Cultivar and Maturity Effects on the Quality Attributes and Ethanol Potential of Sweet Sorghum

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Abstract

Sweet sorghum is a promising feedstock crop for the manufacture of biofuels and bioproducts. Its large starch content may be an opportune source of untapped fermentable sugars to add economical value to sweet sorghum juices and processing by-products. In this study, four commercial cultivars, *Dale*, *M81E*, *Theis*, and *Top 76-6* were grown in Louisiana (over 2 years) and studied at the milk (Mi), soft dough (SD), hard dough (HD), physiological (PM), and post-physiological (PPM) maturity stages to determine the value of sweet sorghum starch and the optimal cultivar, maturity, and environment ideal for processing and fermentation juice quality. Juice quality and physical crop attributes essential to processing and fermentation were strongly influenced by environmental and cultivar interactions ($P < 0.0001$); however, total sugars were specifically affected by cultivar and maturity interactions. Although a moderately-strong correlation between total sugars and juice Brix was found ($R = 0.767$, $P < 0.0001$, $n = 151$), the use of Brix as an in-field harvesting indicator to predict stalk maturity is not recommended since it does not consider cultivar influences. Total sugars and total starch in extracted juice were greatest at HD maturity in *Top 76-6* and *M81E*, respectively. Added-value (~6-17%) can be theoretically obtained from starch if fermented in sweet sorghum juices, and possibly more when including other starch-rich processing by-products and seed-heads that can be recycled back into fermentations. *Top 76-6* was the best cultivar for syrup manufacturing; *Dale* and *M81E* were most attractive for fermentations only if their high starch content are economically incorporated into the manufacturing and fermentation processes.

Keywords: cultivars; maturity; quality attributes; starch; ethanol yields; processing characteristics.

Abbreviations

Mi	Milk physiological maturity stage
SD	Soft dough physiological maturity stage
HD	Hard dough physiological maturity stage
PM	Physiological maturity stage
PPM	Post-physiological maturity stage

Brix	Per cent dissolved refractometric solids
TN/TOC	Total oxidizable and non-oxidizable organic nitrogen/carbon
YAN	Yeast assimilable nitrogen
EtOH	Ethanol
G	Genotype/Cultivar interaction
M	Maturity interaction
E	Environmental interaction
ICUMSA	International Commission for Uniform Methods of Sugar Analysis
NTU	Nephelometer turbidity units
HPAEC-IPAD	High performance anion exchange chromatography with integrated pulsed amperometric detection

1. Introduction

Biochemicals produced from renewable plant biomass like sweet sorghum (*Sorghum bicolor* L. Moench) are an attractive alternative to scarce fossil fuel stores. Sweet sorghum is a high biomass and sugar yielding crop. It is a **widely adopted crop** that is easily cultivated from seed, requires low input, has a wide geographic suitability that allows for substantial breeding potential, and has potential for plantings each cropping season in the southeast U.S. (Tew et al., 2008).

Although sweet sorghum has been traditionally used for forage or small-scale syrup production, several private-sector U.S. and international consortiums are now developing a large-scale industry to supply both the food-grade syrup market and the anticipated bioprocessing demand (Eggleston et al., 2015a). While most of these groups are still in the planning stages, a new large-scale processing plant in Sikeston, Missouri, i.e., Heckemeyer Mill, has started the large-scale production of food and industrial-grade syrup as well as potable alcohol, and in the immediate future, various biochemical and bioproducts (Eggleston et al., 2015a). Heckemeyer Mill is currently capable of crushing up to ~45 ton per day and producing at least 8,000 gal of juice (Eggleston et al., 2015a). There have also been some reports indicating the potential to incorporate sweet sorghum into existing sugarcane factories, specifically during the crop off-season, with sweet sorghum being cultivated on fallowed sugarcane beds before the season starts (Kim & Day, 2011; Linton et al., 2011; Ricaud, 1970; Tew et al., 2008).

One processing obstacle encountered at Heckemeyer Mill, was the high concentration of starch, particularly insoluble starch, in sweet sorghum juice, which was attributable to forage harvesting and/or type of cultivar. Insoluble starch is a natural juice impurity constructed of entangled, soluble glucopolymers, which makes this physical starch form very difficult to solubilize (Chen & Chou, 1993). A high amount of starch is known to complicate downstream syrup processing because the formation of swollen granules can increase syrup viscosity. Furthermore, insoluble starch can readily settle on the evaporator coils and burn, yielding an unsellable syrup product (Eggleston et al., 2015a). Removing the seed-heads, which contain larger granules of starch, prior to juice extraction, can markedly reduce insoluble starch

concentrations downstream and in the final syrup product. Extracted sweet sorghum juice, however, still contains large quantities of starch, but they are much smaller than those found in the primary and auxiliary seed-heads (Eggleston et al., 2015b). Fortunately, twice sedimentation and clarification can remove most of the small and large insoluble starch granules allowing for the production of acceptable food-grade or non-food-grade, commercial syrup (Eggleston et al., 2015a). This results in processing by-products, i.e., sediment, clarification mud, seed-heads, and bagasse, rich in soluble and insoluble starch, which have potential to supplement fermentation as a source of untapped fermentable sugars (Eggleston et al., 2015a).

Most sweet sorghum research has focused mainly on crop production characteristics and, to a limited extent, process optimization (Andrzejewski et al., 2013b; Eggleston et al., 2015a) with very little on quality attributes. Thus, more research is needed to underpin the cultivar, maturity, and environmental effects that impact juice **quality, e.g.,** starch quantity and quality, and its potential to augment fermentation yields, harvesting, processing, and end-products for this commercial industry (Andrzejewski et al., 2013a, 2013b; Eggleston et al., 2015a, 2015b). The objectives of this study, therefore, were (i) to characterize the agronomic, biomass, and juice quality traits of four commercial sweet sorghum cultivars (ii) at five crop maturities over 2 years, and (iii) to evaluate the fermentable potential of sweet sorghum juice sugars with and without untapped sugars from hydrolyzed starch.

2. Experimental

All chemicals and standards were analytical grade and purchased from Sigma Aldrich Company (St. Louis, MO).

2.1 Weather Data

Temperature and rainfall were taken from the USDA Sugarcane Research Unit, Houma, LA weather station (KLAHOUMA8) for the 2013 and 2014 study. Average monthly temperatures and rainfall are shown in Fig. 1.

2.2 Sampling Methods and Juice Extraction for Maturity and Cultivar Study in Schriever, LA

Field studies were conducted in 2013 and 2014 at the USDA-ARS Sugar Research Laboratory's Ardoyne Research Farm (Schriever, LA). The sweet sorghum evaluated included four "mid to late-maturing" inbred sweet sorghum cultivars (*Dale*, *M81E*, *Theis*, and *Top 76-6* or *Topper*) (Broadhead et al., 1970, 1974, 1981; Day et al., 1995). The 2013 study was planted in a randomized complete block design on May 9, 2013 on Schriever clay in twin rows on top of a conventional 1.8 m wide, single raised beds measuring 107 m long as established for fallow sugarcane-sweet sorghum field conversion, with four replicates. The 2014 study was planted on April 24, 2014 on Cancienne silt loam soil using the same plot design and at the same location. Each main plot was planted at a density of approximately 140,000 plants/ha. Following planting, metolachlor plus atrazine at 1.42 plus 1.2 kg/ha was applied to all plots. Upon reaching the four leaf growth stage, pendimethalin (1.6 kg/ha) was added to provide additional pre-emergence control of grass and broadleaf weeds. Prior to planting, plots were fertilized using liquid fertilizer injected on both sides of the bed at a rate of 90 kg/ha N, 22 kg/ha K₂O, and 45 kg/ha P₂O₅ as typical for sweet sorghum. Physical agronomic attributes and juice quality were measured bi-weekly at milk (Mi), soft dough (SD), hard dough (HD), physiological maturity (PM), and post-physiological maturities (PPM). Harvestable stalk counts were measured when stalks reached Mi

stage and normalized to one hectare. On each harvest date, sampling consisted of 30 stalks (three bundles of 10 stalks) selected at random, and hand-harvested using a cane knife from each plot. Seed-heads (top and axillary) were removed to simulate what will likely occur during industrial harvesting with a sugarcane combine harvester equipped with a top cutter. Each stalk bundle and seed heads were weighed separately to determine fresh weight yields and grain yields (Mg/ha), respectively, and to determine crop yields. Stalk heights and diameters (3rd, 7th, and 12th internodes) of ten representative whole stalks were measured using a 12-foot measuring table and digital calipers, respectively. After measuring the physical dimensions of the stalk on each harvest date and physiological crop maturity (Table 1), a bundle with leaves intact was crushed twice through a SquierTM (Buffalo, NY) three-roller mill at Ardoyne Farm (Schriever, LA). First and second expressed juices were collected for each bundle and combined. The juice was filtered through a 0.6 mm mesh filter. Juice was weighed, placed on ice, and transported to the USDA-ARS-SRRC laboratory in New Orleans, LA for further processing. Aliquots of juice were also immediately stored in a -80 °C freezer until further analysis.

2.3 Brix, pH, Color, Turbidity, Assimilable Nitrogen, Phosphate, Conductivity Ash, Total Organic Carbon, Total Nitrogen

Brix was measured in triplicate with a temperature-controlled refractometer (model TCR 15-30; Index Instruments, FL) to an accuracy of ± 0.01 Brix. Juice pH was measured in triplicate with a Metrohm Brinkman 716 DMS Titrino (Riverview, FL) and a Mettler Toledo (Columbus, OH) xerolyte electrode. Color was determined in triplicate according to the official ICUMSA method GS2/3-9 (2011), in which 25 mL of filtered juice (0.45 μ m filter) was mixed with 35 mL of triethanolamine/ hydrochloric acid buffer (pH 7) and absorbance read at 420 nm. Color was also measured at pH 4 and pH 9 after adjusting the solution with 0.1 M HCl and 0.1 M NaOH, respectively. Nephelometer turbidity (NTU) was measured in triplicate using a Hach 2100N turbidimeter (Loveland, CO). Yeast assimilable nitrogen (YAN) was analyzed in triplicate (Abernathy et al., 2009). Phosphate (P₂O₅) was determined in triplicate using a colorimetric assay based on the measurement of phosphate-molybdenum blue complex at 700 nm (ICUMSA, 1994). Conductivity ash was measured in triplicate on filtered juices (0.45 μ m filter) without dilution, using a YSI 3200 conductivity meter (Yellow Springs, OH). The total organic carbon (TOC)/nitrogen (TN) content of the filtered juices (0.45 μ m filter) were performed in triplicate with a Torch Combustion TOC/TN analyzer (Teledyne Tekmar, Mason, OH) after adjusting the total soluble solids content to 10g L⁻¹ and diluted 1:100 with deionized water. All analyses were conducted at room temperature unless otherwise stated in the methods.

2.4 Fermentable Sugars

Glucose, fructose, and sucrose in juice samples were determined in four replicates using high performance anion exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD) following the method reported by Andrzejewski et al. (2013b). Prior to analysis, juice was filtered through a 0.45 μ m PVDF filter and diluted (1:5000, v/v) with deionized water before analysis with a DionexTM ICS5000+ (Dionex-Thermo Scientific, Sunnyvale, CA) with a CarboPac PA-1TM analytical (250 x 4 mm) and guard columns (50 x 4 mm) and 100 mM NaOH eluent. Mannitol (20 ppm) was used as an internal standard. Total fermentable sugars or invert sugars (SO, glucose and fructose, g L⁻¹) were determined stoichiometrically using the simplified formula in Eq. 1. Juice purity (%) was also determined by dividing total sugars (sucrose + glucose + fructose) by Brix.

$$\text{Total Fermentable Sugars (SO, g L}^{-1}\text{)} = ([\text{Sucrose}] \times 1.053) + [\text{Glucose}] + [\text{Fructose}] \quad \text{Eq. (1)}$$

2.5 Total, Soluble, and Insoluble Starch Content and Granule Size in Juices

Total, soluble, and insoluble starch was determined according to the microwave-assisted sonication/iodometric method developed by Cole et al. (2016). Starch was assayed in triplicate, and concentrations are quoted as average g starch per kg stalk. Starch granule size was measured using the method reported by Eggleston et al. (2015b) with a digital microscope at 100x with a built-in 30 MP camera (Omax, Kent, WA) after mixing 50 μL of juice with 50 μL filtered iodine (0.22 μm). Diameters of at least 40 granules were measured to an accuracy of $\pm 1 \mu\text{m}$ and sorted into histogram populations. Mean, mode, and range of starch sizes were calculated. Total starch concentration in raw sweet sorghum juice was converted to fermentable glucose using Eqs. 2 and 3.

$$\begin{aligned} \text{Total Starch (mg L}^{-1}\text{)} = \\ (\text{Total Starch (g kg}^{-1}\text{)} \times \text{stalk weight (kg stalk}^{-1}\text{)} \times \\ 1000 \text{ (mg g}^{-1}\text{)}) / \text{extracted juice volume (L stalk}^{-1}\text{)} \end{aligned} \quad \text{Eq. (2)}$$

$$\text{Untapped Fermentable Glucose from Total Starch (mg L}^{-1}\text{)} = \text{Starch (mg L}^{-1}\text{)} \times 1.10 \quad \text{Eq. (3)}$$

2.6 Values for Potential Ethanol and Profits

Theoretical values of ethanol (mL EtOH L^{-1} juice) in raw sweet sorghum juices were determined using fermentable sugars only (SS, hydrolyzed sucrose + glucose + fructose) or with the untapped glucose added from starch (SO) using on Eqs. 2-4.

$$\text{Theor. EtOH (mL L}^{-1}\text{)} = \text{SS or SO (g L}^{-1}\text{)} \times 0.6455 \text{ (mL EtOH g Fermentable Sugars}^{-1}\text{)} \quad \text{Eq. (4)}$$

2.7 Statistics

Each cultivar was analyzed and compared within years using two-way (cultivar and maturity) ANOVA in JMP SAS 11.2; whereas, two-year juice quality traits and yields were evaluated using multivariate (environment, cultivar, and maturity) analysis. Means were separated using Tukey's Honest Significant Difference Test to a maximum probability (P) of 0.05. Pearson product-moment correlation (R) coefficients were used to assess the relationships among all cultivar quality results.

3. Results and Discussion

3.1 Environmental Effects on Sweet Sorghum Growth

The number of days between planting and Mi maturity differed by cultivar and ranged between 77–94 days, generally following the order: *Dale* > *M81E* > *Theis* > *Top 76-6* (Table 2). Maturity rates and wet crop yields varied between the two years, which was attributable to environmental (climate) differences (Fig. A1), although soil type may have had an effect. In 2014, average temperatures were cooler and rainfall greater than in 2013 (Table 2). Although daily temperatures ranged from 23 to 32 °C (2013) and 17 to 29 °C (2014) from planting to Mi

stage, average temperatures were 26.5 and 25.8 °C for 2013 and 2014 harvest seasons, respectively (Table 3). Average rainfall was greater ($P<0.05$) from planting to Mi stage in 2014 than in 2013 (Table 3). As a result, in 2014 the number of days needed to reach Mi stage among the cultivars was prolonged ($P<0.05$) by 11-27%, with *Top 76-6* and *Dale* the least affected and *M81E* the most. This result is not uncommon as sweet sorghum planted in lower temperatures grows much more slowly than in warmer climates (Guiying et al., 2000; Theivasigamani et al., 2013). Results in Table A1 show that a moderately strong correlation occurred between the number of days needed to reach Mi maturity and rainfall ($R=0.666$). The rainfall pattern observed in each year of this study indicated that crop maturation could have been mutually and adversely affected by the low total and large clustered rainfall observed in 2014 (Table 3). Similar results have been observed at Delta Biorenewables (Memphis, TN) in which low and/or clustered rainfall (>0.2 in) substantially impacted crop yield (Randy Powell, Delta Biorenewables personal communication). This may also explain the year-to-year difference for the average number of days in the field for *Dale*, *M81E*, *Theis*, and *Top 76-6* (Table 2).

3.2 Cultivar and Maturity Effects on Sweet Sorghum

3.2.1 Physical Crop Attributes

Physical crop attributes among the sweet sorghum cultivars studied in 2013 and 2014 were significantly different at the 5% probability level (Tables 2 and 3), and agree with previous studies (Broadhead, 1972; Han et al., 2012a, 2012b). Fresh crop yields in 2014 (43-53%) were lower ($P<0.05$) than in 2013 (52-74%) and had a moderately strong correlation ($R=0.607$) with temperature (Table A1). This is likely due to the consistent rainfall, drier soil, and warmer temperature in 2013, which have been reported to positively affect germination, seedling emergence, booting, tillering, and crop yields in southeastern USA (Duncan & Gardner, 1984; Han et al., 2012a, 2012b, 2012c; Prasad et al., 2007). Environment also affected wet biomass yields, which were much greater in 2013 than 2014 ($P<0.05$) (Tables 2 and 3). Irrespective of cultivar, average wet biomass (Tables 2 and 3) were comparable to previously reported results in LA (Ricaud, 1970; Ricaud & Arcenaux, 1990; Tew et al., 2008; Zhao et al., 2009) and mostly affected by cultivar x environmental (G x E) interactions ($P<0.0001$) (Table 4). Stalk biomass, however, decreased ($P<0.05$) with increasing maturity and was attributed to senescing of green leaves and dehydration (Han et al., 2012a). Individual stalk wet weight were influenced more by cultivar (<0.0001) than maturity and environment (Table 4); *Theis* had the heaviest ($P<0.05$) stalk wet weights for both years (Table 2). It should be noted that auxiliary stalks (side tillers) may have contributed to the cultivar effect observed on stalk weights and crop yields although special care was taken to manually remove them before taking all biophysical measurements. More research is needed to evaluate the effect cultivar, maturity, and environmental interactions have on the presence of sweet sorghum auxiliary stalks and augmented starch yields. However, it is known that more auxiliary stalks will increase the total starch concentration in extracted juices and decrease processing efficiency (Eggleston et al, 2015b), and therefore, be of interest as an untapped fermentable starch resource as well. Table A1 also showed that stalk wet weight was preferentially affected more by ($R=0.881$) than rainfall ($R=0.427$), which agrees with climate results reported by Wortman and Regassa (2011) and Erickson et al. (2011). Stalk heights, on the other hand, decreased ($P<0.05$) in the following order: *Theis* $>$ *M81E* $>$ *Dale* $\approx$ *Top 76-6* (Table 2) and were taller ($P<0.05$) in 2013 than 2014. Stalk height is strongly affected by the environment (<0.0001) (Table 4), which may explain the 9-11% average height reduction between years.

Internodal stalk diameters were affected mostly by G x E interactions (<0.0001), however, as the diameter narrowed in the direction towards the stalk top, cultivar (<0.0001) became more prevalent (Table 4). Similar findings have been reported by Audilakshmi et al. (2010) and Eggleston et al. (2017), in which average stalk diameters correlated strongly with stalk wet weight ($R=0.896$) but weakly with height ($R<0.300$) (Table A1).

3.2.2 Juice Extraction

The average amount of juice extracted from sweet sorghum stalks ranged from 29 to 35% and 30 to 38% in 2013 and 2014, respectively (Table 2), and agrees with results reported by Cifuentes et al. (2014). The greatest overall juice extraction yield by cultivar and maturity was found to be *M81E* and at SD, respectively (Table 2); the percent moisture retained in the bagasse varied slightly, but significantly ($P<0.05$) among each cultivar (Table 2). This finding was attributed to stalk hardness, which has been found to increase with increasing maturity as it is known that, in practice, early season crop is almost always easier to mill than late season crop (Randy Powell, Delta Biorenewables, personal communication). This may indicate a slight, but possible, cultivar effect on roller-mill extraction yields which must be confirmed with more studies. Overall, juice extraction was relatively low and was mostly influenced by environment and maturity (E x M) interactions (Table 4). Extraction yields could be improved with imbibition water or other extraction methods such as diffusion (Eggleston et al., 2013; Jia et al., 2013; Powell, 2016).

3.2.3 Brix and Fermentable Sugars

Juice Brix, a measure of soluble solids content in the extracted juices, was variable and irreproducible between years, maturity, and cultivar ($P<0.05$). On average, Brix values were ~21% higher in 2013 than in 2014 (Tables 2 and 3) and reflected the usual juice Brix observed in southeastern LA (Han et al., 2012a; Kim and Day, 2011). Higher Brix values, however, have been reported in other locations across the U.S. with these same cultivars (Matt Heckemeyer, Heckemeyer Mill, personal communication; Randall Powell, Delta Biorenewables, personal communication; Teetor et al., 2011; Wang et al., 2014). The low juice Brix and respective differences between 2013 and 2014 values observed in this study, as compared to other locations, was most likely due to differences in environment and agronomy practices. Brix of the extracted juice from the cultivars also decreased ($P<0.05$) *Dale* $\geq$ *Theis* $\geq$ *Top 76-6* $>$ *M81E* (Table 2) and varied slightly ($P<0.05$) among crop maturities, with the highest Brix at PM (Table 3). Brix values, as expected, correlated strongly with days to maturity ($R=0.799$), wet biomass weight ($R=0.740$), and crop yield ($R=0.749$), but showed moderate to weak correlations with other crop physical attributes (Table A1). Brix was greatly influenced by G x E at the 0.01% probability level (Table 4), which explains the variable juice Brix observed between harvest years and geographic locations.

Juice purities (% total sugars divided by Brix) ranged between 65-81% and 51-78% for 2013 and 2014, respectively (Table 2), which are typical for sweet sorghum. More specifically, total sugars were also higher ($P<0.05$) in 2013 than in 2014, despite the high variability among results and inconsistent Brix values between years (Fig. 1). Total sugars, however, were greatest at HD and most abundant in *Top 76-6* for both years (Fig. 1). *Top 76-6* also had the highest amount of invert sugars (glucose and fructose) in 2013, but in 2014 had equally abundant invert sugars as *Theis* and *M81E* ($P<0.05$). As expected, sucrose was the dominant sugar in all of the mature 2013 and 2014 sweet sorghum juices, but was lower in 2014 (Fig. 1). This agrees with

Teetor et al. (2011) and Han et al. (2012a) who also reported sucrose dominance in mature sweet sorghum. However, in contrast to findings by Han et al. (2012a), glucose never exceeded the sucrose concentration in immature sweet sorghum in this study. Among the three soluble sugars for all cultivars studied, sucrose correlated the strongest with juice Brix ($R=0.850$), most likely because it was the most abundant (Table A1). Correlations listed in Table A1 also show that temperature strongly affected total sugar content in sweet sorghum juices ($R=0.897$), which may be due to preferential production of sucrose ($R=0.813$). The markedly lower total sugars extracted in sweet sorghum juices in 2014 may have been due to the colder weather and different rainfall patterns. Interestingly, glucose, fructose, and sucrose concentrations were individually influenced by G x E interactions (<0.0001) whereas, total sugars were most affected by G x M interactions (<0.0001) (Table 4). Results in Table A1 show that fermentable sugars only correlated weakly and moderately with Brix ($R=0.569$) and Brix sugars ($R=0.720$), respectively (Table A1). Therefore, although a common practice, using juice Brix as an in-field harvesting indicator to predict stalk maturity may present bias to sweet sorghum cultivars that were bred specifically for crystalline sugar (high sucrose) rather than syrup production (high glucose) (Lime, 1979), because it does not account for the environmental or maturity effects that are unique to each fermentable sugar or the total sugar that varies crop-to-crop and measures all soluble components in juice not accurately measuring sugars. Thus, it is important to develop an in-field, breeding, or industrial method that accurately measures all fermentable sugars rather than soluble impurities in sweet sorghum juice that can effectively evaluate syrup and fermentation potential.

3.2.4 Starch

Extracted juices in 2013 contained nearly two-fold more starch than in 2014 (Fig. 2), with starch yields among the four sweet sorghum cultivars ranging from 50-315 g/kg plant or 0.8-21.3 g L⁻¹ juice (Fig. 2). This was greater than the 76-154 g/kg starch range reported by Alves et al. (2014). Difference among starch quantities in juice may be due to the different starch methods used to measure total, soluble, and insoluble starches in sweet sorghum juice (Cole et al., 2016) in addition to cultivar, crop maturity, harvesting environment (USA vs Brazil), or part of the plant (Alves et al., 2014). In this study, it is also likely that the total starch determined in the juices included the insoluble starch contributed from axillary seed-heads (side tillers) and/or green leaves that were still intact during roller-milling of the stalks. Eggleston et al. (2017) showed that axillary seed-heads significantly increased sweet sorghum starch content and were detrimental to processing. It was also shown that that protein rich green leaves among other trash actually facilitated juice settling and clarification despite augmenting juice color and turbidity (Eggleston et al., 2015a, 2015c).

Starch was lowest at Mi stage for both years, before doubling at SD (Fig. 2). A moderate inverse correlation between the number of days needed for the crop to reach Mi maturity and total starch per plant mass ($R=-0.770$) was also observed (Table A1). The concentration of starch in extracted juices was more affected by G x E interactions (<0.0001) than maturity (Table 4), which may be attributable to cultivar-specific photosynthetic pathways that govern the production of starch but have not been proven (Bihmidine et al., 2013; Shakoor et al., 2014; Slewinski, 2012). Total starch in sweet sorghum juices decreased significantly by cultivar: *Dale* > *M81E* > *Theis* > *Top 76-6* (Fig. 2), and were slightly affected by rainfall ($R=-0.577$) but not temperature ($R=0.315$) (Table A1). Fig. 2 also shows that *M81E* produced 2-3 times more total starch than *Dale* in 2013, but was susceptible to the unfavorable rainfall and colder climate in

2014 and suffered a dramatic decrease in total starch content, which misrepresents the attainable untapped fermentable potential of *M81E* in Fig. 3. Total starch concentration (Table A1) correlated strongly and inversely with glucose ($R=-0.893$) and may be related to glucose utilization during starch biosynthesis ($R=-0.684$).

Majority of the total starch in the extracted juices **was insoluble** (Fig. A2) varying ($P<0.05$) between 83-96% among the cultivars studied; results agree with previous reports (Eggleston et al., 2015a, 2017). Insoluble starch sizes ranged from 0.50 to 9.6 μm among cultivars and maturity stages with average diameters from 2.5 to 3.5 μm (Fig. A3). In this study, $\geq 80\%$ of the insoluble starch in the extracted juice measured was $<5 \mu\text{m}$ (Fig. A3). Starch size increased ($P<0.05$) by cultivar *Theis* $\leq$ *Top 76-6* $\leq$ *Dale* $<$ *M81E* (Fig. A3-A). The largest starch granules were observed at the SD, HD, and PM stages (Fig. A3-B) and attributable to axillary seedheads. It is known that both starch quantity and granule size (~ 1.0 - $20.0 \mu\text{m}$, but mostly $<2.6 \mu\text{m}$) can negatively affect processing at later maturities, particularly juice turbidity and viscosity (Andrzejewski et al., 2013b; Eggleston et al., 2015a, 2017). A few reports have also discussed the negative impact green leaves and seedheads have on sweet sorghum juice quality, processing, and sugar yields (Eggleston, et al., 2013, 2015a, 2017; Viator et al., 2014), but none have reported the influence of these materials on starch quantities in sweet sorghum juices. Future studies are now needed to evaluate how green leaves and axillary seedheads affect starch content in juices by maturity, cultivar, and extraction method. Results obtained will be very useful in determining whether milling, diffusion, or mashes are most suitable for extracting fermentable sugars and untapped sugars from starch to maximize fermentation yields.

3.2.5 Other Sweet Sorghum Juice Quality Traits

Other quality attributes such as juice pH, conductivity ash, yeast assimilable nitrogen (YAN), total organic nitrogen/carbon (TN/TOC), soluble phosphate, and turbidity were studied because of their critical roles in processing and/or fermentation yields (Table A3). In this study, there was no significant difference in juice pH among the four cultivars or at the different maturities (Tables 2 and 3), which could be attributable to seasonal differences or juice quality, as pH was strongly affected by E x M interactions (Table 4). Results listed in Table A1 show that moderate correlations existed between pH and wet biomass ($R=0.634$), sucrose concentration ($R=0.642$), TOC ($R=0.493$), and temperature ($R=0.615$). Weak correlations between conductivity ash and the other juice qualities (Table A1) suggest that charged juice solutes may be an insignificant indicator of the overall sweet sorghum juice quality. More studies are needed to understand the role E x G x M interactions have on specific inorganic and organic ions in extracted sweet sorghum juices and its effect on saccharification and liquefaction of starch and processing by-products and/or their subsequent fermentation. This is especially important since it is known that sweet sorghum is rich in aconitic acid, which is capable of complexing to divalent ions and affecting the availability of certain processing chemical aids, enzyme cofactors, or fermentation nutrients/inhibitors (Amorim et al., 2011). Since inhibitors may be concentrated in processing by-products despite the large quantities of starch, fermentation may still be challenged reducing the overall untapped fermentation potential and economical value of starch.

Yeast assimilable nitrogen (YAN) and total nitrogen (TN) in the extracted juices were governed by E x M interactions (<0.0001) (Table 4), in which the greatest TN and YAN amounts were extracted at SD maturity (Table A3). Raw juices contained medium to high TN and low YAN (Table A3) and indicates that these cultivars, if grown in southeastern LA under similar environmental conditions, may require YAN supplementation during raw juice fermentation. The

total organic carbon (TOC) in the extracted juices, which measures all organic carbon matter that may be utilized during fermentation, fermentation inhibitors (e.g., acids, alcohols, indigestible matter, etc.), as well as non-fermentable sugars, was influenced more by the environment rather than cultivar or maturity stage ($P < 0.05$). Likewise, phosphate ranged from 43-84 ppm and according to Andrzejewski et al., (2013a), it may not be enough for clarification or fermentation without additional supplementation from phosphoric acid. Phosphate concentration was governed mainly by maturity (< 0.0001), although a cultivar effect (< 0.0001) was present (Table 4) which also agreed with Andrzejewski et al. (2013b). As expected, juice turbidity was affected by plant impurities, i.e., soil, stalk, leaves, pith or microbes, that can be extracted with sweet sorghum juice by harvesting and roller-milling (Andrzejewski et al., 2013a, 2013b; Billa et al., 1997; Eggleston et al., 2014) and did not vary significantly ($P > 0.05$) between harvesting years (Table A3). In fact, it was found that raw juice turbidity was affected mostly by cultivar interactions (< 0.0001), without much effect from crop maturity or environment (Table 4). Nonetheless, turbid plant impurities in processing by-products can still have an adverse affect on fermentation yields and the utilization of untapped starch particularly if they contain microbes or enzymes that inhibit saccharification and/or fermentation processes.

3.3 Theoretical Ethanol Yields from Fermentable Soluble Sugars and Untapped Fermentable Glucose from Starch

Theoretical ethanol (EtOH) yields predicted from (i) invert sugars + hydrolyzed sucrose (sugars only, SO) and (ii) SO + hydrolyzed starch (sugars with starch sugars, SS) in extracted sweet sorghum juice varied significantly ($P < 0.05$) by cultivar and maturity (Fig. 3). When SO was considered, average EtOH values decreased by cultivar in this order: *Top 76-6* > *Dale* > *Theis* > *M81E* (Fig. 3). *Top 76-6* and *Theis* demonstrated steady increases in theoretical EtOH from SD to PPM (Fig. 3). In dramatic contrast, *Dale* and *M81E* exhibited inconsistent EtOH yields with increasing maturity and HD and PPM were better maturities for higher juice fermentation yields (Fig. 3). However, the high volume of EtOH estimated at PPM is likely due to a concentration effect on sugars caused by the older, dehydrated stalk, as these values were based on the total extractable juice volume. For all cultivars, theoretical EtOH increased from ~6 to 17% when SS was considered, in which the highest theoretical volume of EtOH was attributed to *Dale*, but the highest % increase in EtOH was attributed to *M81E* (Fig. 3). This result was expected since both *Dale* and *M81E* contained the most amount of starch per 100 mL juice.

Starch in raw sweet sorghum juices and processing by-products can theoretically augment the commercial value of sweet sorghum juice and bioproduct yields, but will vary by cultivar. Environment, cultivar, and maturity all strongly affected theoretical fermentation and bioconversion, but maturity had the least impact. Higher starch containing cultivars, *Dale* and *M81E* exhibited the greatest increases in theoretical EtOH yields from starch, and thus, showed potential to augment bioproduct yields, e.g., ethanol. More research is warranted to consider whether saccharification, liquefaction, and fermentation of untapped fermentable glucose from starch are economically feasible to current sweet sorghum bioprocessing efforts considering the new year-to-year E x G x M interactions and is the focus of an upcoming study. Research is also needed to determine the effects of fermentation inhibitors and/or nonfermentable sugars on sweet sorghum bioprocessing. In dramatic contrast, *Top 76-6* was the better cultivar for syrup manufacture, while it did demonstrate some small potential to augment EtOH yields.

4. Conclusions

Four commercial cultivars of sweet sorghum, grown on land following sugarcane cultivation practices in southeast Louisiana, were compared for biomass, agronomic characteristics, juice quality attributes, and potential theoretical ethanol yields with consideration of the untapped fermentable sugars from insoluble starch. Five crop maturities, i.e., milk, soft dough, hard dough, physiological maturity, and post-physiological maturity, were studied because they overlap the typical and ideal harvest period for sweet sorghum. Numerous crop growth indicators were found to be moderately or strongly correlated to juice quality attributes, specifically days to reach milk maturity, Brix, and total juice sugars, and were largely affected by environmental and cultivar interactions. The amount of starch in the extracted juices was mostly in the insoluble form (>95%), varied ($P<0.05$) by cultivar, and was inversely related to the days required to reach milk maturity. This indicated that a cultivar and maturity effect governs the untapped fermentable value of starch. Other quality attributes such as bagasse moisture, stalk height, assimilable nitrogen, and pH were implicated by environmental and maturity interactions which can adversely affect processing and/or fermentation. Total sugars and juice phosphates were influenced by cultivar and maturity interactions, which may explain why previously reported sweet sorghum juice clarification and evaporation were dramatically affected by cultivar and maturity. Theoretical ethanol yields, considering glucose and fructose only, indicated that *Top 76-6* is a better cultivar for syrup production since it (i) contains the least amount of starch, (ii) can likely be harvested at all crop maturities, and (iii) is less likely to have processing problems. Cultivars *Dale* and *M81E*, however, are more attractive for fermentation since their high starch content can be used to augment the economical value of sweet sorghum from 3-31%. Optimal harvesting maturities, based on two year averages and all cultivars, occurred between SD and HD maturities. More studies are now warranted to understand the value of starch in fermentation processes, from processing products and by-products, and whether or not other juice extraction methods enable better quality juices, change starch quantities in juices, or are affected by the same parameters as roller-milling. Considering that there is a strong cultivar effect on processing and starch augmentation of ethanol yields, in addition to the quantity of other critical fermentation parameters, more studies are also needed to understand the economical value of these crops across the entire manufacturing and biorefinery streams.

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Table 1. Dates that sweet sorghum was harvested in Schriever, Louisiana, by cultivar and maturity, in 2013 and 2014.

	Harvest Dates per Crop Maturity				
<i>Cultivar</i>	Mi	SD	HD	PM	PPM
<i>Dale</i>	8/1/2013 7/30/2014	8/15/2013 8/13/2014	8/29/2013 8/27/2014	9/12/2013 9/10/2014	9/26/2013 9/24/2014
<i>M81E</i>	8/15/2013 8/27/2014	8/29/2013 9/10/2014	9/12/2013 9/24/2014	9/26/2013 10/7/2014	10/17/2013 10/22/2014
<i>Theis</i>	8/15/2013 8/20/2014	8/29/2013 9/2/2014	9/12/2013 9/16/2014	9/26/2013 9/30/2014	10/17/2013 10/14/2014
<i>Top 76-6</i>	8/29/2013 8/27/2014	9/12/2013 9/10/2014	9/26/2013 9/24/2014	10/17/2013 10/7/2014	10/31/2013 10/22/2014

Table. 2. Agronomic, crop performance, physical, and juice quality attributes of sweet sorghum by cultivar during 2013 and 2014 seasons.

Parameter	2013*				2014				Two-Year Average			
	Dale	M81E	Theis	Top 76-6	Dale	M81E	Theis	Top 76-6	Dale	M81E	Theis	Top 76-6
Days to Milk	83 ^C	97 ^B	97 ^B	111 ^A	96 ^C	123 ^A	116 ^B	123 ^A	90 ^C	110 ^B	107 ^B	117 ^A
Days in Field	139 ^C	161 ^B	161 ^B	174 ^A	150 ^C	178 ^A	170 ^B	178 ^A	145 ^B	170 ^A	166 ^{AB}	176 ^A
Crop Yield (Stalks/ha)	89,100 ^B	103,100 ^A	72,500 ^C	90,000 ^B	73,500 ^A	70,700 ^B	64,000 ^C	60,000 ^D	81,300 ^A	86,900 ^A	68,250 ^A	75,000 ^A
Crop Yield (%)**	64 ^B	74 ^A	52 ^C	64 ^B	53 ^A	51 ^B	46 ^C	43 ^{CD}	58 ^{AB}	62 ^A	49 ^C	54 ^B
Biomass (Mg/ha)	68 ^{BC}	84 ^A	64 ^C	71 ^B	43 ^B	43 ^B	45 ^B	48 ^A	55 ^B	63 ^A	55 ^B	60 ^{AB}
Stalk Weight (g/ 10 stalks)	7,599 ^B	8,128 ^B	8,798 ^B	7,933 ^B	5,800 ^C	6,176 ^C	7,029 ^B	8,046 ^A	6,699 ^B	7,152 ^B	8,012 ^A	7,990 ^A
Extraction Efficiency (%)	29.2 ^C	32.4 ^B	32.0 ^B	35.4 ^A	30.4 ^C	30.8 ^C	32.3 ^B	38.2 ^A	29.8 ^D	31.6 ^C	33.4 ^B	36.8 ^A
Bagasse Moisture (%)	64.3 ^A	60.3 ^B	62.5 ^{AB}	57.0 ^C	66.8 ^A	65.0 ^B	62.2 ^C	65.3 ^B	65.6 ^A	63.0 ^B	62.3 ^B	61.7 ^B
Juice Yield (L/ha)	19,830 ^C	29,081 ^A	20,942 ^C	26,435 ^B	13,209 ^C	13,626 ^C	15,917 ^B	18,091 ^A	18,359 ^C	25,646 ^A	20,007 ^B	24,495 ^A
Juice Brix	13.73 ^A	13.59 ^A	12.91 ^B	13.93 ^A	12.51 ^A	8.67 ^D	10.98 ^B	9.92 ^C	13.01 ^A	10.98 ^C	11.91 ^B	11.59 ^B
Juice pH	5.82 ^A	5.90 ^A	6.26 ^A	6.08 ^A	5.33 ^B	5.24 ^C	5.41 ^A	5.19 ^C	5.40 ^{AB}	5.33 ^B	5.53 ^A	5.32 ^B
Juice Purity (%)	75.5 ^B	69.5 ^C	80.9 ^A	80.4 ^{AB}	51.7 ^C	77.7 ^A	62.7 ^B	69.5 ^B	63.4 ^B	72.7 ^{AB}	72.5 ^{AB}	75.9 ^A
Juice Turbidity (NTU)	4,100 ^A	3,691 ^B	3,857 ^{AB}	2,094 ^C	5,678 ^A	2,491 ^B	2,420 ^B	2,766 ^B	4,889 ^A	3,091 ^B	3,138 ^B	2,430 ^C
Height (cm)	339 ^B	370 ^A	372 ^A	341 ^B	313 ^C	328 ^B	368 ^A	311 ^C	325 ^C	351 ^B	371 ^A	325 ^C
3rd Node Diameter (cm)	1.96 ^B	2.06 ^A	2.00 ^B	1.98 ^B	1.76 ^C	1.86 ^B	1.86 ^B	2.08 ^A	1.86 ^C	1.96 ^B	1.94 ^B	2.03 ^A
7th Node Diameter (cm)	1.65 ^C	1.81 ^A	1.76 ^B	1.67 ^C	1.38 ^C	1.54 ^B	1.53 ^B	1.83 ^A	1.52 ^C	1.68 ^B	1.65 ^B	1.75 ^A
12th Node Diameter (cm)	1.14 ^D	1.29 ^B	1.35 ^A	1.20 ^C	0.94 ^C	1.21 ^B	1.23 ^B	1.43 ^A	1.04 ^C	1.25 ^B	1.29 ^A	1.32 ^A
Average Diameter (cm)	1.58 ^B	1.72 ^A	1.70 ^A	1.62 ^B	1.36 ^C	1.54 ^B	1.54 ^B	1.78 ^A	1.47 ^C	1.63 ^B	1.62 ^B	1.70 ^A
Average Temperature (°C)	2.66 ^{AB}	2.66 ^A	2.66 ^A	2.65 ^B	2.59 ^A	2.58 ^A	2.59 ^A	2.58 ^A	2.62 ^A	2.62 ^A	2.63 ^A	2.62 ^A
Average Rainfall (cm)	24.7 ^C	26.0 ^B	26.0 ^B	27.4 ^C	17.8 ^B	19.2 ^B	23.3 ^A	22.0 ^A	21.5 ^B	22.6 ^B	24.7 ^A	24.7 ^A

*Mean results followed by the same upper case letter within rows are compared within year or two-year average and are not significantly different at the 5% probability level. **Crop yield (%) was calculated by dividing the number of stalks per hectare by 140,000.

Table. 3. Agronomic, crop performance, physical, juice quality attributes of sweet sorghum by maturity during 2013 and 2014 seasons.

	*2013					2014					Two-Year Average				
Parameter	Mi	SD	HD	PM	PPM	Mi	SD	HD	PM	PPM	Mi	SD	HD	PM	PPM
<i>Days in Field</i>	96 ^D	110 ^{CD}	123 ^{BC}	139 ^{AB}	157 ^A	115 ^D	127 ^{CD}	141 ^{BC}	155 ^{AB}	169 ^A	105 ^D	119 ^{CD}	132 ^{BC}	147 ^B	163 ^A
<i>Crop Yield (Stalks/ha)</i>	88,675 ^A					67,026 ^B					77,851 [‡]				
<i>Crop Yield (%)</i>	63 ^A					48 ^B					56 [‡]				
<i>Biomass (Mg/ha)</i>	84 ^A	76 ^B	70 ^{BC}	68 ^{CD}	61 ^D	44 ^B	47 ^A	47 ^A	39 ^C	46 ^{AB}	64 ^A	62 ^{AB}	59 ^{ABC}	54 ^C	55 ^C
<i>Stalk Weight (kg/10 stalks)</i>	9.51 ^A	8.61 ^B	7.91 ^C	7.60 ^C	6.95 ^D	6.69 ^A	7.16 ^A	7.14 ^A	5.98 ^B	6.78 ^A	8.10 ^A	7.89 ^B	7.52 ^{BC}	6.79 ^C	6.88 ^C
<i>Extraction Efficiency (%)</i>	30.0 ^B	35.3 ^A	34.3 ^A	31.2 ^B	30.5 ^B	31.3 ^B	33.0 ^{AB}	35.5 ^A	33.5 ^{AB}	35.0 ^A	30.6 ^A	34.1 ^{AB}	34.9 ^A	32.4 ^{BC}	32.4 ^{BC}
<i>Bagasse Moisture (%)</i>	64.5 ^A	62.6 ^A	58.7 ^B	54.2 ^C	64.3 ^A	61.4 ^C	67.0 ^A	63.7 ^B	66.9 ^A	66.0 ^A	62.9 ^B	64.8 ^A	61.4 ^C	62.1 ^{BC}	65.3 ^A
<i>Juice Yield (L/ha)</i>	25,752 ^B	27,994 ^A	25,191 ^B	21,557 ^C	19,360 ^D	13,587 ^B	15,843 ^A	17,222 ^A	13,367 ^B	16,078 ^A	23,048 ^B	25,294 ^A	23,420 ^B	19,737 ^C	18,745 ^C
<i>Juice Brix</i>	11.36 ^D	13.33 ^C	14.38 ^{AB}	14.75 ^A	14.03 ^B	11.21 ^A	10.46 ^B	10.55 ^B	10.61 ^B	9.91 ^C	11.23 ^D	11.78 ^C	12.19 ^{AB}	12.30 ^A	11.95 ^{BC}
<i>Juice pH</i>	5.62 ^B	5.84 ^{AB}	6.27 ^A	6.14 ^A	6.19 ^A	5.31 ^{AB}	5.35 ^A	5.31 ^{AB}	5.22 ^C	5.26 ^{BC}	5.36 ^A	5.42 ^A	5.45 ^A	5.35 ^A	5.36 ^A
<i>Juice Purity (%)</i>	80.6 ^A	72.1 ^C	80.1 ^A	64.8 ^D	76.6 ^B	63.0 ^B	52.4 ^C	58.3 ^{BC}	57.9 ^{BC}	72.1 ^A	71.8 ^A	62.3 ^C	69.2 ^{AB}	61.3 ^{BC}	74.4 ^A
<i>Juice Turbidity (NTU)</i>	4,480 ^A	3,071 ^C	2,694 ^D	3,888 ^B	3,011 ^C	2,540 ^D	3,747 ^B	3,065 ^C	4,437 ^A	2,907 ^C	3,833 ^B	3,296 ^C	2,818 ^D	4,071 ^A	2,976 ^{CD}
<i>Height (cm)</i>	337 ^C	364 ^A	366 ^A	357 ^B	354 ^B	334 ^A	323 ^B	336 ^A	324 ^B	324 ^B	335 ^C	343 ^A	351 ^A	340 ^B	340 ^B
<i>3rd Node Diameter (cm)</i>	2.14 ^A	2.03 ^B	2.03 ^B	1.91 ^C	1.90 ^C	1.77 ^D	1.87 ^C	1.94 ^{AB}	1.92 ^{BC}	1.98 ^A	1.96 ^A	1.95 ^{ABC}	1.99 ^A	1.91 ^C	1.93 ^{BC}
<i>7th Node Diameter (cm)</i>	1.83 ^A	1.76 ^B	1.76 ^B	1.61 ^C	1.64 ^C	1.49 ^C	1.60 ^A	1.60 ^{AB}	1.54 ^{BC}	1.65 ^A	1.66 ^A	1.68 ^A	1.68 ^A	1.57 ^B	1.64 ^A
<i>12th Node Diameter (cm)</i>	1.83 ^A	1.32 ^B	1.20 ^C	1.15 ^{CD}	1.13 ^D	1.12 ^D	1.28 ^A	1.25 ^{AB}	1.16 ^{CD}	1.20 ^{BC}	1.27 ^A	1.30 ^A	1.23 ^B	1.15 ^C	1.16 ^C
<i>Average Diameter (cm)</i>	1.42 ^A	1.72 ^A	1.68 ^A	1.55 ^A	1.59 ^A	1.45 ^A	1.60 ^A	1.59 ^A	1.52 ^A	1.61 ^A	1.63 ^A	1.64 ^A	1.63 ^A	1.54 ^B	1.58 ^{AB}
<i>Average Temperature (°C)</i>	26.5 ^B	26.6 ^A	26.7 ^A	26.6 ^A	26.3 ^C	25.8 ^B	26.0 ^A	26.1 ^A	25.8 ^B	25.6 ^C	26.2 ^B	26.3 ^{AB}	26.4 ^A	26.3 ^{AB}	25.9 ^C
<i>Average Rainfall (cm)</i>	24.0 ^E	25.3 ^D	26.1 ^C	26.7 ^B	28.2 ^A	16.2 ^D	18.1 ^C	19.5 ^C	23.3 ^B	25.8 ^A	20.4 ^D	21.7 ^{CD}	22.8 ^C	25.0 ^B	27.0 ^A

*Mean results followed by the same upper case letter within rows are compared within year and are not significantly different at the 5% probability level.

Table 4. F-statistics for effects of harvest year or environment (E), cultivar (G), and maturity (M) date on four commercial sweet sorghum cultivars and their biophysical and juice quality traits.

F-Ratio (Pr>F)							
Parameter	Harvest Year (E)	Maturity (M)	Cultivar (G)	E x M	E x G	G X M	E x G x M
Temperature	20,592.4 (<0.0001)	921.9 (<0.0001)	122.5 (<0.0001)	99.0 (<0.0001)	42.1 (<0.0001)	414.3 (<0.0001)	79.3 (<0.0001)
Biomass	445.5 (<0.0001)	10.3 (<0.0001)	14.8 (<0.0001)	5.9 (<0.0001)	17.3 (<0.0001)	1.2 (0.3186)	1.4 (0.1924)
Stalk Wt.*	78.1 (<0.0001)	11.2 (<0.0001)	18.8 (<0.0001)	5.6 (<0.0001)	16.9 (<0.0001)	1.2 (0.2111)	1.3 (0.2679)
Moisture	66.4 (<0.0001)	11.2 (<0.0001)	22.0 (<0.0001)	32.8 (<0.0001)	5.1 (0.0073)	8.4 (<0.0001)	10.7 (<0.0001)
Height	107.8 (<0.0001)	6.4 (0.0004)	21.4 (<0.0001)	8.2 (<0.0001)	2.7 (<0.0001)	3.6 (<0.0001)	6.5 (<0.0001)
3 rd Node Diam.	19.6 (<0.0001)	1.5 (0.3273)	7.3 (0.0023)	7.4 (<0.0001)	7.8 (0.0012)	0.7 (0.8226)	0.7 (0.6761)
7 th Node Diam.	43.3 (<0.0001)	4.0 (0.0164)	17.2 (<0.0001)	4.1 (0.0027)	19.6 (<0.0001)	0.5 (0.8919)	1.3 (0.2649)
12 th Node Diam.	4.8 (0.0287)	6.3 (0.0002)	26.1 (<0.0001)	8.2 (<0.0001)	15.8 (<0.0001)	0.9 (0.4851)	1.6 (0.1135)
Juice Yield	41.7 (<0.0001)	66.4 (<0.0001)	234.8 (<0.0001)	24.7 (<0.0001)	128.3 (<0.0001)	31.2 (<0.0001)	46.3 (<0.0001)
Brix	2,406.4 (<0.0001)	71.9 (<0.0001)	157.5 (<0.0001)	147.7 (<0.0001)	165.0 (<0.0001)	55.3 (<0.0001)	48.1 (<0.0001)
Gluc.*	294.9 (<0.0001)	70.7 (<0.0001)	182.7 (<0.0001)	151.3 (<0.0001)	355.1 (<0.0001)	111.4 (<0.0001)	43.4 (<0.0001)
Fruc.*	1,468.1 (<0.0001)	312.3 (<0.0001)	390.9 (<0.0001)	247.7 (<0.0001)	645.7 (<0.0001)	158.5 (<0.0001)	54.7 (<0.0001)
Suc.*	3,630.5 (<0.0001)	162.2 (<0.0001)	45.1 (<0.0001)	74.7 (<0.0001)	231.0 (<0.0001)	80.7 (<0.0001)	37.2 (<0.0001)
Total Sug *	4,706.7 (<0.0001)	145.0 (<0.0001)	80.5 (<0.0001)	81.0 (<0.0001)	53.8 (<0.0001)	228.6 (<0.0001)	76.4 (<0.0001)
Total Starch	1,434.7 (<0.0001)	318.5 (<0.0001)	1,026.5 (<0.0001)	51.8 (<0.0001)	532.5 (<0.0001)	88.0 (<0.0001)	56.3 (<0.0001)
Insoluble Starch	1,064.1 (<0.0001)	307.4 (<0.0001)	957.7 (<0.0001)	51.1 (<0.0001)	558.4 (<0.0001)	86.5 (<0.0001)	61.4 (<0.0001)
Soluble Starch	3,837.5 (<0.0001)	111.8 (<0.0001)	168.9 (<0.0001)	16.9 (<0.0001)	282.9 (<0.0001)	93.8 (<0.0001)	74.4 (<0.0001)
Turb.*	47.9 (<0.0001)	1,090.0 (<0.0001)	5,657.0 (<0.0001)	1,172.1 (<0.0001)	2,728.1 (<0.0001)	2,188.2 (<0.0001)	614.8 (<0.0001)
N*	1,784.2 (<0.0001)	86.3 (<0.0001)	235.5 (<0.0001)	106.6 (<0.0001)	91.2 (<0.0001)	47.9 (<0.0001)	41.2 (<0.0001)
P*	8.7 (0.0046)	76.3 (<0.0001)	53.9 (<0.0001)	2.8 (0.0326)	37.6 (<0.0001)	41.6 (<0.0001)	11.2 (<0.0001)
pH	3,431.9 (<0.0001)	83.9 (<0.0001)	102.2 (<0.0001)	122.2 (<0.0001)	55.1 (<0.0001)	18.4 (<0.0001)	29.7 (<0.0001)

* parameter abbreviations: stalk weight (St. Wt.), diameter (Diam.), glucose (Gluc.), fructose (Fruc.), sucrose (Suc.), total sugars (Total Sug.), turbidity (turb.), assimilable nitrogen (N), and phosphate (P).

Fig. 1. Estimated sugars from Brix and actual total fermentable sugars (glucose, fructose, and sucrose) in sweet sorghum juice extracted during 2013 and 2014 seasons at milk (Mi), soft dough (SD), hard dough (HD), physiological (PM), and post-physiological (PPM) maturities. Estimated sugar content from Brix was determined according to the Circular of the National Bureau of Standards (Bates, 1974)

Fig. 2. Total starch per wet plant mass (g/kg) and in extracted sweet sorghum juice (g/100 mL) by cultivar (A-D) and maturity. Letters of the same case among maturity are not statistically different at the 5% probability level. Yearly averages (Avg) denoted with more than one asterisk (*) are statistically significant at the 5% probability level.

Fig. 3. Comparison of theoretical ethanol volume (mL/L juice) from fermentations of invert sugars only (SO) and invert sugar supplemented with hydrolyzed starch sugars (SS) per cultivar, maturity, and two year averages. Asterisks (*,**) indicate significant differences between total SO and SS at the 5% probability level.

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Figure 1

Figure 2

Figure 3

Table A1. Agronomic, crop performance, physical, and juice quality attributes of sweet sorghum by cultivar during 2013 and 2014 seasons.

Parameter	2013 [*]				2014				Two-Year Average			
	<i>Dale</i>	<i>M81E</i>	<i>Theis</i>	<i>Top 76-6</i>	<i>Dale</i>	<i>M81E</i>	<i>Theis</i>	<i>Top 76-6</i>	<i>Dale</i>	<i>M81E</i>	<i>Theis</i>	<i>Top 76-6</i>
<i>Days to Milk</i>	83 ^C	97 ^B	97 ^B	111 ^A	96 ^C	123 ^A	116 ^B	123 ^A	90 ^C	110 ^B	107 ^B	117 ^A
<i>Days in Field</i>	139 ^C	161 ^B	161 ^B	174 ^A	150 ^C	178 ^A	170 ^B	178 ^A	145 ^B	170 ^A	166 ^{AB}	176 ^A
<i>Crop Yield (Stalks/ha)</i>	89,100 ^B	103,100 ^A	72,500 ^C	90,000 ^B	73,500 ^A	70,700 ^B	64,000 ^C	60,000 ^D	81,300 ^A	86,900 ^A	68,250 ^A	75,000 ^A
<i>Crop Yield (%)**</i>	64 ^B	74 ^A	52 ^C	64 ^B	53 ^A	51 ^B	46 ^C	43 ^{CD}	58 ^{AB}	62 ^A	49 ^C	54 ^B
<i>Biomass (Mg/ha)</i>	68 ^{BC}	84 ^A	64 ^C	71 ^B	43 ^B	43 ^B	45 ^B	48 ^A	55 ^B	63 ^A	55 ^B	60 ^{AB}
<i>Stalk Weight (g/ 10 stalks)</i>	7,599 ^B	8,128 ^B	8,798 ^B	7,933 ^B	5,800 ^C	6,176 ^C	7,029 ^B	8,046 ^A	6,699 ^B	7,152 ^B	8,012 ^A	7,990 ^A
<i>Extraction Efficiency (%)</i>	29.2 ^C	32.4 ^B	32.0 ^B	35.4 ^A	30.4 ^C	30.8 ^C	32.3 ^B	38.2 ^A	29.8 ^D	31.6 ^C	33.4 ^B	36.8 ^A
<i>Bagasse Moisture (%)</i>	64.3 ^A	60.3 ^B	62.5 ^{AB}	57.0 ^C	66.8 ^A	65.0 ^B	62.2 ^C	65.3 ^B	65.6 ^A	63.0 ^B	62.3 ^B	61.7 ^B
<i>Juice Yield (L/ha)</i>	19,830 ^C	29,081 ^A	20,942 ^C	26,435 ^B	13,209 ^C	13,626 ^C	15,917 ^B	18,091 ^A	18,359 ^C	25,646 ^A	20,007 ^B	24,495 ^A
<i>Juice Brix</i>	13.73 ^A	13.59 ^A	12.91 ^B	13.93 ^A	12.51 ^A	8.67 ^D	10.98 ^B	9.92 ^C	13.01 ^A	10.98 ^C	11.91 ^B	11.59 ^B
<i>Juice pH</i>	5.82 ^A	5.90 ^A	6.26 ^A	6.08 ^A	5.33 ^B	5.24 ^C	5.41 ^A	5.19 ^C	5.40 ^{AB}	5.33 ^B	5.53 ^A	5.32 ^B
<i>Juice Purity (%)</i>	75.5 ^B	69.5 ^C	80.9 ^A	80.4 ^{AB}	51.7 ^C	77.7 ^A	62.7 ^B	69.5 ^B	63.4 ^B	72.7 ^{AB}	72.5 ^{AB}	75.9 ^A
<i>Juice Turbidity (NTU)</i>	4,100 ^A	3,691 ^B	3,857 ^{AB}	2,094 ^C	5,678 ^A	2,491 ^B	2,420 ^B	2,766 ^B	4,889 ^A	3,091 ^B	3,138 ^B	2,430 ^C
<i>Height (cm)</i>	339 ^B	370 ^A	372 ^A	341 ^B	313 ^C	328 ^B	368 ^A	311 ^C	325 ^C	351 ^B	371 ^A	325 ^C
<i>3rd Node Diameter (cm)</i>	1.96 ^B	2.06 ^A	2.00 ^B	1.98 ^B	1.76 ^C	1.86 ^B	1.86 ^B	2.08 ^A	1.86 ^C	1.96 ^B	1.94 ^B	2.03 ^A
<i>7th Node Diameter (cm)</i>	1.65 ^C	1.81 ^A	1.76 ^B	1.67 ^C	1.38 ^C	1.54 ^B	1.53 ^B	1.83 ^A	1.52 ^C	1.68 ^B	1.65 ^B	1.75 ^A
<i>12th Node Diameter (cm)</i>	1.14 ^D	1.29 ^B	1.35 ^A	1.20 ^C	0.94 ^C	1.21 ^B	1.23 ^B	1.43 ^A	1.04 ^C	1.25 ^B	1.29 ^A	1.32 ^A
<i>Average Diameter (cm)</i>	1.58 ^B	1.72 ^A	1.70 ^A	1.62 ^B	1.36 ^C	1.54 ^B	1.54 ^B	1.78 ^A	1.47 ^C	1.63 ^B	1.62 ^B	1.70 ^A
<i>Average Temperature (°C)</i>	2.66 ^{AB}	2.66 ^A	2.66 ^A	2.65 ^B	2.59 ^A	2.58 ^A	2.59 ^A	2.58 ^A	2.62 ^A	2.62 ^A	2.63 ^A	2.62 ^A
<i>Average Rainfall (cm)</i>	24.7 ^C	26.0 ^B	26.0 ^B	27.4 ^C	17.8 ^B	19.2 ^B	23.3 ^A	22.0 ^A	21.5 ^B	22.6 ^B	24.7 ^A	24.7 ^A

^{*}Mean results followed by the same upper case letter within rows are compared within year or two-year average and are not significantly different at the 5% probability level. ^{**}Crop yield (%) was calculated by dividing the number of stalks per hectare by 140,000.

Table A2. Agronomic, crop performance, physical, juice quality attributes of sweet sorghum by maturity during 2013 and 2014 seasons.

	*2013					2014					Two-Year Average				
<u>Parameter</u>	Mi	SD	HD	PM	PPM	Mi	SD	HD	PM	PPM	Mi	SD	HD	PM	PPM
<i>Days to Milk</i>			n.a.					n.a.					n.a.		
<i>Days in Field</i>	96 ^D	110 ^{CD}	123 ^{BC}	139 ^{AB}	157 ^A	115 ^D	127 ^{CD}	141 ^{BC}	155 ^{AB}	169 ^A	105 ^D	119 ^{CD}	132 ^{BC}	147 ^B	163 ^A
⁺ <i>Crop Yield (Stalks/ha)</i>			88,675 ^A					67,026 ^B					77,851 [‡]		
⁺ <i>Crop Yield (%)</i>			63 ^A					48 ^B					56 [‡]		
<i>Biomass (Mg/ha)</i>	84 ^A	76 ^B	70 ^{BC}	68 ^{CD}	61 ^D	44 ^B	47 ^A	47 ^A	39 ^C	46 ^{AB}	64 ^A	62 ^{AB}	59 ^{ABC}	54 ^C	55 ^C
<i>Stalk Weight (kg/10 stalks)</i>	9.51 ^A	8.61 ^B	7.91 ^C	7.60 ^C	6.95 ^D	6.69 ^A	7.16 ^A	7.14 ^A	5.98 ^B	6.78 ^A	8.10 ^A	7.89 ^B	7.52 ^{BC}	6.79 ^C	6.88 ^C
<i>Extraction Efficiency (%)</i>	30.0 ^B	35.3 ^A	34.3 ^A	31.2 ^B	30.5 ^B	31.3 ^B	33.0 ^{AB}	35.5 ^A	33.5 ^{AB}	35.0 ^A	30.6 ^A	34.1 ^{AB}	34.9 ^A	32.4 ^{BC}	32.4 ^{BC}
<i>Bagasse Moisture (%)</i>	64.5 ^A	62.6 ^A	58.7 ^B	54.2 ^C	64.3 ^A	61.4 ^C	67.0 ^A	63.7 ^B	66.9 ^A	66.0 ^A	62.9 ^B	64.8 ^A	61.4 ^C	62.1 ^{BC}	65.3 ^A
<i>Juice Yield (L/ha)</i>	25,752 ^B	27,994 ^A	25,191 ^B	21,557 ^C	19,360 ^D	13,587 ^B	15,843 ^A	17,222 ^A	13,367 ^B	16,078 ^A	23,048 ^B	25,294 ^A	23,420 ^B	19,737 ^C	18,745 ^C
<i>Juice Brix</i>	11.36 ^D	13.33 ^C	14.38 ^{AB}	14.75 ^A	14.03 ^B	11.21 ^A	10.46 ^B	10.55 ^B	10.61 ^B	9.91 ^C	11.23 ^D	11.78 ^C	12.19 ^{AB}	12.30 ^A	11.95 ^{BC}
<i>Juice pH</i>	5.62 ^B	5.84 ^{AB}	6.27 ^A	6.14 ^A	6.19 ^A	5.31 ^{AB}	5.35 ^A	5.31 ^{AB}	5.22 ^C	5.26 ^{BC}	5.36 ^A	5.42 ^A	5.45 ^A	5.35 ^A	5.36 ^A
<i>Juice Purity (%)</i>	80.6 ^A	72.1 ^C	80.1 ^A	64.8 ^D	76.6 ^B	63.0 ^B	52.4 ^C	58.3 ^{BC}	57.9 ^{BC}	72.1 ^A	71.8 ^A	62.3 ^C	69.2 ^{AB}	61.3 ^{BC}	74.4 ^A
<i>Juice Turbidity (NTU)</i>	4,480 ^A	3,071 ^C	2,694 ^D	3,888 ^B	3,011 ^C	2,540 ^D	3,747 ^B	3,065 ^C	4,437 ^A	2,907 ^C	3,833 ^B	3,296 ^C	2,818 ^D	4,071 ^A	2,976 ^{CD}
<i>Height (cm)</i>	337 ^C	364 ^A	366 ^A	357 ^B	354 ^B	334 ^A	323 ^B	336 ^A	324 ^B	324 ^B	335 ^C	343 ^A	351 ^A	340 ^B	340 ^B
<i>3rd Node Diameter (cm)</i>	2.14 ^A	2.03 ^B	2.03 ^B	1.91 ^C	1.90 ^C	1.77 ^D	1.87 ^C	1.94 ^{AB}	1.92 ^{BC}	1.98 ^A	1.96 ^A	1.95 ^{ABC}	1.99 ^A	1.91 ^C	1.93 ^{BC}
<i>7th Node Diameter (cm)</i>	1.83 ^A	1.76 ^B	1.76 ^B	1.61 ^C	1.64 ^C	1.49 ^C	1.60 ^A	1.60 ^{AB}	1.54 ^{BC}	1.65 ^A	1.66 ^A	1.68 ^A	1.68 ^A	1.57 ^B	1.64 ^A
<i>12th Node Diameter (cm)</i>	1.83 ^A	1.32 ^B	1.20 ^C	1.15 ^{CD}	1.13 ^D	1.12 ^D	1.28 ^A	1.25 ^{AB}	1.16 ^{CD}	1.20 ^{BC}	1.27 ^A	1.30 ^A	1.23 ^B	1.15 ^C	1.16 ^C
<i>Average Diameter (cm)</i>	1.42 ^A	1.72 ^A	1.68 ^A	1.55 ^A	1.59 ^A	1.45 ^A	1.60 ^A	1.59 ^A	1.52 ^A	1.61 ^A	1.63 ^A	1.64 ^A	1.63 ^A	1.54 ^B	1.58 ^{AB}
<i>Average Temperature (°C)</i>	26.5 ^B	26.6 ^A	26.7 ^A	26.6 ^A	26.3 ^C	25.8 ^B	26.0 ^A	26.1 ^A	25.8 ^B	25.6 ^C	26.2 ^B	26.3 ^{AB}	26.4 ^A	26.3 ^{AB}	25.9 ^C
<i>Average Rainfall (cm)</i>	24.0 ^E	25.3 ^D	26.1 ^C	26.7 ^B	28.2 ^A	16.2 ^D	18.1 ^C	19.5 ^C	23.3 ^B	25.8 ^A	20.4 ^D	21.7 ^{CD}	22.8 ^C	25.0 ^B	27.0 ^A

*Mean results followed by the same upper case letter within rows are compared within year and are not significantly different at the 5% probability level. ⁺Mean results followed by the same upper case letter within rows are compared between year and are not significantly different at the 5% probability level. [‡] n.a.=not applicable

Table A3. Pearson correlation coefficients (R) for two year average weather, agronomy, and juice quality attributes for the four cultivars at five physiological maturities. Results are significant at the 5% probability level. Weak correlations values <0.300 are not listed.

Parameters	DTM	Temp.	Rain	Wet Biomass	Yield	Wet Stalk Wt.	Moisture	Height	Avg. Diam.	Extr. Effic.	Juice Yield
DTM*	1.000										
Temp.*		1.000									
Rain	0.666	0.417	1.000								
Wet Biomass	-0.505	0.887		1.000							
Yield	-0.595	0.608		0.883	1.000						
Wet Stalk Wt.*		0.881	0.427	0.695		1.000					
Moisture	0.040	-0.844	-0.517	-0.759	-0.574	-0.656	1.000				
Height		0.637		0.557	0.358	0.545	-0.722	1.000			
Avg. Diam.*	0.170	0.709	0.592	0.528		0.896	-0.476	0.301	1.000		
Extr. Effic.*	0.697		0.646		-0.369	0.392	-0.336		0.540	1.000	
Juice Yield		0.898		0.947	0.768	0.768	-0.856	0.521	0.641		1.000
Brix	-0.799	0.588	-0.361	0.740	0.749	0.398	-0.521	0.389			0.661
Gluc.*	0.502		0.391	-0.607	-0.788		0.400	-0.431			-0.527
Fruc.*	-0.332	0.508				0.489			0.306		
Suc.*	-0.542	0.813		0.946	0.917	0.534	-0.808	0.567			0.899
Total Sug.*	-0.497	0.897		0.828	0.668	0.688	-0.708	0.436	0.428		0.779
Juice Purity		0.740	0.637	0.478		0.590	-0.509	0.302	0.578		0.457
Total Starch	-0.770	0.314	-0.577	0.674	0.812			0.342		-0.527	0.507
Starch Size			-0.321	0.897	0.922						0.590
Turb.*	-0.727	-0.322	-0.980				0.512		-0.450	-0.656	
TOC*	-0.707	0.577	-0.392	0.722	0.730	0.386	-0.552	0.421			0.676
TON*	-0.466		-0.355			0.453		0.343			
N*	-0.601	0.671		0.535	0.400	0.491	-0.524	0.549			0.427
Cond. Ash*		0.682	0.541	0.708	0.503	0.673	-0.558		0.833	0.378	0.784
P*	-0.325	-0.409	-0.510	0.002	0.325	-0.469	0.279	-0.367	-0.438	-0.210	-0.061
pH	-0.558	0.867	0.038	0.788	0.613	0.678	-0.724	0.654	0.339		0.729

* parameter abbreviations: days to milk (DTM), temperature (Temp.), crop yield (Yield), stalk weight (St. Wt.), average stalk diameter (Avg. Diam.), extraction efficiency (Extr. Effic.), glucose (Gluc.), fructose (Fruc.), sucrose (Suc.), total sugars (Total Sug.), turbidity (turb.), total organic carbon (TOC), total organic nitrogen (TON), assimilable nitrogen (N), conductivity ash (Cond. Ash), and phosphate (P). † In raw juices

Table A3 (continued). Pearson correlation coefficients (R) for two year average weather, agronomy, and juice quality attributes for the four cultivars at five physiological maturities. Results are significant at the 5% probability level. Weak correlations values <0.300 are not listed.

Parameters	Brix	Gluc.	Fruc.	Suc.	Total Sug.	Juice Purity	Total Starch	Starch Size	Turb.	TOC	TON	N	Elec. Cond.	P	pH
Brix	1.000														
Gluc.*	-0.584	1.000													
Fruc.*	0.333	0.482	1.000												
Suc.*	0.850	-0.684		1.000											
Total Sug*	0.767		0.694	0.837	1.000										
Juice Purity			0.694	0.377	0.696	1.000									
Total Starch	0.688	-0.893		0.679	0.334		1.000								
Starch Size	0.430	-0.899	-0.841	0.672	-0.645	-0.982	0.904	1.000							
Turb.*	0.374	-0.366				-0.555	0.616		1.000						
TOC*	0.955	-0.658		0.845	0.697	0.059	0.724		0.392	1.000					
TON*			0.379					-0.400	0.480		1.000				
N*	0.715		0.759	0.615	0.845	0.533		-0.831		0.607	0.367	1.000			
Cond. Ash*				0.509	0.449	0.513		0.858	-0.452				1.000		
P*	0.225	-0.490	-0.510	0.072	-0.250	-0.687	0.431	0.842	0.442	0.118	-0.288	-0.311	-0.068	1.000	
pH	0.794	-0.330	0.586	0.829	0.909	0.576	0.451	0.368	-0.809		0.811	0.345	0.857		-0.385

* parameter abbreviations: days to milk (DTM), temperature (Temp.), crop yield (Yield), stalk weight (St. Wt.), average stalk diameter (Avg. Diam.), extraction efficiency (Extr. Effic.), glucose (Gluc.), fructose (Fruc.), sucrose (Suc.), total sugars (Total Sug.), turbidity (turb.), total organic carbon (TOC), total organic nitrogen (TON), assimilable nitrogen (N), conductivity ash (Cond. Ash and phosphate (P)). † In raw juices

Table A4. Conductivity ash (mS/cm), total organic carbon (TOC, ppm), total organic nitrogen (TON, ppm), assimilable nitrogen (YAN, ppm), and phosphate (ppm) in extracted sweet sorghum juice. Same letters across columns are not statistically different at the 5% probability level.

	Cultivar				Maturity				
	<i>Dale</i>	<i>M81E</i>	<i>Theis</i>	<i>Top 76-6</i>	<i>Mi</i>	<i>SD</i>	<i>HD</i>	<i>PM</i>	<i>PPM</i>
Conductivity Ash (mS/cm)									
2013	6.1 ^{BC}	7.4 ^A	5.8 ^C	6.5 ^B	7.6 ^A	6.2 ^{BC}	6.3 ^{BC}	5.7 ^C	6.6 ^B
2014	4.7	6.0 ^B	5.4 ^B	6.8 ^A	5.1 ^C	6.7 ^A	5.5 ^{BC}	6.1 ^{AB}	5.4 ^{BC}
% Change	-24.1%	-19.4%	-5.9%	4.3%	-33.3%	7.6%	-12.8%	6.1%	-18.5%
Two Year Avg	5.4 ^B	6.7 ^A	5.6 ^B	6.7 ^A	6.3 ^A	6.4 ^A	5.9 ^A	5.9 ^A	6.0 ^A
TOC (ppm)									
2013	56,959 ^B	65,407 ^A	62,213 ^{AB}	65,690 ^A	52,798 ^B	64,529 ^A	63,372 ^A	64,108 ^A	68,030 ^A
2014	59,554 ^A	38,005 ^C	47,539 ^B	42,436 ^{BC}	51,008 ^A	46,336 ^A	46,077 ^A	46,638 ^A	44,358 ^A
% Change	4.6%	-41.9%	-23.6%	-35.4%	-3.4%	-28.2%	-27.3%	-27.3%	-34.8%
Two Year Avg	58,256 ^A	51,706 ^A	54,876 ^A	54,063 ^A	51,903 ^A	55,432 ^A	54,725 ^A	55,373 ^A	56,194 ^A
TON (ppm)									
2013	197.2 ^B	179.6 ^{BC}	263.6 ^A	137.8 ^C	232.0 ^A	275.3 ^A	177.0 ^B	143.8 ^B	144.5 ^B
2014	195.4 ^A	127.6 ^B	191.8 ^A	196.8 ^A	150.3 ^B	191.8 ^A	181.3 ^{AB}	192.0 ^A	174.3 ^{AB}
% Change	-0.9%	-29.0%	-27.2%	42.8%	-35.2%	-30.3%	2.4%	33.6%	20.6%
Two Year Avg	196.3 ^B	153.6 ^C	227.7 ^A	167.3 ^{BC}	191.1 ^B	233.6 ^A	179.1 ^B	167.9 ^B	150.4 ^B
YAN (ppm)									
2013	98.9 ^A	53.7 ^B	99.4 ^A	88.7 ^A	55.5 ^B	109.5 ^A	84.0 ^B	94.2 ^{AB}	82.7 ^B
2014	41.1 ^B	35.7 ^B	63.3 ^A	28.9 ^C	51.9 ^A	51.9 ^A	43.8 ^B	34.6 ^C	29.1 ^C
% Change	-58.4%	-33.5%	-36.3%	-67.4%	-6.5%	-52.6%	-47.9%	-63.3%	-64.8%
Two Year Avg	62.8 ^B	42.4 ^C	76.9 ^A	51.4 ^C	53.2 ^B	73.5 ^A	58.9 ^B	56.9 ^B	49.2 ^B
Phosphate (ppm)									
2013	78.5 ^A	77 ^A	44.2 ^B	64.8 ^A	46.4 ^B	65.1 ^A	71 ^A	71.7 ^A	76.3 ^A
2014	83.8 ^A	60.4 ^B	69 ^B	67.4 ^B	43.4 ^B	70.5 ^A	81.4 ^A	78.1 ^A	77.3 ^A
% Change	6.8%	-21.6%	56.1%	4.0%	-6.5%	8.3%	14.6%	8.9%	1.3%
Two Year Avg	81.7 ^A	67 ^B	59.1 ^B	66.3 ^B	44.6 ^B	68.4 ^A	77.2 ^A	75.6 ^A	76.9 ^A