

## Features of sweet sorghum juice and their performance in ethanol fermentation

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### ARTICLE INFO

#### Article history:

Received 24 August 2009

Received in revised form

24 September 2009

Accepted 3 October 2009

#### Keywords:

Sweet sorghum juice

Ethanol

Fermentation

Sugar profile

Organic acids

### ABSTRACT

As demand for and production of fuel ethanol increase to unprecedented levels, feedstocks for ethanol production will become more diverse. Sweet sorghum is an ideal feedstock for fuel ethanol production in the Southeast and Midwest. Sweet sorghum juices usually contain approximately 16–18% fermentable sugar, which can be directly fermented into ethanol by yeast. Technical challenges of using sweet sorghum for biofuels are a short harvest period for highest sugar content and fast sugar degradation during storage. This study showed that as much as 20% of the fermentable sugars can be lost in 3 days at room temperature because of activities of contaminating bacteria, which lead to significant increases in bacterial count and decreases in pH values. No significant changes in pH value, sugar contents, and sugar profiles were observed in juices stored in a refrigerator. Fermentation efficiencies of fresh juice, autoclaved juice, and concentrated juice with 20% sugar were higher than 93% in the laboratory shake flask batch process. Fermentation of concentrated juices with 25% and 30% sugars were not complete. Significant amount of fermentable sugars remained in the finished beers of these concentrated juices. Glycerol contents in finished beers from concentrated juices were higher than in beers from normal juices. These results help to identify the most important factors affecting the quality of sweet sorghum juice under different processing and storage conditions, enabling development of effective strategies to process the juice, preserve fermentable sugars, and retain the processing properties of the juice during processing, transportation, and storage.

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### 1. Introduction

The US fuel ethanol industry is growing at an unprecedented speed. Ethanol yield reached 9.0 billion gallons in 2008, a 38% increase from 6.5 billion gallons in 2007 according to the renewable fuel association (RFA, <http://www.ethanolrfa.org/industry/statistics/>). Currently, corn is the major feedstock used for fuel ethanol production in the United States (RFA, 2008). Construction of new ethanol facilities also is proceeding rapidly, particularly across the Corn Belt, which is nearly saturated with ethanol facilities. Opportunities for continued expansion of ethanol production exist in other agricultural regions. One area with high potential for increasing contribution is the sorghum production region of the central Plains. Currently, feedstock for commercial ethanol production is ≈95% from corn grain and ≈4% from sorghum grain. Sorghum is a reasonable feedstock for ethanol production and could make a larger contribution to the nation's fuel ethanol requirements. Climate variability and continuing decreases in water availabil-

ity make conserving available energy resources and enhancing sustainable economic development increasingly important. Using dryland areas to grow grain sorghum, forage sorghum, and sweet sorghum can help achieve these goals.

Sweet sorghum is a type of sorghum that has a high concentration of soluble sugars in the plant sap, or juice. Sweet sorghum is attractive for bioethanol production because of its high fermentable sugars and very high yield of green biomass (20–30 dry tons/ha), low requirement for fertilizer, high efficiency in water usage (1/3 of sugarcane and 1/2 of corn), and short growth period (120–150 days); and, it is well adapted to diverse climate and soil conditions. These desirable agricultural characteristics make sweet sorghum a promising alternative feedstock for fuel ethanol production in the southern United States (Gibbons et al., 1986; Prasad et al., 2007; Rooney et al., 2007; Steduto et al., 1997). Sweet sorghum can produce readily fermentable sugars (sucrose, glucose, and fructose) in its juice, starch in its grain, and lignocellulose, that can be used in both current starch-based ethanol plants and future cellulosic ethanol plants. Of the 20–30 dry tons/ha of biomass, approximately 40–45% are fermentable sugars and starch, equivalent to more than 200 bushels/acre of corn yield. If all fermentable sugars in sweet sorghum are converted to ethanol, potential ethanol

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yield could be 600–650 gal/acre. However, normal pressing can recover only  $\approx 50\%$  of the total sugars in the sorghum stalk (Bryan et al., 1985). Increasing the juice yield or making proper use of remaining sugars in the bagasse is crucial for realizing the high ethanol yield of sweet sorghum and is of important economical value.

Studies on many aspects of ethanol production from sweet sorghum have been conducted during the past two decades. Buxton et al. (1999) studied the effects of different agricultural practices on performance of sweet sorghum and demonstrated that double cropping sweet sorghum with winter rye might improve soil and water conservation but not sweet sorghum yield. The effects of different harvest approaches (Worley and Cundiff, 1991) and juice processing techniques (Reidenbach and Coble, 1985; Weitzel et al., 1989) on juice recovery and ethanol yield have been investigated. Several other research groups (Day and Sarkar, 1982; De Mancilha et al., 1984) evaluated performance of several yeast strains in ethanol fermentation of sweet sorghum juices. Day and Sarkar (1982) reported that ethanol productivity varied significantly among different yeast strains; ethanol yields differed among juice batches. However, most tested strains showed a sugar to ethanol conversion efficiency of more than 90% (De Mancilha et al., 1984). Different fermentation techniques also have been tested. Solid-phase fermentation using the shredder mill system generated higher ethanol yield (78% of theoretical yield) than the forage harvest system (75% of theoretical yield) (Bryan et al., 1985). Farm-scale fermentation processes using shredded sweet sorghum in solid-phase fermentation (Gibbons et al., 1986) and sweet sorghum juice in liquid batch fermentation (Kundiya et al., 2006; Oklahoma State University, 2007) have been developed and tested. Fed-batch fermentation had a higher conversion efficiency than batch fermentation (Laopaiboon et al., 2007), and application of immobilized yeast in a fluidized bed reactor not only shortened fermentation time significantly but also increased conversion efficiency (Liu et al., 2008).

No research data on chemical, physical, and microbial changes of sweet sorghum juices as affected by preprocessing and storage condition are available. The objectives of this study were to investigate chemical, physical, and microbial characteristics of sweet sorghum juices under different preprocessing and storage conditions and performance of these juices in ethanol fermentation.

## 2. Materials and methods

### 2.1. Materials

Sweet sorghum (M81E) was planted in May at two Kansas locations (Riley and Doniphan, KS) with four replicates at each location. Plots were non-irrigated dryland with 160 lb/acre nitrogen. Plant populations were between 12,000 and 21,000/acre. Stalks were hand harvested in late October and pressed after heads and leaves were removed. Juices were stored in a refrigerator (4 °C) and freezer (−20 °C) immediately after harvest. The bacterial load and pH values of juices stored in the refrigerator and at room temperature were monitored for 2 weeks to evaluate storage stability of the juices under different temperatures.

Potassium phosphate monobasic, magnesium sulfate, dextrose, hydrochloric acid, and sodium hydroxide were purchased from Fisher Scientific (Fairlawn, NJ). Difco yeast extract was from Becton-Dickinson (Sparks, MD). Sucrose, glucose, and fructose standards were ordered from Supelco (Bellefonte, PA). All chemicals were reagent grade or better.

The dry alcohol yeast Ethanol Red, which was provided by Fermentis in vacuum-packed bags (Lesaffre Yeast Corp., Milwaukee, WI), was used for ethanol fermentation.

### 2.2. Bacterial counts

Sweet sorghum juices were serially diluted with sterile water (1:10 dilution). One milliliter of each diluted suspension was pipetted onto a 3M Petrifilm aerobic count plate and evenly distributed using a plastic spreader. Petrifilms were then incubated at 35 °C for  $48 \pm 3$  h following the manufacturer's instructions (3M Corporate Headquarters, St. Paul, MN) (Garry et al., 2004). At the end of the storage period, bacteria in the juices stored at room temperature tended to be mostly lactic bacteria, which were enumerated by diluting the juices in MRS broth and incubating the Petrifilm plates under the same conditions but in a GasPack jar with an EZ anaerobe pouch. Plates with colony numbers between 25 and 250 were chosen for colony counting.

### 2.3. Ethanol fermentation

One hundred milliliters of each sweet sorghum juice (fresh, autoclaved, or concentrated) were weighed into 250-mL Erlenmeyer flasks and supplemented with 0.3 g of yeast extract per flask. After adjusting pH values to 4.2–4.3 with 2N hydrochloric acid, juices were inoculated with 1.0 mL freshly activated dry yeast (Ethanol Red). Activation of dry yeast was conducted by adding 1.0 g of dry yeast into 19 mL of preculture broth (containing 20 g glucose, 5.0 g peptone, 3.0 g yeast extracts, 1.0 g  $\text{KH}_2\text{PO}_4$ , and 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  per liter) and shaking at 200 rpm in an incubator at 38 °C for 25–30 min. The activated yeast culture had a cell concentration of  $\approx 1 \times 10^9$  cells/mL, which ensured the inoculated juice a yeast concentration of  $\approx 1 \times 10^7$  cells/mL. Ethanol fermentation was performed in an incubator shaker (Model I2400, New Brunswick Scientific Inc., Edison, NJ) at 30 °C for 72 h at 150 rpm. Conversion efficiency was calculated by dividing the actual ethanol yield with theoretical yield of 51.1 g of ethanol generated from 100 g of glucose (Wu et al., 2006).

### 2.4. Analytical methods

Moisture contents of bagasses were determined by drying approximately 2 g of ground bagasse in a forced-air oven at  $105 \pm 3$  °C until constant weight (Sluiter et al., 2005). Concentrations of sucrose, glucose, fructose, and ethanol in juices and finished beers were determined by HPLC with a Rezex RCM-monosaccharide column (300 mm  $\times$  7.8 mm; Phenomenex, Torrance, CA, USA) and a refractive index detector (Shimadzu RID-10A, Columbia, MD, USA). The mobile phase was 0.6 mL/min of deionized water and oven temperature was 80 °C (Wu et al., 2006). Organic acids in stored juices were analyzed by the same HPLC with a Rezex ROA organic acid column (300 mm  $\times$  7.8 mm; Phenomenex, Torrance, CA, USA) and a UV-VIS detector at 210 nm (Shimadzu SPD-10AV VP, Columbia, MD, USA). The mobile phase was 0.6 mL/min of 5 mM sulfuric acid and the oven temperature was 65 °C.

### 2.5. Statistical analysis

Differences between means were compared using the ANOVA function in Microsoft Excel at the 0.05 significance level.

## 3. Results and discussion

### 3.1. Juice yield, sugar profile and sugar contents

Average dry mass yield for sweet sorghum in Riley County (KS) was 24,366 kg/ha; mass ranged from 20,373 kg/ha to 25,750 kg/ha. Dry mass yield for the same sweet sorghum in Doniphan County (KS) ranged from 18,142 kg/ha to 32,024 kg/ha with an average

**Table 1**  
Sugar, grain and total dry mass yields (kg/ha) of sweet sorghum M81E in Riley and Doniphan Counties.

	Sugars in juice	Sugar yield	Grain yield	Total dry mass
RL103	5198.6 (60.9%) <sup>a</sup>	8534.3 (32.2%) <sup>b</sup>	1876.7 (7.08%) <sup>b</sup>	26,498
RL206	3581.4 (53.6%)	6686.3 (32.8%)	1440.8 (7.07%)	20,374
RL304	4226.3 (56.4%)	7489.3 (30.2%)	1808.9 (7.28%)	24,842
RL410	4736.7 (58.7%)	8074.4 (31.4%)	872.1 (3.39%)	25,750
Average	4435.7 (57.4%)	7696.0 (31.6%)	1499.6 (6.21%)	24,366
DP111	6366.8 (65.7%)	9682.9 (30.2%)	2395.9 (7.48%)	32,024
DP209	4974.9 (60.1%)	8283.0 (33.7%)	2710.4 (11.0%)	24,568
DP304	6196.5 (59.4%)	10438.3 (34.1%)	2027.8 (6.62%)	30,640
DP413	3177.8 (58.5%)	5429.9 (29.9%)	1287.2 (7.10%)	18,142
Average	5179.0 (60.9%)	8458.5 (32.0%)	2105.3 (8.06%)	26,344

<sup>a</sup> Percentage of total sugars in the stalk.

<sup>b</sup> Percentage of the total dry mass.

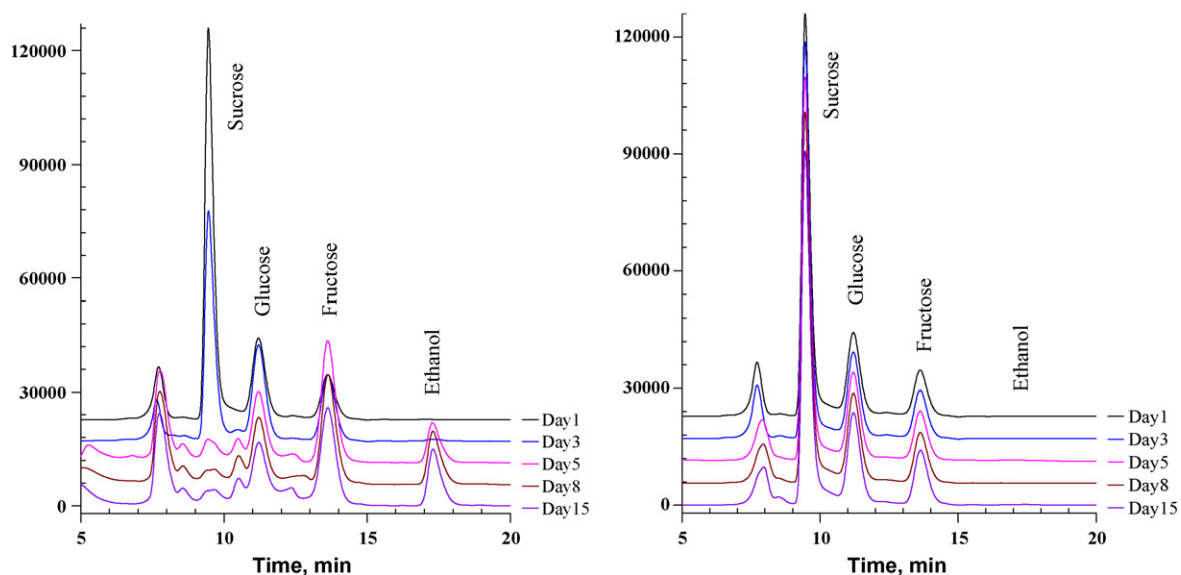
of 26,343 kg/ha (Table 1). Although yields varied numerically in different plots, there was no significant difference between average yields harvested from the two counties. Yields from the two test locations were in the upper range of reported dry mass yields (Smith et al., 1987; Weitzel et al., 1989).

Weitzel et al. (1989) reported juice yields between 46% and 54% if non-stripped stalks were pressed by roller mills, and yield increased to 58% if stalks were stripped before pressing. In the present study, all stalks were stripped before pressing. Average juice yields were 57.4% and 60.9% for sorghum grown in Riley and Doniphan Counties, respectively (Table 1), which is comparable to reported juice yields from roller mills. This means that approximately 40% of fermentable sugars in sweet sorghum are still in the bagasse. Increasing juice yield or finding ways to make use of residual sugars in bagasse will be of great economical value when sweet sorghum is used as a feedstock for fuel ethanol production.

Using a screw press could increase sugar yield in juice to 63–70%, about 10% higher than the roller mill pressing process (Weitzel et al., 1989). If combined with pith and rind-leaf separation, total sugar yield in juice could reach 75%. This is an extra 400–600 L of ethanol per hectare of sweet sorghum from an average sugar yield of 8000 kg/ha based on a modest 90% of the theoretical sugar to ethanol conversion efficiency. A recent patent application (Badalov, 2008) claimed more than

95% recovery of sugars from sweet sorghum stem using two-step emulsifiers and double press operation. A procedure used in Northeastern China (Lu et al., 1994) using three-roller squeezer juice-extracting system could extract more than 97% of the juice (not sugar) from sweet sorghum stem. If this process is commercialized, ethanol yield per acre from sweet sorghum (total of 565 gallons from a modest yield of 8000 kg sugar and 1750 kg grain per hectare, approximately 485 gallons from juice and 80 gallons from grain) will be a lot higher than that from corn (464 gallon/acre assuming 160 bushels/acre and 2.9 gallons per bushel), which will make sweet sorghum a more attractive energy crop.

Fermentable sugars in sweet sorghum are mainly sucrose, glucose, and fructose. Contents of total fermentable sugars in juices from Riley County sorghum stalks ranged from 13.77% to 15.89% with an average of 15.14% and standard deviation of 0.94%. Sugar contents in juices from Doniphan County sorghum stalks ranged from 14.44% to 16.87% with an average of 15.57% and standard deviation of 1.02%. There was no significant difference between average sugar contents in juices from Riley County and Doniphan County sorghum. Relative percentages of each sugar were approximately 70%, 20%, and 10% for sucrose, glucose, and fructose, respectively. Sugar content and profile in sweet sorghum juice of different varieties can be very different (Prasad et al., 2007). Fortunately, the



**Fig. 1.** HPLC chromatograms showing the change of sugar profile over time at room (left) and refrigerator temperature (right).

sorghum variety (M81E) used in this study had consistent high sugar content and a similar sugar profile in both growing locations.

### 3.2. Sugar content and profile changes during storage

At room temperature ( $\approx 25^\circ\text{C}$ ), sugar content and profile of sweet sorghum juice changed dramatically over time. Average sugar losses for Riley County samples were 12.3%, 31.4%, 46.3%, and 52.8% after 3, 5, 8, and 15 days, respectively, and the Doniphan samples lost 29.6%, 38.6%, and 44.5% of fermentable sugars after 3, 6, and 13 days, respectively. Sucrose content decreased quickly during storage and essentially disappeared after 5 days, whereas fructose content slightly increased over time (Fig. 1, left). Ethanol (Fig. 1, left) and organic acids (Fig. 2) started to appear after 5 days at room temperature, demonstrating that sweet sorghum juice cannot be stored at room temperature.

When stored in a refrigerator, sugar losses were less than 1% and 3% after 1 and 2 weeks of storage, respectively. Average reduction in sugar content in Riley County juice samples was 0.16%, 0.53%, 0.65%, and 2.3% after 3, 5, 8, and 15 days, respectively, sugar losses in Doniphan County samples were 0.9%, 1.0%, and 2.9% after 3, 6, and 13 days, respectively. Although sugar loss increased over time, fermentable sugar contents in the refrigerated juices were reduced less than 1% in a week, which was not significantly different from starting sugar contents. There was no noticeable change in sugar profile in the refrigerated juices within the 2-week testing period (Fig. 1, right). No significant difference in ethanol yields and sugar conversion efficiencies was observed for refrigerated juices during the 2-week storage period (data not shown).

Originally, there was essentially no acetic acid and only trace amounts of lactic acid and formic acid in the juices (Fig. 2). After 3–5 days of room temperature storage, noticeable amounts of lactic acid, acetic acid, and ethanol (Fig. 2) were detected in all juices, but the amount of formic acid remained the same, obviously a metabolic result of heterofermentative lactic acid bacteria. By the end of the 2-week storage period, formic acid contents in the juices were still the same, the amounts of acetic acid and ethanol showed a very slight increase, but concentrations of lactic acid increased dramatically to 5–10 times the concentrations of formic and acetic acids (Fig. 2). This suggested that the activity of heterofermentative lactic acid bacteria almost stopped. However, homolactic acid bacteria were active during the second week of storage at room temperature; this is evident because metabolic products of hexoses by heterofermentative lactic acid bacteria are lactic acid, acetic acids, ethanol and carbon dioxide, and the product of homofermen-

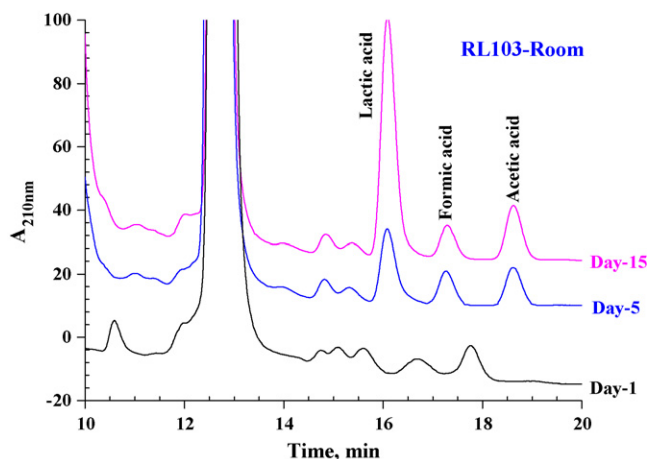


Fig. 2. HPLC chromatograms showing accumulation of organic acids in sweet sorghum juice at room temperature over time.

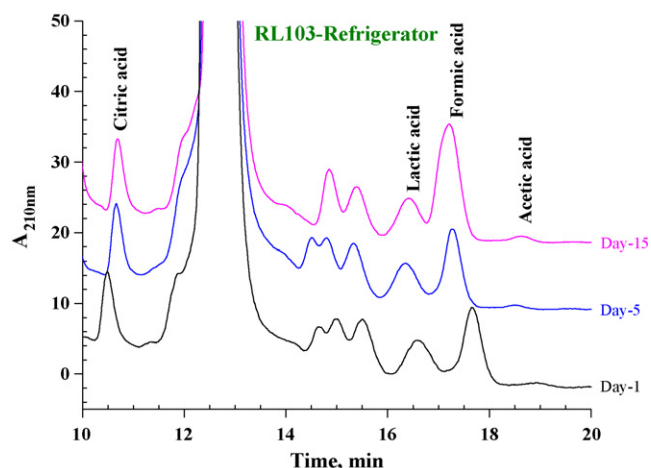


Fig. 3. HPLC chromatograms of organic acids in juice stored at refrigerator temperature.

tative lactic acid bacteria is lactic acid (Axelsson, 2004; Hofvendahl and Hahn-Hägerdal, 2000). Bacterial count results supported this.

Under refrigerated temperature, no significant change in organic acid profile was observed in juices during the 2-week storage period. Concentrations of formic acid and lactic acid remained the same, and no noticeable acetic acid was detected in juices (Fig. 3).

### 3.3. Change in pH value and bacterial counts during storage

The pH values of juices stored at room temperature decreased from an average of 4.7 on day 1 to 3.8 after 1 week and remained at  $\approx 3.8$  during the second week. The pH values of refrigerated juices increased slightly from 4.7 to 5.1. Because lowering temperature can increase the pH value of a weak acid solution and the original pH values of juices were measured at room temperature, pH of the refrigerated juices essentially were not changed during the 2-week storage period if effects of lower temperature ( $15\text{--}20^\circ\text{C}$  lower) on pH value were excluded.

Bacteria counts in juice samples during the 2-week period are shown in Fig. 4. Bacterial counts in juices stored at room temperature increased by 30–300-fold in the first week and then declined to 20–200-fold of original levels after 2 weeks of storage. Bacteria in the original juices might be very diverse, only a few species can be active under the low pH ( $\approx 4.7$ ) and anaerobic (still and sealed

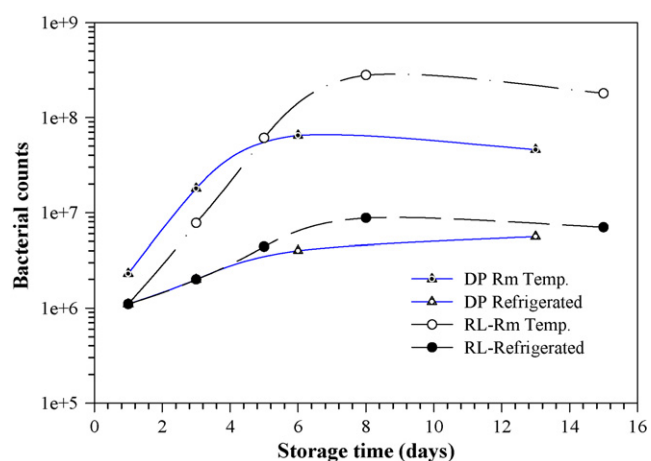


Fig. 4. Average bacterial counts in Doniphan (DP) and Riley (RL) County juices during storage.

bottles) conditions. Judged by the viscous appearance (extracellular polysaccharides), large amount of gas, and ethanol and organic acids (lactic acid and acetic acid) profile (Fig. 1, left and Fig. 2), bacteria active during the first week were heterofermentative lactic acid bacteria (Cerning, 1990). More than 95% of bacteria in the juice after 1 week were homofermentative, as indicated by the colony characteristics on the 3M Petridishes. This was confirmed by the chromatographs in Fig. 2.

Bacterial counts in the refrigerated juices increased to about 5–10-fold of original counts by the end of the 2-week storage period. As shown by the chromatograms of sugar and organic acid profiles (Fig. 1, right and Fig. 2), activity of bacteria in the refrigerated juices did not cause much change in the sugar and organic acid profiles. Results showed that if bacterial counts and pH values of sweet sorghum juices are reasonably low, juices can be safely stored for 1–2 weeks under refrigerator temperature without significant loss in fermentable sugar and fermentation quality. However, it is hard to predict quality of juice refrigerated for a longer time.

#### 3.4. Fermentation efficiency of juices with different sugar contents

Fermentation efficiencies of frozen juices, autoclaved juices, and concentrated juices with different sugar contents are listed in Table 2.

Fermentation efficiencies of frozen juices were a little higher than those of the autoclaved juices, which is different from a previous report (Rein et al., 1989). Rein et al. (1989) reported fermentation efficiencies for unheated raw juices of 17.9% to 41.1% and for heated (30 min at 60 or 85 °C) juices of higher than 90%. Several factors could have contributed to the higher efficiency of frozen fresh juice in the present study. First, hand harvest and leaf-stripping resulted in a significantly low bacterial load ( $<10^6$ /mL vs. the reported  $10^8$ /mL) in juices; second, the low initial pH (average of 4.7 vs. the reported  $\approx 6.0$ ) kept most contaminated bacteria from actively growing during handling; and third, adjusting pH to 4.2 before inoculation with yeast further prevented contaminated bacteria from competing with the inoculated yeast ( $1 \times 10^7$ /mL). Autoclaving juices could cause loss of some heat-sensitive nutrients and generate inhibitors, which can lower fermentation efficiencies of autoclaved juices.

Fermentation efficiencies of concentrated juices were significantly lower than those of the frozen or autoclaved juices, except those with 20% sugar contents (Table 3). The lower fermentation efficiencies from concentrated juices with high sugar contents could be due to the inhibiting effects of high ethanol concentration, aconitic acid, or the combination of both on yeast.

There were essentially no fermentable sugars left in the finished beer of normal sweet sorghum juices (fresh, frozen, or autoclaved),

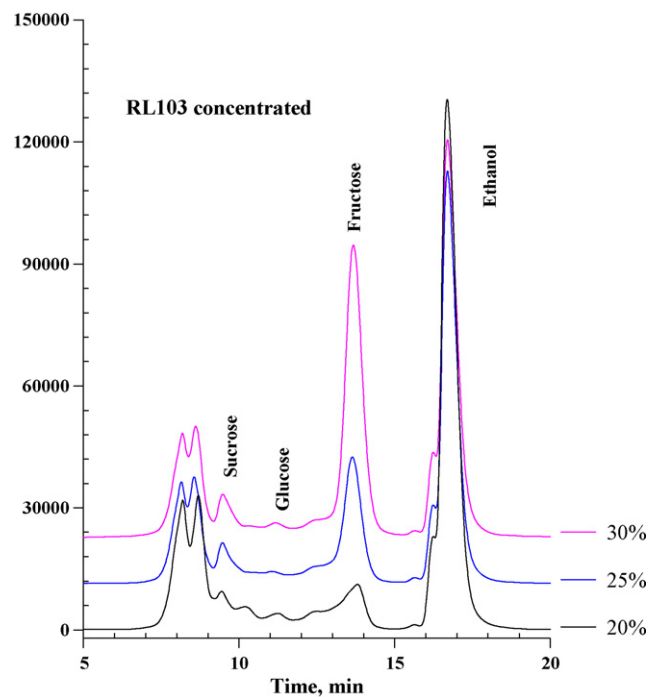


Fig. 5. Profile of residual sugars in finished beer from concentrated juices with different sugar contents.

and residual sugars in the finished beer from concentrated juices with 20% sugars were very low. A significant amount of residual sugars (approximately 4–17% of the original sugars) remained in the finished beers from concentrated juices with 25% and 30% sugars (Table 3 and Fig. 5). The residual sugar amounts in the finished beers of higher original sugar contents were similar to those (1.8–8.5%, w/v) reported by Laopaiboon et al. (2009) in high gravity sweet sorghum juice fermentation. This indicates that normal yeast used for ethanol production (brewing and distillers yeast), although can ferment essentially all the fermentable sugars (glucose and maltose) of similar concentrations in normal SSF process of maize mash (Devantier et al., 2005), may not be able to convert all the fermentable sugars in concentrated sweet sorghum juices into ethanol.

The major portion of the residual sugars in finished beers from concentrated juices was fructose. There was little sucrose and barely detectable glucose in finished beers (Fig. 5). This indicated that, among the three kinds of sugars in the concentrated sweet sorghum juices, sucrose and glucose were consumed by the yeast; but considerable amount of fructose (1.0–5.1%, w/v) was still in the finished beers from concentrated juices (25% and 30% sugars) and

Table 2  
Average fermentation efficiency of different juices (mean  $\pm$  standard deviation).

	Frozen juice	Autoclaved juice	Concentrated juices		
			20%	25%	30%
Riley juices	94.6 $\pm$ 1.1%	93.8 $\pm$ 0.8%	93.3 $\pm$ 3.0%	86.4 $\pm$ 3.9%	72.4 $\pm$ 7.5%
Doniphan juices	94.3 $\pm$ 2.7%	91.6 $\pm$ 1.1%	93.8 $\pm$ 1.9%	89.4 $\pm$ 3.1%	77.0 $\pm$ 4.4%

Table 3  
Residual sugars and glycerol contents in finished beers from concentrated juices.

	Residual sugars (%)			Glycerol (%)		
	20%	25%	30%	20%	25%	30%
Riley juices	0.35 $\pm$ 0.11	1.66 $\pm$ 0.25	5.13 $\pm$ 1.12	0.32 $\pm$ 0.03	0.46 $\pm$ 0.03	0.53 $\pm$ 0.03
Doniphan juices	0.22 $\pm$ 0.08	1.02 $\pm$ 0.39	4.13 $\pm$ 0.85	0.33 $\pm$ 0.04	0.49 $\pm$ 0.07	0.63 $\pm$ 0.07

remained essentially unchanged even 1 month after the completion of normal fermentation process. As previous research showed that common ethanol fermentation yeasts, strains of *Saccharomyces cerevisiae*, utilize sugars in mixtures of fermentable sugars in a certain order, most brewing yeasts utilize sugars in sugar mixtures in the order of sucrose, glucose, fructose, maltose, and matotriose (Meneses et al., 2002). Because of *S. cerevisiae*'s preference in utilizing sucrose and glucose to fructose (Berthels et al., 2004), sucrose and glucose are always first consumed and converted into ethanol before fructose is used if a feedstock with mixed sugars like sweet sorghum juice is used for ethanol fermentation. If the concentrations of sucrose and glucose are not too high as that presented in the original sweet sorghum juices (~15%, and <25%, w/v), the yeast although under inhibitory conditions of moderate ethanol concentration but can still manage to convert the remaining fructose in the fermentation broth into ethanol in time after all the sucrose and glucose have been utilized, therefore the final fermentation efficiency is reasonably high. However, in the concentrated juice cases, because the sugar contents were significantly higher than (about 10% higher) normal juices, ethanol concentrations in the fermentation broth was so high (~13%, w/v) that it completely represses the fermentation activity of the yeast to further ferment fructose when all the sucrose and glucose were consumed. When sucrose is utilized by yeasts, it is hydrolyzed into glucose and fructose by invertase. Fructose will stay in the broth as long as there is still glucose in the broth. Therefore, residual fructose concentration in the finished beer could be higher than that of the initial concentrated juice.

Several approaches may be used to solve the residual fructose problem in high gravity ethanol fermentation of concentrated sweet sorghum juices: using yeast strains with enhanced fructose metabolism capacity or tolerant to higher ethanol concentrations, or employing fermentation processes that alleviate the unfavorable repression effects of high ethanol and sugar concentrations. Normal *Saccharomyces* strains used in the fuel ethanol production are effective in utilizing glucose, but not so effective with fructose. The winemaking yeast strains, especially those used for making dry wines, are more effective in turning fructose in grape must into ethanol than most baker's yeasts or brewery yeasts (Guillaume et al., 2007). Grape juices usually contains approximately equal amount of glucose and fructose (glucose to fructose ratio of 0.74–1.05). Although the ability of winemaking yeast to utilize fructose in the late stage of fermentation differs among strains, the residual fructose concentrations in the finished wine are very low (ranging from 0.15–0.7%) (Reynolds et al., 2001). These numbers are much lower than those in the finished beers from concentrated juices in the present study.

Most yeast strains can ferment juices or broths with up to approximately 20% sugars (~10–12% ethanol, v/v) with high efficiencies in batch fermentation process (Belloch et al., 2008). With over 25% sugars, normal brewery yeasts will always leave significant amount of residual sugars in the finished beers (Bvochora et al., 2000; Laopaiboon et al., 2009). Some ethanol, osmo-tolerant yeast strains could ferment high sucrose and fructose juices with high efficiencies (Bertolini et al., 1991; Meneses et al., 2002).

Glycerol contents in finished beers from normal sweet sorghum juices were around 0.2%, whereas glycerol contents in finished beers from concentrated juices were significantly higher (Table 3). This also contributed to the lower fermentation efficiencies of concentrated juices.

#### 4. Conclusion

Sweet sorghum variety M81E had reasonably good biomass yields (18,000–32,000 kg/ha) at both Riley and Doniphan Counties in 2007. Sugar and grain accounted for ~40% of total dry mass yield.

Sugar contents and profiles of the sweet sorghum juices were suitable for ethanol fermentation. Juice samples from both locations showed fermentation efficiencies of 93–94% in laboratory flask shaking tests. The low pH values (average of 4.7) and low bacterial contamination levels ( $\leq 1 \times 10^6$ /mL) might have contributed to good stability under refrigerator temperature. Storing unprocessed sweet sorghum juices can be a challenge. At room temperature, up to 12–30% fermentable sugars can be lost in 3 days, 40–50% in 1 week. To achieve high fermentation efficiency in batch process, sugar contents in juices should not exceed 20%. Otherwise, both the high sugar content and the resulting high ethanol concentration will exert inhibitory effects on yeast, which will result in incomplete fermentation of fructose and higher glycerol contents in finished beers. Use of winemaking yeast strains and immobilization technique may improve fermentation efficiency of concentrated sweet sorghum juices. It is difficult to quantitatively correlate pH value and bacteria count with fermentation quality of juices during storage.

#### Acknowledgement

The authors would like to thank the Sun Grant Initiative, South Central Region, for providing the fund for this research under the project "evaluating sweet sorghum hybrids as a bioenergy feedstock".

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