Adding sweet sorghum juice into current dry-grind ethanol process for improving ethanol yields and water efficiency

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Abstract: Sweet sorghum is a promising energy crop due to its low fertilizer and water requirements, short growth period, and high biomass yield. However, the challenge for sweet sorghum as a feedstock for ethanol production is its short harvest period and the extreme instability of its juice, both of which make achieving a year-round production process difficult. One way to solve this challenge and to meet the growing demand of bio-renewable ethanol is to incorporate sweet sorghum juice into the current dry-grind ethanol process. In the dry-grind process, the whole grain kernel is milled and fermented to produce ethanol. In this study, sweet sorghum juice with varying grain sorghum flour contents was liquefied, saccharified, fermented, and distilled to produce ethanol. Ethanol yield from sweet sorghum juice with the optimum grain sorghum flour loading was about 28% higher than that from the conventional ethanol process. Enzymatic hydrolysis with this process could be reduced by 30 min. The fermentation performance of sweet sorghum juice with grain flour using a raw starch hydrolyzing enzyme was also investigated, and ethanol yield was about 21% higher than that from the conventional process. This innovative technology enabling ethanol production from sweet sorghum juice could improve ethanol yield, save energy, and significantly decrease water use in the current dry-grind ethanol process.

Keywords: sweet sorghum, biomass, ethanol yield, hydrolyzing time, conversion efficiency

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1 Introduction

According to the Renewable Fuels Association, the U.S. ethanol industry produced a total of 13.3 billion gallons of ethanol, representing 57% of the world's output in 2013. Over 98% of the renewable fuel produced in the same year was made from corn[1]. Ethanol production for blends such as E10, E15, E85, and mid-level blends is required to reach 36 billion gallons by 2022 according to the Renewable Fuel Standard (RFS) adopted by the U.S. Congress in 2005 and expanded in 2007[2]. To meet the growing demand for ethanol, potential energy crops such as wheat[3], hybrid poplar[4], and sweet sorghum could be integrated into current dry-grind ethanol production to help achieve the RFS target.

Sweet Sorghum (*Sorghum bicolor* L. *Moench*) is a promising energy crop that has high water and nitrogen-use efficiency, short growing seasons (110- 160 d), pest and disease tolerance, and high biomass productivity (45-80 t/hm^2), depending on variety and growing location[5-7]. The thick stalk and juicy internodes maintain stem juiciness until maturity, and the plant has good residue digestibility when used for lignocellulosic ethanol production[7]. Fully matured stalks contain up to

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70% water, and the remaining solid biomass is made of structural cellulose, hemicellulose, and non-structural carbohydrates (sucrose, glucose, and fructose)[8]. Unlike sugarcane, sweet sorghum also produces grain in the panicle and the grain represents 10%-30% of the total biomass. Sweet sorghum is not regarded as a food crop in the United States and can grow on diverse marginal lands. Sweet sorghum is drought-tolerant and can be cultivated in regions where other crops fail[9]. Approximately 40%-50% of sweet sorghum dry mass comprises fermentable sugars and starch (equivalent to corn yield of about 14 t/hm^2). If all of these sugars and starches are converted to ethanol, potential ethanol yield could reach 5 600-6 000 L/hm^2 compared with corn ethanol yield from 4 000-4 300 L/hm^2[10].

Sweet sorghum is considered a more efficient and cost-effective source of energy than corn because it requires less nitrogen and water[11]. As a competitive biofuel feedstock source for ethanol production, sweet sorghum has been shown to be adaptable to environmentally friendly processing, resulting in ethanol-blended fuel with lower sulfur content and a high octane rating. In addition, an ethanol-gasoline mixture of up to 25% can be used without engine modification[12-14].

The juice from sweet sorghum is extracted by mechanically crushing the stalk using roller mills, screw presses or diffusers, which results in over 95% recovery of fermentable sugars[8,15,16]. The typical composition of the fermentable juice in sweet sorghum is 53%-85% sucrose, 9%-33% glucose and 6%-21% fructose. Sugar cane juice, on the other hand, could contain 90% sucrose, 4% glucose and 6% fructose[17]. Thus, sweet sorghum is a competitive feedstock for ethanol production. The bagasse obtained after juice extraction can be combusted to generate electricity, fodder for cattle, soil fertilizer or lignocellulosic ethanol feedstock[16,18,19]. The greatest challenge in using sweet sorghum as a feedstock for ethanol production is its short harvest period and the extreme instability of the juice: up to 50% of total fermentable sugars in sweet sorghum juices would be lost if stored at room temperature for one week. This loss is due to the fact that microorganisms metabolize the sugars into organic acids and ethanol at room temperature[10]. The lack of constant feedstock supply makes it difficult for the sweet-sorghum-based ethanol industry to achieve a year-round production process, especially in temperate production environments. A possible solution to this problem is to incorporate sweet sorghum juice into the current dry-grind ethanol process.

The objective of this study is to develop a new processing technology for the current ethanol industry using sweet sorghum for ethanol production with improved energy, water efficiency and ethanol yield, and to meet the challenge of using sweet sorghum as an energy crop. Most ethanol plants require approximately 3 liter of water per liter of ethanol produced[20,21]. Using sweet sorghum juice could significantly reduce the amount of water consumed per liter of ethanol produced and could lessen conflicts over water in the Midwest, where increasing water utilization by agricultural processing facilities, livestock operations, and urban areas heightens shortages.

In this study, the performance of ethanol fermentation by granular starch hydrolysis enzymes (GSHE) on sorghum grain flour is investigated as well. Granular starch hydrolysis, also described as native or raw starch hydrolysis, converts starch to fermentable sugars at lower starch gelatinization temperatures[22]. Previous investigators have reported various studies on using GSHE to hydrolyze starch granules without prior cooking and liquefaction and simultaneous fermentation of sugars by yeast to produce ethanol[22-24]. It is also known that the granular starch hydrolysis process decreases energy input by 10%-20%[23], may increase the capacity of conversion equipment because of lower slurry viscosity, and reduces the formation of undesirable Maillard reaction products[25,26].

2 Materials and method

2.1 Materials

Sweet sorghum juice from sweet sorghum hybrid TX09052 was used in this study. TX09052 is an experimental sweet sorghum hybrid developed in the Texas A&M Agrilife Research sorghum breeding program. This hybrid was grown in College Station, Texas and at the soft dough stage of maturity; stalks were harvested and crushed using a three-roller mill (Ampro Sugar Cane Mill). Extracted juice was strained and immediately frozen at a temperature of -23°C. Prior to use, it was thawed to below room temperature. To separate remaining solid materials from the liquid, the juice was centrifuged by a Sorvall RC 6+ Centrifuge (Thermo Fisher Scientific, Asheville, NC) and concentrated to 18% sugar content by a vacuum evaporation process at room temperature. Cleaned grain sorghum samples were milled into flour through a 0.5 mm screen in an Udy cyclone mill (Udy Corp., Fort Collins, CO, USA) and used for ethanol fermentation.

2.2 Starch content analysis

The starch content of the sorghum grain was analyzed using a total starch kit (Megazyme International) following an accepted method[27].

2.3 Ethanol fermentation of varying grain sorghum loadings with sweet sorghum juice

Samples of grain sorghum flour (30.0 g dry base db) were weighed into a clean 250 mL Erlenmeyer flask and mixed with 100 mL of preheated (about 60°C) enzyme solution containing 0.1 g of KH_2PO_4 and 20 μL of Liquozyme (alpha-amylase, Novozymes, Franklinton, NC) to form an evenly suspended slurry. Additional samples of grain sorghum flour (6.0 g, 9.0 g, 12.0 g, and 15.0 g) were also weighed into clean 250 mL Erlenmeyer flasks and mixed with 100 mL of preheated (60°C to 70°C) sweet sorghum juice; each flask contained 0.1 g of KH_2PO_4, and 20 μL of Liquozyme (240 KNU/g, about 1.15 g/mL) (alpha-amylase, Novozymes, Franklinton, NC). One hundred milliliters of sweet sorghum juice was measured into another clean 250 mL Erlenmeyer flask and mixed with 0.1 g of KH_2PO_4. For starch liquefaction, the flasks were transferred to a 70°C water-bath shaker operating at about 180 r/min. The temperature of the water bath was gradually increased from 70°C to 90°C in a 30 min period, kept at 90°C for a few minutes, and then, lowered to 85°C; liquefaction continued for 60 min. Flasks were then removed from the water bath, and materials sticking on the inner surface of the flasks were pushed back into the mashes with a spatula. The spatula and inner surface of the flasks were rinsed with 3-5 mL of distilled water. After cooling to room temperature (25°C to 30°C), the pH of the mashes was adjusted to around 4.2 with 2 mol/L HCl.

2.4 Preparation of Inoculum

Dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4 and 0.5 g of $MgSO_4{\cdot}7H_2O$ per liter) and incubated at 38°C for 30 min in an incubator operating at 200 r/min. The activated yeast culture had a cell concentration of 1×10^9 cells/mL.

2.5 Simultaneous saccharification and fermentation (SSF)

The SSF process started with the addition of 1.0 mL of activated yeast culture, 100 μL of Spirizyme, (750 AGU/g, about 1.15 g/mL) (Novozymes, Franklinton, NC), and 0.30 g of yeast extract into mashes in each flask. Flasks were sealed with an S-airlock with mineral oil. Fermentation was conducted at 30°C for 72 h in an incubator shaker operating at 150 r/min. Fermentation performance was monitored by weighing the fermentation flasks over the 3 d incubation period at 4, 8, 18, 24, 32, 44, 56 and 72 h of fermentation. The weight loss was due to the evolution of CO_2 during the fermentation process ($C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2\uparrow$).

2.6 Distillation

After the fermentation process (72 h), the finished mash was transferred to a 500 mL distillation flask. The Erlenmeyer flask was washed several times with 100 mL of distilled water. Two drops of antifoam agent were added to the distillation flask before the flask was placed on a heating unit to prevent foaming during distillation. Distillates were collected into a 100 mL volumetric flask immersed in ice water. When distillates in the volumetric flask approached the 100 mL mark (about 99 mL), the volumetric flask was removed from the distillation unit. Distillates in the volumetric flask were equilibrated for a few hours in a 25°C water bath. The ethanol concentration was determined by HPLC following the method described by Wu et al.[28]. Fermentation efficiencies were calculated as the actual ethanol yield divided by the theoretical ethanol yield. The theoretical ethanol yield was determined using the total starch contents in the samples, assuming 0.511 g ethanol from 1 g of starch[29].

2.7 Ethanol fermentation with granular starch hydrolyzing enzyme (GSHE)

Samples of grain sorghum flour (6.0, 9.0, 12.0 and 15.0 g) were weighed into clean 250 mL Erlenmeyer flasks. One hundred milliliters of sweet sorghum juice was also measured into another clean 250 mL Erlenmeyer flask. Flasks containing sorghum grain flour were mixed with warm sweet sorghum juice (60°C to 70°C) to hydrate the starch granules. Samples were treated with 60 μL granular starch hydrolyzing enzyme (STARGEN 002, Novozymes, Franklinton, NC, USA), and pH was adjusted to 4.2 by 2 mol/L HCl. Flasks were then set in a water bath at 48°C for 2 h. The SSF process started with the addition of 1.0 mL of activated yeast culture and 0.30 g of yeast extract in each flask. Fermentation was conducted following the procedure mentioned above.

2.8 Statistical analysis

All experiments were performed at least in duplicate. The tabular results presented were the mean values of repeated experimental data. Regression analysis was conducted in Microsoft Excel with the linear regression function.

3 Results and discussion

3.1 Ethanol fermentation of sweet sorghum with varying sorghum grain loading

Figure 1 shows the comparison of ethanol yields of sweet sorghum juice with varying grain sorghum loadings. Fermentation of the juice-only sample was completed after 32 h of fermentation and yielded 11.33% ethanol (v/v), with a high conversion efficiency of 93.15%. Sweet sorghum juice containing 6.0 g of grain sorghum flour and the control, 32.0 g flour and water (instead of juice) had similar ethanol performance and offered comparable ethanol yields of 14.36% and 14.05% (v/v) after the 72 h, respectively (Table 1).

Although fermentation of the control was complete after about 65 h, the process continued for 12.0 and 15.0 g samples through 72 h. Among the grain sorghum flour samples, the 15.0 g loading showed the highest yield (18.05% (v/v)) and the lowest conversion efficiency (90.93%) (Table 1). Fermentation results showed that ethanol fermentation efficiency decreased as flour loading increased, corroborating the results obtained by previous investigators[30]. Samples with lower starch loading would give higher fermentation efficiency, if the same amount of yeast were used for the ethanol conversion from sugar[31]. Decreasing efficiencies may be attributed to higher viscosity with increasing starch content[28,31-33]. Sweet sorghum juice is viscous and exhibits pseudoplastic behavior[34]; ground grain sorghum mash is also known to be viscous[33].

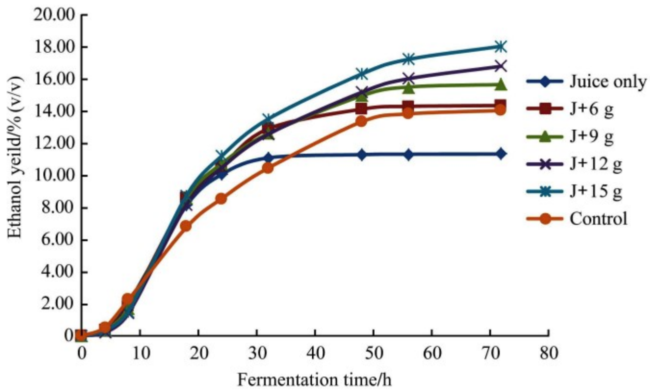

Figure 1 Comparison of ethanol yields of sweet sorghum juice (100 mL) with varying grain sorghum flour loadings

Table 1 Ethanol yields and fermentation efficiencies of sweet sorghum juice with varying grain sorghum loading

	Juice sugar content /%	Flour starch content /%	Theoretical ethanol yield/% (v/v)	Actual ethanol yield/% (v/v)	Ethanol fermentation efficiency /%
Juice only	18.89	0	12.12	11.29[a]	93.15[b]
Juice + 6.0 g flour	18.89	71.57	15.21	14.36[b]	94.41[a]
Juice + 9.0 g flour	18.89	71.57	16.75	15.67[c]	93.55[b]
Juice+ 12.0 g four	18.89	71.57	18.29	16.81[d]	91.91[c]
Juice + 15.0 g flour	18.89	71.57	19.95	18.05[e]	90.48[d]
Control- 30.0 g flour (db)	0	71.70	15.48	14.05[b]	90.75[d]

Note: Means in the same column followed by different superscript letters indicate significant differences ($P \leqslant 0.05$).

In this study, the sample with 15.0 g of grain sorghum displayed the highest ethanol yield of 18.05% (v/v), a 28.47% increase compared with the control (14.05% (v/v)), greater than average yield from highly irrigated sorghum (14.10% (v/v))[30], and greater than average ethanol yield (14.44% (v/v)) from 70 sorghum genotypes and elite hybrids[28]. Samples with high yields also had high conversion efficiency, which agreed with previous studies of ethanol fermentation from grain starch.

The highest yield found in this study was greater than the results obtain from modified and conversional dry-grind processes using four different corn types, as published by Kullar et al.[35]. They reported the highest final ethanol yields of 15.7% (v/v) for wet fractionation, 15.0% (v/v) for dry fractionation and 14.1% (v/v) for the conventional process. Results from this research showed that incorporating sweet sorghum juice into dry-grind ethanol production allows high gravity fermentation and therefore, results in high ethanol yield.

3.2 Ethanol fermentation with varying enzymatic hydrolysis times

Based on the results obtained from the above study, the optimal ethanol fermentation of sorghum mashes from sweet sorghum juice by altering starch enzymatic hydrolysis time was investigated. Four flasks consisting of homogenous slurries of 15.0 g grain sorghum flour and 100 mL sweet sorghum juice were liquefied, saccharified, and fermented by *S. cerevisiae* to produce ethanol following the above procedure. Starch enzymatic hydrolysis among the flasks was conducted for periods of 30, 45, 60 and 90 min. The ethanol yields of the samples after the 72 h fermentation period are displayed in Figure 2 and Table 2 compares the yields and efficiencies. As shown in Figure 2, no significant difference in ethanol yields occurred among the four samples. Ethanol yields were comparable and ranged from 17.84% (v/v) for the 30 min hydrolysis sample to 18.05% (v/v) for the 90 min sample (Table 2), which corresponded to similar efficiencies of 89.12% to 90.93%, respectively. In this section, the hydrolysis time of 60 min was as the control. The difference in ethanol yields between the 30 min sample and the 60 min sample was 0.49%, and the change in yield between the 45 min and 60 min samples was 0.48%. Similar to the graphical representation, the conversion efficiencies in Table 2 also demonstrated little difference among the samples.

Results indicate that enzymatic hydrolysis for ethanol production from sweet sorghum juice with grain sorghum starch can be shortened to 30 min to save time and conserve energy in the dry-grind ethanol fermentation process.

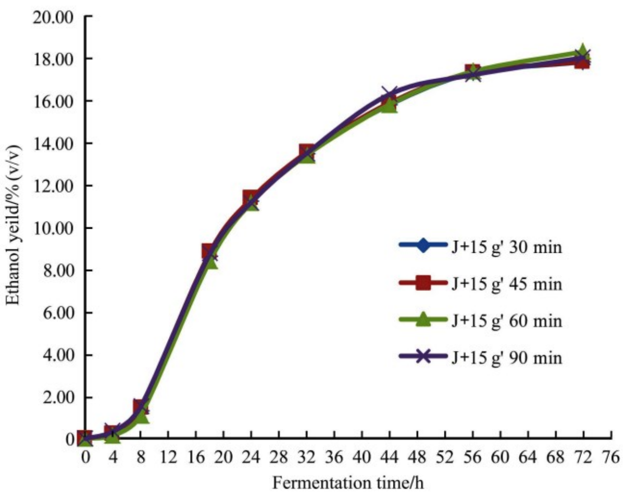

Figure 2 Effect of hydrolysis time on ethanol yield from mixture of sweet sorghum juice (100 mL) and grain sorghum flour (15.0 g)

Table 2 Ethanol yields and fermentation efficiencies of mixture of sweet sorghum juice (100 mL) and grain sorghum flour (15.0 g) with varying hydrolysis times

Hydrolysis time/min	Juice sugar content/%	Flour starch content/%	Theoretical ethanol yield/% (v/v)	Actual ethanol yield% (v/v)	Ethanol fermentation efficiency/%
30	18.89	71.57	19.95	17.84[a]	89.42[c]
45	18.89	71.57	19.95	17.85[a]	89.47[c]
60	18.89	71.57	19.95	18.33[a]	91.88[a]
90	18.89	71.57	19.95	18.05[a]	90.48[b]

Note: Means in the same column followed by different superscript letters indicate significant differences ($P \leqslant 0.05$).

3.3 Ethanol fermentation by GSHE

Ethanol yield performances of sweet sorghum juice and grain sorghum flour by the granular starch-hydrolyzing enzyme, Stargen 002, are presented in Figure 3. Samples had similar yield performance until after 18 h of fermentation, when differences in ethanol yields emerged. Significant differences in ethanol yields among the samples were noticed at the end of the fermentation process (72 h) and varied from 10.73% to 16.97% (v/v). Conversion efficiencies also ranged from 87.66% to 94.65% (Table 3). However, samples that contained 9.0 g and 15.0 g of grain sorghum flour loading showed comparable yield performance throughout the entire fermentation process. From observing the yield curves, it can be concluded that fermentation of the juice-only sample was completed in approximately 24 h and produced the lowest ethanol yield (10.73%, v/v), the highest conversion efficiency (94.85%). The high conversion efficiency of the juice alone can be attributed to the lesser amount of sugars available for the same amount of yeast conversion to ethanol compared with the other samples. The 15.0 g loading showed the highest ethanol yield of 16.97% (v/v), representing a yield increase of 20.78% compared with the control (Table 2). Results indicated that sorghum starch content had a significant effect on ethanol yield. Ethanol concentration increased with increasing sorghum flour loading. The yield obtained from this study also was greater than the ethanol yield produced from the modified and conversional dry-grind process reported by Kullar et al[33].

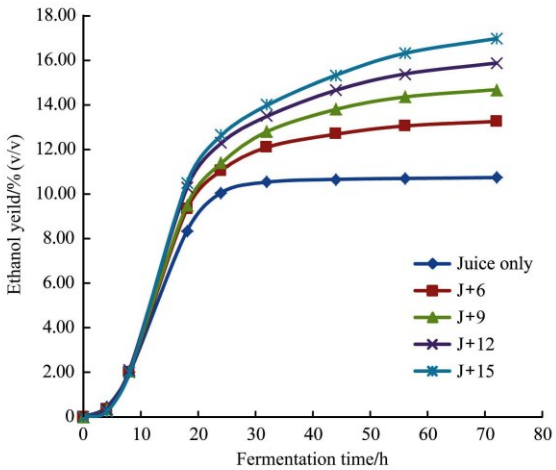

Figure 3 Comparison of ethanol yield performances from sweet sorghum juice (100 mL) with varying grain sorghum flour loadings by granular starch hydrolysis enzymes

Table 3 Comparison of ethanol yields and fermentation efficiencies of sweet sorghum juice (100 mL) with varying grain sorghum loading by GSHE

	Juice sugar content/%	Flour starch content/%	Theoretical ethanol yield /% (v/v)	Actual ethanol yield /% (v/v)	Ethanol fermentation efficiency/%
Juice only	17.5	0	11.33	10.73[a]	94.65[a]
Juice + 6.0 g flour	17.5	71.57	14.42	13.24[b]	91.82[b]
Juice + 9.0 g flour	17.5	71.57	15.96	14.67[c]	91.92[b]
Juice + 12.0 g flour	17.5	71.57	17.51	15.87[d]	90.63[c]
Juice + 15.0 g flour	17.5	71.57	19.05	16.70[e]	87.66[d]

Note: Means in the same column followed by different superscript letters indicate significant differences ($P \leq 0.05$).

4 Conclusions

Results showed incorporating sweet sorghum juice into the current dry-grind ethanol process can improve ethanol yield, save energy and increase water efficiency. High-gravity fermentation can be applied when using sweet sorghum juice instead of water for ethanol fermentation. Ethanol yield from the mixture of sweet sorghum juice and sorghum flour was about 28% higher than from the conventional method, and ethanol yield increased as flour loading increased. The results of this study also showed that the enzymatic hydrolysis time could be reduced by 30 min, which will help conserve water and energy. In addition, sweet sorghum juice enhances the potential for ethanol production from starch-based materials by granular starch-hydrolyzing enzymes.

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