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Ethanol production potential of sweet sorghum assessed using forage fiber analysis procedures

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Abstract

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is widely recognized as a highly promising biomass energy crop with particular potential to complement sugarcane production in diversified cropping systems. Agronomic assessments have led to identification of four cultivars well suited for such sugarcane-based production systems in southern Louisiana. Sweet sorghum biofuel production systems are currently being developed, and research producing large sample numbers requiring ethanol yield assessment is anticipated. Fiber analysis approaches developed for forage evaluation appear to be useful for screening such large numbers of samples for relative ethanol yield. Chemical composition, forage fiber characteristics, digestibility, and ethanol production of sweet sorghum bagasse from the four cultivars were assessed. Measures of detergent fiber, lignin, and digestibility were highly correlated with ethanol production ($P < 0.01$). The best linear regression models accounted for about 80% of the variation among cultivars in ethanol production. Bagasse from the cultivar Dale produced more ethanol per gram of material than any of the other cultivars. This superior ethanol production was apparently associated with less lignin in stems of Dale. Forage evaluation measures including detergent fiber analyses, *in vitro* digestibility, and an *in vitro* gas production technique successfully identified the cultivar superior in ethanol yield indicating their usefulness for screening sweet sorghum samples for potential ethanol production in research programs generating large sample numbers from evaluations of germ plasm or agronomic treatments. These screening procedures reduce time and expense of alternatives such as hexose sugar assessment for calculating theoretical ethanol yield.

Keywords: biofuel conversion, digestibility, ethanol production, fiber analysis, *in vitro* gas production, lignin, *Sorghum bicolor*, sweet sorghum bagasse

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Introduction

Sweet sorghum has been evaluated for biofuel feedstock potential since the 1980s because of its capability of producing sugar-enriched juice and high biomass yields. Sweet sorghum has particular potential for biofuel production in areas where sugarcane is currently produced because sugarcane is harvested only during a rather short period primarily between November and February. Harvest of sweet sorghum should be possible from as early as late July for early cultivars until frost, which is typically after the start of sugarcane harvest. Sweet sorghum can extend the season of sugar mill operation each year to enhance economic viability of this established industry, while contributing to development of the new biofuels industry. Thus, sweet sorghum has been identified as a particularly promising complementary crop for diversification of sugarcane croplands. Ethanol yields of 4.8 g per 100 g of fresh stalks have been obtained from soluble sugars in expressed juice along with additional production from the residual non-soluble material of 5.1 g of ethanol per 100 g of fresh stalks (Mamma *et al.*, 1995). The extractable juice component of sweet sorghum provides a non-food source of readily fermentable sugars comparable to the carbohydrates of

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maize grain and juice of sugarcane, which are food resources currently used for ethanol production. High biomass production of sweet sorghum also provides a cellulosic source of ethanol in sufficient quantity to further enhance economic opportunities for this crop. Along with recent worldwide interest in sweet sorghum, widespread agronomic evaluations across the United States have demonstrated that it has wide adaptability and efficient production potential (Smith *et al.*, 1987; Smith & Buxton, 1993). Various assessments of the chemical composition of this crop and their potential relationships with ethanol production have been reported (Smith *et al.*, 1987; Smith & Buxton, 1993; Billa *et al.*, 1997; Dolciotti *et al.*, 1998; Zhao *et al.*, 2009). Substantial differences have been reported in chemical constituents of both soluble and structural components between a sweet sorghum cultivar and a non-sweet sorghum hybrid (Dolciotti *et al.*, 1998). Differences in chemical composition among genotypes, years, and harvest times along with a genotype-by-year (environment) interaction have also been reported for sweet sorghum (Zhao *et al.*, 2009). Hydrolysis of structural components of biofuel feedstocks for conversion to fuels is affected by aspects of chemical composition and treatment including arabinose to xylose ratio, penetration of pretreatment chemicals, cellulose structure (crystalline vs. amorphous), and lignin/hemicellulose contents, and their distributions (Kurakake *et al.*, 2001; Park *et al.*, 2010; Vandenbrink *et al.*, 2010).

Because of the laborious and time-consuming methods for determining chemical composition and ethanol yields, recent interest has developed in finding more efficient methods for screening large numbers of samples for ethanol production potential. Large sample numbers are generated by research programs evaluating plant genetic improvement efforts and agronomic treatments for increased production efficiency. Content of fibers resistant to acid detergent (acid detergent fiber, ADF) and neutral detergent (neutral detergent fiber, NDF) have been commonly used to indicate relative digestibility and intake, respectively, of forage samples. Biological degradation during digestion by ruminant livestock is somewhat similar to enzymatic hydrolysis procedures used to release sugars from cell wall polysaccharides of biofuel crops for ethanol production, with a key exception that rumen microbes utilize both glucans and xylans. Currently available technologies with potential for practical application to ethanol production from biomass feedstocks utilize primarily cellulose-derived glucose because enzyme and fermentation technology for cellulose conversion to ethanol are available and further developments are required for effective use of xylans. Future approaches for conversion of xylans from the hemicellulose fraction of cell walls to ethanol should make rumen microbe-based bioassays even more closely correlated with ethanol production than relationships based on current approaches. The application of an *in vitro* ruminal fermentation assay measuring gas production was proposed as a potential rapid feedstock evaluation method to provide rank order of ethanol production among treatments based on fermentability (Weimer *et al.*, 2005). This approach is currently used in forage quality analysis (Seo *et al.*, 2009). The ability of pure cultures of the rumen bacterium *Ruminococcus albus* to ferment sweet sorghum soluble sugars along with cellulose and hemicellulose for hydrogen production (Ntaikou *et al.*, 2008) provides some confirmation for the use of a ruminal assay approach with sweet sorghum biofuel evaluation. The application of forage evaluation procedures for assessment of ethanol production from biomass materials was recently reported by Lorenz *et al.* (2009), who determined that 95% of the variation in ethanol yield among genetically divergent maize (*Zea mays* L.) samples could be explained by a regression model consisting of neutral detergent fiber (NDF) and NDF digestibility (NDFD).

The cultivars Dale, M81-E, Theis, and Topper have recently been suggested as suitable cultivars for use as energy crops in southern Louisiana based on agronomic performance (Tew *et al.*, 2008). Evaluations of chemical differences potentially affecting ethanol production among these four cultivars have not been reported, although calculations of theoretical ethanol yields based on estimated hexose sugar yields of juice and estimated fiber yields of bagasse were reported (Tew *et al.*, 2008). The cultivars Dale, M81-E, Theis, and Topper did not differ in this theoretical ethanol yield. This potential ethanol yield calculation did not consider effects of plant cell wall structural differences on conversion of plant fiber to ethanol. Of particular interest is the structural composition of the bagasse and relationships of structural components with ethanol production. Because of the potential usefulness of a rapid and inexpensive screening procedure to assess ethanol yield differences among sweet sorghum samples in research programs, the primary objective of this research was to assess potentially useful chemical fiber analysis procedures and biological assays of forage digestibility to provide indications of relative ethanol yield within a set of sweet sorghum samples. Samples from replicated field plots of the four cultivars identified for use in Louisiana were subjected to evaluations of chemical composition, ethanol production, forage fiber characterization, and forage digestibility to provide this assessment. A secondary objective was to determine any differences in ethanol yield among cultivars assessed and identify associations of plant chemical composition with any detected differences in ethanol yield.

Materials and methods

Field production of sweet sorghum samples

Stem samples for analysis of sugar composition of sweet sorghum juice, chemical composition of the residue (bagasse) following mechanical juice expression, bagasse ethanol yield, and bagasse fiber characteristics were obtained from field plots at the Southeast Research Station near Franklinton, Louisiana in 2008. The four cultivars Dale, M81-E, Theis, and Topper were planted in a randomized complete block experimental design with four replications. Plots were planted in April at 2 kg ha^{-1} of seed on a Tangi silt loam (fine-silty, mixed thermic Typic Fragiudult) soil. Each experimental unit consisted of a plot four rows wide and 15 m long with 91-cm row spacing. Nitrogen fertilizer was applied at a rate of 33 kg ha^{-1} when sweet sorghum was planted. Another 33 kg ha^{-1} of nitrogen fertilizer was applied when plants were approximately 30 cm tall. Plants were harvested in September at the hard-dough stage of seed maturation. Stems were hand clipped from the two center rows in each plot at approximately a 6-cm stubble height. Leaves were stripped from stems and seed heads were removed, with only stems subjected to further analysis.

To confirm cultivar responses to fiber analyses, a second set of stem samples from plots at the Hill Farm Research Station in northwestern Louisiana were collected and processed by standard forage quality evaluation procedures. As with the plots at the Southeast Research Station described in the preceding paragraph, the four cultivars Dale, M81-E, Theis, and Topper were grown in field plots in a randomized complete block experimental design with four replications. Plots were planted in April 2008 on a Darley gravelly loamy fine sand (clayey, kaolinitic, thermic Typic Hapludult) at the same seeding rate as at the Southeast Research Station. Plots were 1.5 by 6 m planted on 18-cm row spacing. Nitrogen fertilizer was applied at 67 kg N ha^{-1} in late May. Harvest was in September when plants were in the hard-dough stage. Stems were not processed through the three-roller mill to remove the juice as was done with samples from the Southeast Research Station, but harvest and sample preparation for fiber analyses were otherwise similar to procedures used to prepare the bagasse samples from the Southeast Research Station with dried, ground samples subjected to the fiber analysis procedures.

Juice analysis for soluble components

Ten stems taken from each experimental unit of the planting at the Southeast Research Station were pressed through a three-roller sugarcane mill three times. Juice was filtered and immediately analyzed for concentration of solids (°Bx,% w/w) by refractometry in a standard sugarcane Brix analysis. Sugar yield was determined by the equation: sugar yield = fresh stem yield × Bx/100 × 0.90. Individual fermentable sugars including sucrose, glucose, and fructose were determined using an HPLC system (Agilent 1200 series) equipped with an HPX-87K column (Bio-Rad, Hercules, CA) and a differential refractive index (DRI) detector (G1362A Agilent). Totals of the individual sugars from the HPLC analysis were considered as total fermentable sugars.

Bagasse sample pretreatment

Samples of 500 g each from the bagasse of each experimental unit were subjected to lime ($Ca(OH)_2$) pretreatment. These samples were weighed fresh and dried for 72 h at 55 °C. Dried samples were ground to pass through a 2-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) and then ground to pass a 1-mm screen using a Cyclotec 1093 sample mill (Foss Tecator, Hoganas, Sweden). Each 500-g dried bagasse sample was treated with 100 g of $Ca(OH)_2$ and 8 L of water at 121 °C for 1 h. These pretreated bagasse samples were split into two equal portions for structural sugar composition (fermentable sugars) analysis and cellulosic ethanol conversion analysis.

Analysis of bagasse for fermentable sugars

Analysis of fermentable sugars from the cell wall structural components of pretreated bagasse samples was conducted to characterize the relative composition among sweet sorghum cultivars. Duplicate samples were analyzed for only one replication of the field experiment. Pretreated bagasse samples of 10 g each were hydrolyzed with 72% sulfuric acid for 1 h at 30 °C in a water bath and then diluted to 4% sulfuric acid and autoclaved for 1 h at 121 °C. After cooling, samples were filtered, and the supernatant was neutralized and analyzed for reducing sugar composition by HPLC. The HPLC system was equipped with an HPX-87Pb column (Bio-Rad, Hercules, CA, USA) and a differential refractive index (DRI, Schimatzu, Japan) detector.

Ethanol production from bagasse

Analysis of ethanol production was conducted by procedures of NREL (2008), with modifications described below. The NREL approach has been considered the most economically viable approach for commercial conversion of lignified cellulosic biomass materials to ethanol currently available, even though only the glucose-containing portion (cellulose) of the cell wall polysaccharides is converted to ethanol. This cellulosic ethanol conversion analysis involves a 24-h hydrolysis and a 24-h fermentation process. The 24-h hydrolysis was performed on pretreated bagasse samples on a dry weight basis of 6 g of sample per 100 g of total reaction weight with an enzyme mixture of 30 FPU g^{-1} glucan of Spezyme CP (Genencor, Danisco US Inc., Rochester, NY) and 16 CBU of Novozyme 188 (Sigma-Aldrich, Inc., St. Louis, MO) in 50 mM sodium citrate buffer (pH 4.8) incubated at 50 °C for 24 h. Actual dry weight of each sample was 200 g. Fermentation was conducted with inoculums of 1.0×10^8 yeast (*Saccharomyces cerevisiae* D_5A) cells mL^{-1} at 30 °C in a rotary shaker at 200 rpm. Ethanol was analyzed using gas chromatography (GC, Hewlett Packard 5890 series II) with a wax column (Zebron ZB Wax Plus) and GC-flame ionization detector (GC-FID, 280 °C).

Fiber analysis

Dried, ground samples of both bagasse from the Southeast Research Station and stems, which had not been pressed to remove juice, from the Hill Farm Research Station were subjected to standard forage fiber analyses. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using standard methods (Robertson & Soest, 1981). Duplicate 0.5-g samples were analyzed for *in vitro* true digestibility (IVTD) using procedures of Goering & Soest (1970).

In vitro fermentation gas production from bagasse

Ground sweet sorghum bagasse samples (500 mg) were weighed into 250 mL incubation bottles. Buffered mineral solution (Goering & Soest, 1970) was prepared, and 16 mL of the solution was distributed to the incubation bottles in a water bath at 39 °C. Rumen fluid was collected after the morning feeding from a ruminally fistulated Holstein heifer fed alfalfa and grass hay twice (7:00 and 18:00 h) daily. Rumen fluid was obtained with a 300 mL plastic beaker and transferred into two pre-warmed air-tight thermos containers. Collected rumen fluid was immediately moved into a laboratory and filtered through four layers of cheesecloth and flushed with CO_2. Rumen fluid was combined with the remainder of the prepared buffered mineral solution, which was maintained in a water bath at 39 °C. All handling was under continuous blanket flushing with CO_2. About 24 mL of buffered rumen fluid was dispensed into 250-mL incubation bottles containing the sweet sorghum bagasse samples. After assembling each bottle to a module (Ankom Technology, Macedon, NY), capable of communicating with a computer using radio frequency transmission, bottles were gently shaken. The whole incubation was processed at 39 °C, and gas readings for each bottle were recorded at 30-min intervals. Rate and extent of gas production were determined for each bagasse sample by fitting the gas production data to the dual pool logistic equation (Schofield *et al.*, 1994):

$$V = V_{F1}(1 + \exp(2 + 4\mu_{m1}/V_{F1} \times (\lambda_1 - t)))^{-1} + V_{F2}(1 + \exp(2 + 4\mu_{m2}/V_{F2} \times (\lambda_2 - t)))^{-1}$$

where V is the amount of gas production at time t, and V_{F1} and V_{F2} are the final gas production volumes corresponding to complete substrate digestion for a rapidly fermenting pool and a slowly fermenting pool, respectively. μ_{m1} and μ_{m2} are the points of inflection of the gas curve for the two pools, respectively λ_1 and λ_2 are the lag times of the two pools.

Statistical analysis

Analysis of variance (ANOVA) was conducted using the Proc Mixed procedure of SAS version 9.2 [SAS Institute (2004) SAS/STAT User's Guide (Ver. 9.1.3 Service Pack 4). SAS Institute Inc., Cary, North Carolina]. Responses assessed were ethanol yield, sugar composition, and measures of fiber composition. The cultivar effect was considered as a fixed effect, and block and the block by cultivar interaction effect were considered random. Differences between means were tested using Satterthwaite approximation for the denominator degrees of freedom as an option. Pearson correlations were assessed between fiber characteristics and ethanol production using Proc Corr of SAS, and both linear regression and stepwise regression procedures of SAS were also used to further assess these relationships. Means were used in the statistical analysis when duplicate laboratory analyses were conducted. Gas production data fitting a nonlinear model were analyzed using Proc NLIN of SAS (SAS Institute 2004), which was developed by Dr. P. J. Weimer (personal communication). Four runs of gas measurements with variation less than ±5% were obtained for the analysis.

Results

Juice composition

Carbohydrate composition of juice differed among samples of the different cultivars, with both ratios of sucrose to reducing sugar and Brix values reflecting these differences (Table 1). Differences among cultivars in individual sugars were greater than the differences in total sugars, indicating that sugar composition may be a distinguishing characteristic for some cultivars. Dale ranked highest in Brix and total sugar concentration and had the highest ($P < 0.05$) sucrose concentration. Although Theis had the highest ($P < 0.05$) glucose concentration, it ranked lowest in sucrose, Brix and total sugars. Sucrose concentration was greater than the concentrations of the other sugars combined for all cultivars except Theis. Fructose was only a minor proportion of the sugars present. The low range in total sugars and Brix values among cultivars suggests that any substantial differences in biomass productivity

Table 1 Degree brix and fermentable carbohydrate composition (sucrose, glucose, and fructose) of the juice mechanically extracted from four sweet sorghum cultivars grown at the Southeast Research Station

Item	Dale	M81-E	Topper	Theis
Brix°	16.0a	15.0ab	15.6a	14.5b
		Solid,%		
Sucrose	9.50a (59.3)	7.69b (51.5)	7.88b (50.4)	5.30c (36.6)
Glucose	3.62b (22.7)	3.83b (25.6)	4.09b (26.2)	5.12a (35.5)
Fructose	2.01ab (12.6)	1.71b (11.4)	1.90b (12.1)	2.30a (15.9)
Total sugar	15.1a	13.2bc	13.9b	12.7c

Numbers followed by the same letter within a row are not significantly ($P > 0.05$) different according to pair-wise comparison.

Number in the parentheses is percent of this carbohydrate component in brix.

Table 2 Monosaccharide compositions and ethanol production from the bagasse of four sweet sorghum cultivars pretreated with $Ca(OH)_2$ and followed by enzymatic hydrolysis

Item	Dale	SE	M81-E	SE	Topper	SE	Theis	SE
				Composition† after lime pretreatment (%)				
Glucose	65.4	0.5	58.3	0.8	58.5	0.7	60.0	1.1
Xylose	25.7	0.7	24.8	0.3	24.6	0.2	25.6	0.1
Arabinose	3.3	0.1	2.9	0.1	3.0	0.1	3.1	0.1
				24h hydrolysis + 24 h fermentation				
Ethanol yield‡, g/g pretreated bagasse	0.23 a¶		0.16 b		0.13 b		0.10 c	

†Compositional analysis results were obtained from the direct evaluation of cell wall material and include monosaccharides not accessible for ethanol conversion primarily due to lignification.
‡Ethanol measurements were obtained after fermentation with *Saccharomyces cerevisiae* and conducted separately from cell wall compositional analysis.
¶Means of ethanol yield followed by the same letter within a row are not significantly ($P > 0.05$) different according to pair-wise comparison.

could readily offset an advantage in juice quality among these cultivars.

Fermentable Sugar and Ethanol from the Bagasse

Compositions of fermentable monomer sugars released from the pretreated sweet sorghum bagasse samples were generally similar among cultivars (Table 2). Lack of replication precludes determination of significant differences, although Dale was slightly higher in glucose concentration than were the other cultivars. The high proportion of glucose for all cultivars reflects the dominance of cellulose among the structural polysaccharides. Hemicellulose apparently consisted primarily of arabino-xylans as indicated by the proportions of arabinose and xylose.

Bagasse from Dale produced the most ($P < 0.05$) ethanol (Table 2), and that from Theis produced the least ($P < 0.05$), with Theis producing less than half as much ethanol (0.10 vs. 0.23 g) per gram of bagasse as Dale. Ethanol production from the bagasse of the other two cultivars was similar to each other and intermediate between Dale and Theis.

Fiber analysis

Distinct differences were obtained among samples of the different cultivars in all fiber analysis measures of the bagasse (Table 3). Dale was lowest ($P < 0.05$) in NDF, ADF, and ADL and highest ($P < 0.05$) in IVTD among the four cultivars. Theis ranked highest in all fiber measures and was lowest ($P < 0.05$) in IVTD. The superior fiber, IVTD characteristics, and structural carbohydrates of bagasse from Dale were confirmed by the

Table 3 Mean NDF (neutral detergent fiber), ADF (acid detergent fiber), cellulose, hemicellulose, ADL (acid detergent lignin), and IVTD (*in vitro* true digestibility) of bagasse obtained from four sweet sorghum cultivars grown at the Southeast Research Station

Item	Dale	M81-E	Topper	Theis
% DM				
NDF	60.0c	66.6b	67.9ab	71.0a
ADF	36.1c	40.2b	40.9ab	43.1a
Cellulose	30.5c	32.1bc	33.1ab	34.4a
Hemicellulose	23.9b	26.4a	27.0a	27.8a
ADL	3.3c	5.2b	5.0b	6.0a
IVTD	70.1a	61.4b	61.3b	57.5c

Means followed by the same letter within a row are not significantly ($P > 0.05$) different according to pair-wise comparison.

Table 4 Mean NDF (neutral detergent fiber), ADF (acid detergent fiber), cellulose, hemicellulose, ADL (acid detergent lignin), and IVTD (*in vitro* true digestibility) in whole stems of four sweet sorghum cultivars grown at the Hill Farm Research Station

Item	Dale	M81-E	Topper	Theis
% DM				
NDF	40.4b	48.9a	41.7b	46.0a
ADF	22.1c	27.5a	22.4c	25.3b
Cellulose	19.8b	23.6a	19.8b	22.3a
Hemicellulose	18.2c	21.4a	19.2bc	20.7ab
ADL	2.1b	3.7a	2.5b	3.1ab
IVTD	81.3a	73.0c	79.1ab	75.7bc

Means followed by the same letter within a row are not significantly ($P > 0.05$) different according to pair-wise comparison.

whole stem samples from the Hill Farm Research Station (Table 4). Dale again ranked lowest in NDF, ADF, and ADL and highest in IVTD, however, none of these values was statistically ($P > 0.05$) different from the corresponding value for Topper.

In vitro fermentation gas production and modeling

The fermentation gas accumulation curves displayed a nonlinear trend over the incubation period (Fig. 1). Gas production levels of cultivars diverged with duration of the incubation period. The logistic model consisting of a fast fermenting fraction and a slow fermenting fraction, as determined using accumulated fermentation gas, is defined in terms of digestion kinetics for each cultivar in Table 5. After the first 5 h or so of fermentation, gas production values for the four cultivars produced distinct patterns with Dale ranking highest in gas production (Fig. 1). Topper and M81-E were intermediate and produced almost identical amounts of gas from about 5 h until about 17 h. Although Theis ranked lowest in gas production throughout the incubation period, gas production from M81-E was similar to that of Theis after about 50 h. Gas production kinetics from the model of dual logistic function (Table 5) provides insights into the responses of the four cultivars in Fig. 1. Distinct contrasts were obtained in the gas accumulation patterns for all cultivars between the fast fermenting fraction and the slow fermenting fraction. Both the fast fermenting and slow fermenting carbohydrate pools of Dale were numerically larger than those of the other cultivars. The greater lag time of M81-E for the fast fermenting fraction is reflected during the first 5 h, which is the only time gas production of M81-E was below that of Theis as illustrated in Fig. 1. The reduced gas production rate of M81-E, when an asymptote was reached at about 17 h, appears to be related to the distinctly smaller size of the slow fermenting fraction (Table 5), which determined the gas production rate after the fast fermenting fraction had been depleted.

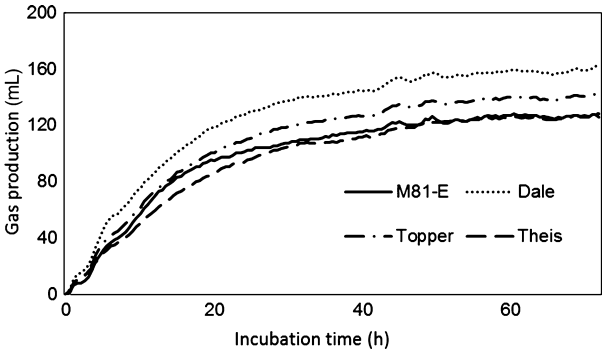

Fig. 1 Fermentation gas (CH_4 and CO_2) accumulation from bagasse of four sweet sorghum cultivars incubated with rumen fluid and buffer solution.

Table 5 Digestion kinetics for fermentation gas (primarily CO_2 and CH_4) production during *in vitro* rumen fluid incubation of bagasse (cellulosic material obtained after passing stems through a three-roller mill three times to remove the juice) from four cultivars of sweet sorghum

Item	Dale	M81-E	Topper	Theis
	In vitro ruminal assay			
Fast fermenting fraction, mL g^{-1} DM	102.8	91.0	89.0	86.2
Fast rate, mL h^{-1}	0.9	0.8	0.8	0.6
Fast lag, h	0.3	1.5	0.8	0.5
Slowly fermenting fraction, mL g^{-1} DM	56.2	37.4	51.6	42.2
Slow rate, mL h^{-1}	0.2	0.1	0.2	0.1
Slow lag, h	7.5	13.5	8.5	13.4
Fast pool,%	64.5	70.9	62.9	67.2

Relationships of fiber with ethanol production

Highly significant ($P < 0.001$) negative correlations were obtained between ethanol production and measures of fiber (NDF, ADF, cellulose, and ADL) as shown in Table 6. Highly significant ($P < 0.001$) positive correlations were obtained between ethanol production and both IVTD and NDFD. Significant ($P < 0.01$) correlations were also obtained for all combinations of fiber and digestibility measures (Table 6), limiting discernment of specific cause-effect relationships. A linear regression model with IVTD provided the best prediction of bagasse ethanol yield with an R^2 of 0.814 ($P < 0.01$). The best two-variable model from stepwise regression was provided by cellulose and NDFD with an R^2 of 0.832 ($P < 0.01$).

Discussion

Differences among cultivars in soluble hexose sugars paralleled Brix order ranking. Rankings previously reported for these four cultivars by Tew *et al.* (2008) differed somewhat from our rankings. Our lowest ranked cultivar Theis was ranked highest in total soluble sugars at the final sampling date by Tew *et al.* (2008). Dale, our highest ranking cultivar, also ranked highest at all earlier sampling dates of Tew *et al.* (2008). The differences perhaps reflect interactions among cultivars with environmental influences on soluble sugar composition and quantity through their effects on both photosynthesis and assimilation of photosynthates. Smith & Buxton (1993) obtained highly significant ($P < 0.01$) effects due

Table 6 Correlation coefficients of ethanol production potential and fiber/digestibility measures of bagasse obtained from four sweet sorghum cultivars

	NDF	ADF	Cellulose	ADL	IVTD	NDFD	Ethanol
NDF	1	0.99 **	0.96 **	0.84 **	−0.85 **	−0.88 ***	−0.84 ***
ADF		1	0.96 **	0.87 **	−0.93 **	−0.89 ***	−0.85 ***
Cellulose			1	0.73 **	−0.85 **	−0.83 ***	−0.80 ***
ADL				1	−0.93 **	−0.85 ***	−0.82 ***
IVTD					1	0.96 ***	0.90 ***
NDFD						1	0.82 ***
Ethanol							1

NDF (neutral detergent fiber); ADF (acid detergent fiber); ADL (acid detergent lignin); IVTD (*in vitro* true digestibility); NDFD (NDF digestibility).
, * significant at the 0.01 and 0.001 probability levels, respectively.

to both year and location along with interactions of cultivars with environmental effects on sucrose concentration of sweet sorghum cultivars. Although the differences we obtained in individual sugar proportions of expressed juice were statistically significant, environmental effects and their interactions with cultivar limit the application of these results beyond the growing conditions of this research. Theoretical ethanol yields have been calculated based on conversion of soluble sugars to ethanol and used to report results of sweet sorghum soluble sugar comparisons in terms of ethanol yield (Smith *et al*., 1987; Tew *et al*., 2008). Actual ethanol production from fermentation of soluble sugars in sweet sorghum has been found to depend upon fermentation conditions, particularly the microorganisms employed (Bryan, 1990). Therefore, growing conditions, which can affect biomass yield and individual sugar composition, along with the fermentation process may have greater effects on ethanol production from the soluble sugars than will the cultivar. In addition, plant maturity and interactions of maturity with cultivar can apparently also contribute to differences in rank order among cultivars as shown by the switch in order of ranking of Dale and Theis between some sampling dates reported by Tew *et al.* (2008).

Cell wall carbohydrate compositions of sweet sorghum were reported previously by Zhao *et al.* (2009), with both genotype and maturity affecting cellulose (average of 19.9% of stem dry matter) and hemicellulose (average of 16.5% of stem dry matter) contents. Our higher glucose levels (near 60%, Table 2), which primarily come from cellulose, are presented as a proportion of cell wall monosaccharides rather than whole stem, precluding direct comparisons with the results of Zhao *et al.* (2009). The high levels of the cellulose-derived monosaccharide glucose, rather than of hemicellulose-derived monosaccharides arabinose and xylose,are consistent with the results of the previous research (Zhao *et al*., 2009). Arabinose and xylose are often found in grass cell walls as arabino-xylans of various monosaccharide proportions. Our arabinose:xylose proportions are within the range previously reported for various structural carbohydrate fractions extracted from cell wall materials of C_4 grasses (Pitman *et al*., 1981; Pitman & Moore, 1985). Although glucose, but not xylose, is readily metabolized by naturally occurring *Saccharomyces* yeast used in ethanol production, possibilities for use of xylose are developing. A genetically engineered *Saccharomyces* yeast line was capable of metabolizing xylose (Sedlak & Ho, 2004). Conversion of xylose to products other than ethanol was proposed as a promising option (Aita & Salvi, 2009).

Substantial differences were obtained in fiber and digestibility analyses between bagasse from the Southeast Research Station and whole stem samples from the Hill Farm Research Station. Environmental conditions differed distinctly during growth of these two sample groups and apparently affected cell wall development and chemical composition. Lack of soil moisture limited plant growth throughout the growing period on the sandy soil at the Hill Farm Research Station where the whole stem samples were produced. Plant growth was not subjected to such growth limitations at the Southeast Research Station where the bagasse samples originated. Wilson (1983) previously reported a beneficial effect of growth-limiting moisture on digestibility of C_4 grasses as occurred between our two sample sets. Even

with the substantial differences in environmental conditions during growth, the high digestibility and low fiber levels of stems of Dale relative to the other cultivars occurred with both sample sets. Somewhat paradoxically, the cultivar with the highest cellulose concentration also had the lowest digestibility (IVTD) among the four cultivars for both the bagasse and whole stems, even though this was a different cultivar for each group of samples. The high-cellulose, low-IVTD cultivar in each sample set was also highest in lignin (ADL). Such a negative relationship between cellulose and IVTD was also detected by correlation analysis ($r = -0.847$, $P < 0.01$). This relationship also reflects the positive correlation between ADL and cellulose ($r = 0.727$, $P < 0.01$) and the negative relationship between ADL and IVTD ($r = -0.932$, $P < 0.01$). Both cellulose ($r = -0.802$, $P < 0.001$) and ADL ($r = -0.823$, $P < 0.001$) were also negatively correlated with ethanol yield. Vogel (2008) noted that during secondary cell wall development, 'lignin…essentially fills the pores between the polysaccharides.' Thus, as cellulose is added in the process of cell wall production, lignin is also added in close association and readily limits degradation of cellulose and other cell wall polysaccharides.

Proportions of monosaccharides upon hydrolysis of carbohydrates from bagasse of the different sweet sorghum cultivars (Table 2) did not indicate sufficient differences in structural carbohydrates for major differences in ethanol yield among cultivars. Thus, the ethanol recovery from bagasse of Dale of more than twice that from Theis (Table 2) was unexpected. As already noted from the correlations above, fiber analyses indicate that lignin, rather than the carbohydrate components themselves, is perhaps a key factor. The ADL fraction, which is a measure of lignin, was almost twice as high in Theis (60.3%) as in Dale (32.6%, Table 3). Lorenz *et al.* (2009) reported a similar negative relationship of lignin with ethanol production from maize stover, although they suggested that variation in structural carbohydrate quantity also influenced ethanol yield. The effect of lignin has been considered as primarily structural, however, phenolic compounds such as phenol aldehyde and phenol ketones produced during lignin degradation also act as fermentation inhibitors (Klinke *et al.*, 2004). Along with ADL, IVTD (Table 3) also grouped the four cultivars according to ethanol yield. Since the IVTD procedure involves both cell wall degradation and digestion of carbohydrates, our highest correlation with ethanol production ($r = 0.902$) for IVTD is in agreement with the report by Lorenz *et al.* (2009) that both lignin and structural carbohydrates affect ethanol yield.

Although the detergent fiber measurements were very highly correlated with ethanol production (Table 6), none of the individual measurements accounted for much more than 80% of the variation in ethanol production by linear regression analyses. The best fit was for IVTD with an R^2 of 0.814. Stepwise regression provided the best two-variable model of cellulose and NDFD with only slightly better prediction value ($R^2 = 0.832$). A model of NDF and NDFD explained 95% of the variation in maize stover ethanol yield in an evaluation of a diverse maize population (Lorenz *et al.*, 2009). The model of NDF and NDFD for our sweet sorghum cultivars explained 82% of the variation in ethanol yield. Thus, even though fiber characteristics were highly correlated with ethanol production, regression models involving these variables were not able to account for as much of the variation in ethanol production among this group of four sweet sorghum cultivars as previously reported (Lorenz *et al.*, 2009) for maize stover.

The *in vitro* gas production model provided a similar, although not exact ranking order to that of ethanol production for this set of sweet sorghum cultivars. The two extreme cultivars, with very different ethanol yields, were correctly ordered, whereas the two intermediate cultivars with similar ethanol yields were not ranked according to the ethanol yields. The grouping of similar materials and appropriate ordering of distinctly different materials provides possibilities for use in screening large numbers of genotypes or treatments efficiently. Correlation coefficients (r from -0.80 to -0.85) between ethanol production and chemically defined fibrous components were lower than the correlation between ethanol production and IVTD ($r = 0.90$) illustrating that measures including biological fiber degradation contribute at least some to estimation of ethanol production beyond levels of chemical measures alone. Although cellulose provides the substrate for ethanol production, increased cellulose concentration does not necessarily provide a linear increase in ethanol yield. Lignification and phenolic compounds released in lignin degradation may have greater effects than the somewhat limited extent of variation in cellulose concentration among sweet sorghum cultivars and management treatments.

The cultivars of sweet sorghum previously recognized as agronomically suitable for use as energy crops in Louisiana differed in potential for conversion to ethanol. Differences in soluble sugar concentration were not large, but were statistically significant, although not likely of a magnitude to overcome potential yield differences recently reported (Han *et al.*, 2012). Bagasse from the cultivar Dale was superior to the other cultivars in ethanol production, and this ethanol production advantage was apparently associated with cell wall structure, particularly lignification. Although environment substantially affected cell wall chemical composition, digestibility, and their rank order among cultivars, stem material of Dale was considerably higher in digestibility

and lower in fiber quantities than the other cultivars when grown in two contrasting environments. These responses indicate consistently higher ethanol yield potential for Dale than the other three cultivars. Considering the prevalence of environmental effects on chemical composition of sweet sorghum and the different approaches available to hydrolyze cell wall materials, germplasm evaluation from additional environments and processing of the bagasse with specifically identified approaches are needed to confirm the extent of the ethanol yield advantage of this cultivar.

For comparisons of sweet sorghum genotypes or management treatments potentially affecting ethanol yield from bagasse, substantial differences in ethanol yield will likely be necessary for separation of treatments using fiber and digestibility analyses. The requirement for such substantial differences for separation of treatments, however, does not preclude usefulness of the approach since changes in plant structural characteristics must be quite substantial to be particularly meaningful considering the magnitude of agronomic and environmental influences on ethanol production through their effects on biomass yield (Han *et al*., 2012). The biological assays of digestibility provided only slightly improved predictions of ethanol yield compared to the fiber analyses. Relationships detected between ethanol yield and both fiber and digestibility provide opportunity to efficiently screen sets of sweet sorghum samples and to order them when meaningful differences in ethanol yield potential exist. The forage fiber and digestibility analysis procedures were developed to process large batches of samples allowing efficient and cost-effective processing of samples obtained in large quantities. The widely used measures of forage nutritive value can more reliably indicate relative ethanol yield potential of sweet sorghum bagasse than can alternative approaches such as quantification of monomer sugar content or calculation of theoretical ethanol yields based on sugar content, because plant structural characteristics such as lignification affect monomer sugar availability for conversion to ethanol.

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