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Research paper

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.

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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. [40].

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 [20]. 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 [20]. Heckemeyer Mill is currently capable of crushing up to ~45 ton per day and producing at least 8000 gal of juice [20]. 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

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.

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[30,32,35,40].

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 [13]. 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 [20]. 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. 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 [20]. 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 [20].

Most sweet sorghum research has focused mainly on crop production characteristics and, to a limited extent, process optimization [5,20] 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 [4,5,20]. 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. A1.

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*) [9,11,12,16]. 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 K_2O, and 45 kg/ha P_2O_5 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 Squier™ (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 [28], in which 25 mL of filtered juice (0.45 μm filter) was mixed with 35 mL of triethanoloamine/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 2100 N turbidimeter (Loveland, CO). Yeast assimilable nitrogen (YAN) was analyzed in triplicate [1]. Phosphate (P_2O_5) was determined in triplicate using a colorimetric assay based on the measurement of phosphate-molybdenum blue complex at 700 nm [27]. Conductivity ash was measured in triplicate on filtered juices (0.45 μm 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 μm filter) were performed in triplicate with a Torch Combustion TOC/TN analyzer (Teledyne Tekmar, Mason, OH) after adjusting the total soluble solids content to 10 g 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

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