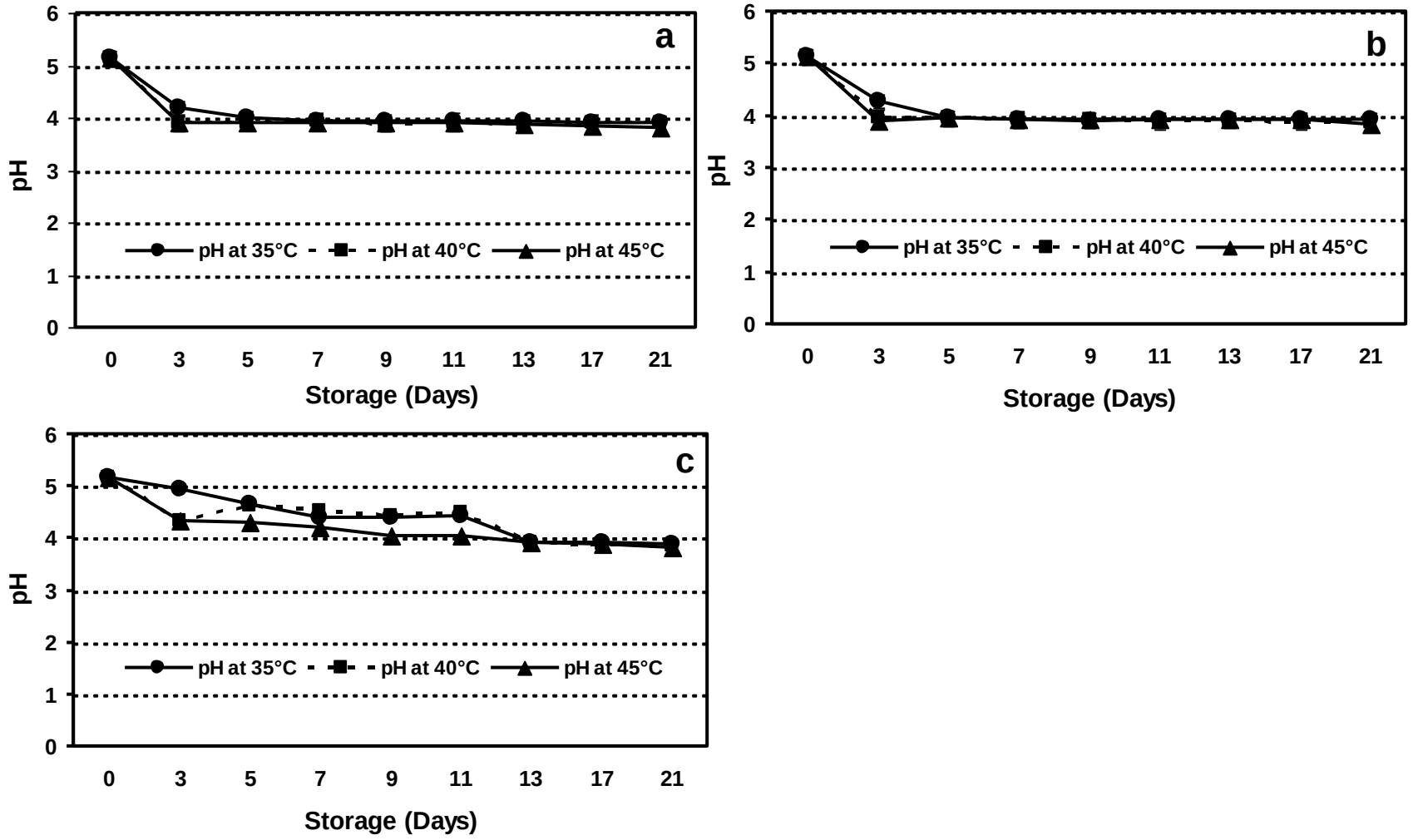

a
b
c
pH
Storage (Days)
pH at 35°C
pH at 40°C
pH at 45°C
0
1
2
3
4
5
6
0
3
5
7
9
11
13
17
21

Figure 4

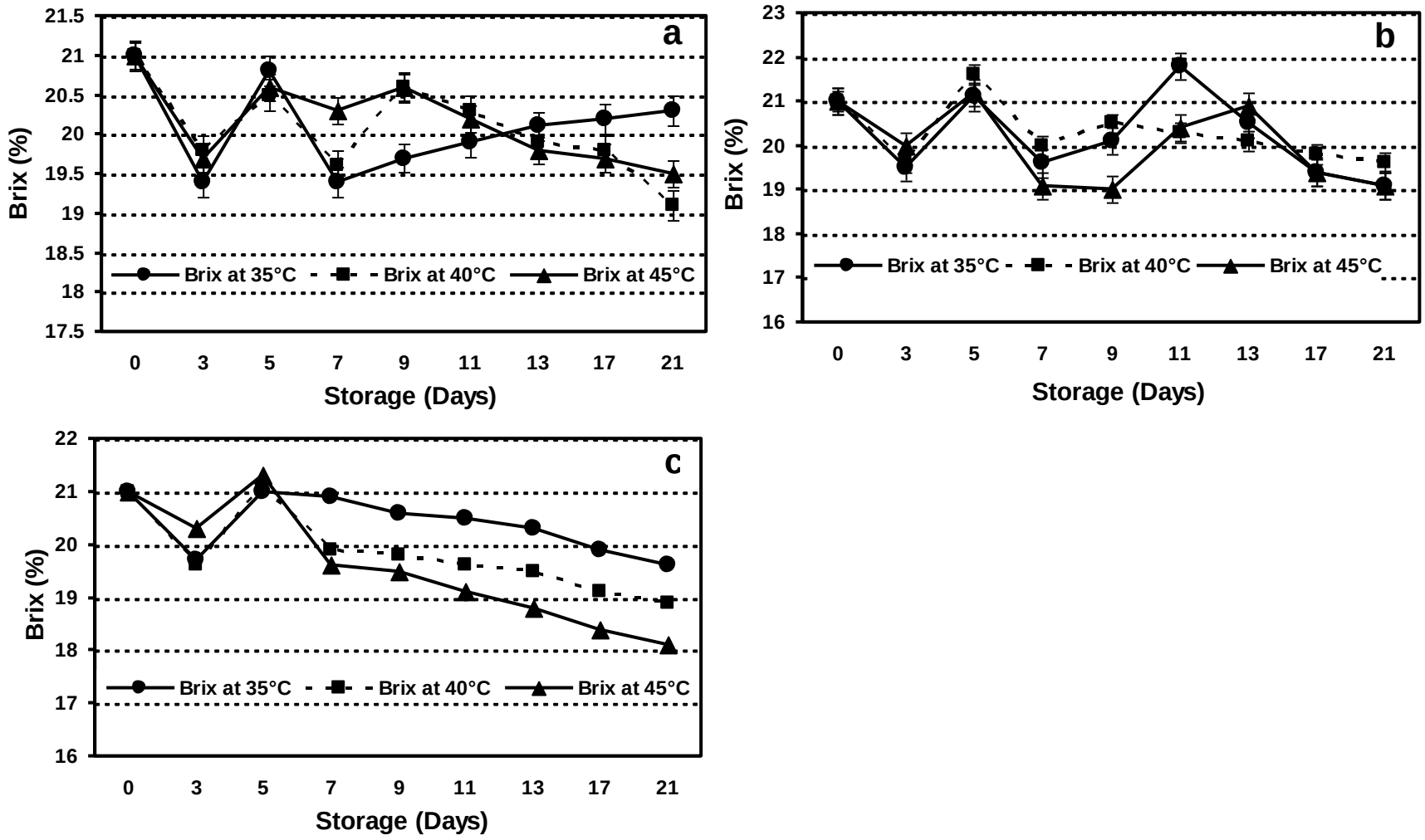
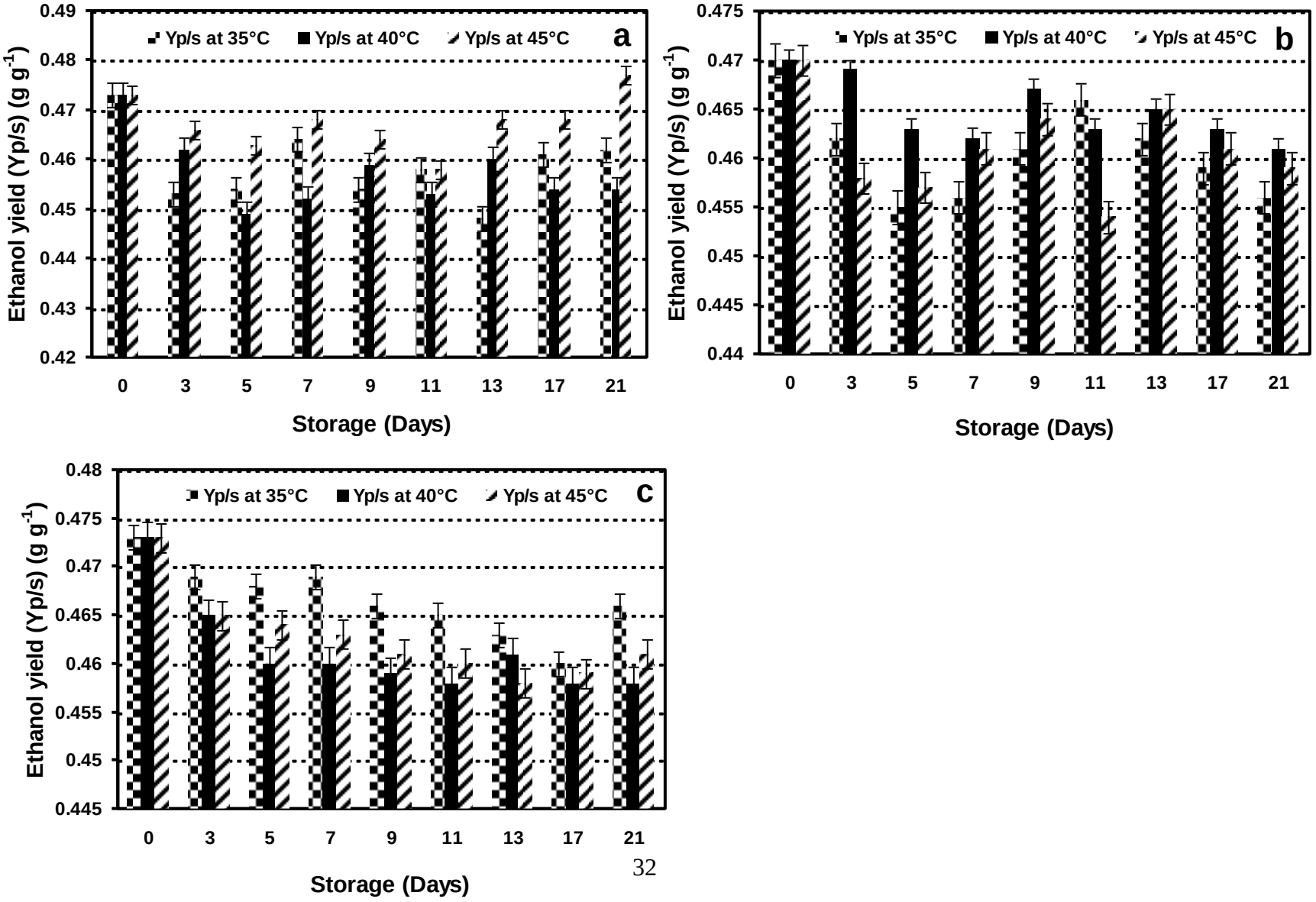

a
Brix (%)
21.5
21
20.5
20
19.5
19
18.5
18
17.5
0 3 5 7 9 11 13 17 21
Storage (Days)
Brix at 35°C
Brix at 40°C
Brix at 45°C
b
Brix (%)
23
22
21
20
19
18
17
16
0 3 5 7 9 11 13 17 21
Storage (Days)
Brix at 35°C
Brix at 40°C
Brix at 45°C
c
Brix (%)
22
21
20
19
18
17
16
0 3 5 7 9 11 13 17 21
Storage (Days)
Brix at 35°C
Brix at 40°C
Brix at 45°C

Figure 5


Yp/s at 35°C
Yp/s at 40°C
Yp/s at 45°C
a
b
c
Ethanol yield (Yp/s) (g g-1)
Storage (Days)
0
3
5
7
9
11
13
17
21