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Estimation methods and parameter assessment for ethanol yields from total soluble solids of sweet sorghum

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ABSTRACT

Estimation methods and evaluation of ethanol yield from sweet sorghum (*Sorghum bicolor* (L.) Moench) based on agronomic production traits and juice characteristics is important for developing parents and inbred lines of sweet sorghum that can be used by the bio-ethanol industry. The objectives of this study were to compare published indirect methods for the calculation of ethanol yields from sweet sorghum and test them against direct ethanol production in laboratory, as well as to determine the relationships among total soluble sugar and juice traits with ethanol concentrations over time. Four sorghum varieties (KKU40, Theis, BJ248 and SPV1411) were compared for juice characters and ethanol yield in a randomized complete block design with four replications. Agronomic and juice traits of sweet sorghum were recorded during flowering and at harvest. Juice of sweet sorghum was fermented by yeast (*Saccharomyces cerevisiae*) to obtain ethanol yields in the laboratory, which were then compared with ethanol yields calculated based upon five calculation methods from the literature. Ethanol yield estimates calculated from published methods were generally higher than laboratory values. However, estimates based upon Somani and Taylor (2003) and on Smith et al. (1987) when multiplying theoretical yields by 80% were not significantly different from laboratory results. Though ethanol yield are strongly correlated with sugar yields, juice traits influenced the rate of fermentation of sugars over time. For example, glucose, fructose and nitrogen content in the juice had a positive effect on ethanol concentration after 12 h of fermentation while multiple juice traits were significantly associated with ethanol concentrations after 24, 36 and 48 h of fermentation.

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1. Introduction

Bio-ethanol is a renewable energy source produced mainly from agricultural crops such as sugarcane (Ratnavathi et al., 2010), maize (*Zea mays* L.), and cassava (*Manihot esculenta* Crantz) (Ali et al., 2008). These feedstocks are generally used for human and animal consumption and for other industries, for which the competition for these feedstocks is high (Jia et al., 2013). Therefore, alternative or supplemental feedstocks for bio-ethanol production are necessary during raw material shortage and for expansion of the industry (Shen et al., 2011). Sweet sorghum is an attractive crop as feedstock for bio-ethanol production (Ratnavathi et al., 2010). The juice from the fresh stems of sweet sorghum contains sucrose, glucose and fructose, which can be directly fermented to produce alcohol (Tew et al., 2008; Sipos et al., 2009). Moreover, the crop can be used as feedstock for producing sugar, syrup, fodder, bedding, roofing, fencing and paper in many areas of the world (Doggett, 1988; Laopaiboon et al., 2007; Liu et al., 2008). Genetically, sweet sorghum is similar to grain sorghum except for genes controlling plant height and sweet juice in the stalks (Ratnavathi et al., 2010). In addition, sweet sorghum is similar to sugarcane due to the high sugar content in the stalk which can range from 16 to 23% Brix (Smith et al., 1987; Murray et al., 2009; Ratnavathi et al., 2011). Sweet sorghum accumulates large amounts of sugar in stem parenchyma cells, beginning after internode elongation is complete (Hoffmann-Thoma et al., 1996), and peaking from anthesis to physiological maturity (Pfeiffer et al., 2010).

Ethanol yield of sweet sorghum can be determined directly from fermentation of sweet sorghum juice with bacteria or yeast (*Saccharomyces cerevisiae*) (Liu et al., 2008; Ratnavathi et al., 2011) or

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ethanol yield can be estimated indirectly from many calculation methods (Ratnavathi et al., 2010; Tew et al., 2008; Somani and Taylor, 2003; Smith and Buxton, 1993; Dalvi et al., 2011). Tew et al. (2008) estimated the ethanol yield of five sweet sorghums (Dale, M81-E, Rio, Theis and Topper) and two non-flowering sorghum × sudangrass forage hybrids by using Brix and the percent sucrose [1.05 (%sucrose) + 1.00 (%Brix − %sucrose)] to estimate hexose and then using a conversion rate of 1.7 kg hexose L^{-1} ethanol. This estimate did not take into account soluble solids in Brix other than sugars. Smith et al. (1987) assumed that fermentation would use every sugar molecule and produce ethanol and carbon dioxide and that 5.83 kg of hexose or 5.54 kg sucrose was needed to convert to one gallon (3.78 L) of ethanol. Smith and Buxton (1993) revised their theoretical ethanol yields by dividing total sugar yield by 5.68, and assuming only 80% efficiency due to other metabolic factors. Hills et al. (1990) reduced all sugars to hexose by multiplying sucrose by the factor 1.05 then uses an ethanol conversion rate of 15.02 lbs (6.81 kg) hexose for each gallon (1.8 kg hexose L^{-1}). This conversion rate of hexose to ethanol is slightly less than that used by Tew et al. (2008). Somani and Taylor (2003) estimated alcohol yield from sweet sorghum juice by using specific gravity (SG) and adjusting sugar estimates by subtracting 3 from each reading in the formula: (Brix − 3) × SG × 0.59 L ethanol kg^{-1} sucrose. This equation takes into account non-sugars in Brix but does not take into account possible differences in the sugar profile. All ethanol estimates of sweet sorghums were useful for comparisons among genotypes or treatments within the studies. However, comparisons of ethanol yields obtained from different estimates with measured ethanol yields from the lab have not been reported for sweet sorghum genotypes. Information is needed to determine the most accurate methods for estimating ethanol yield from juice components of sweet sorghum.

It is also important to determine how variation in juice parameters affects the conversion of sweet sorghum juice to ethanol. The pH of sweet sorghum juice has been shown to range from 4.4 to 5.5 (Davila-Gomez et al., 2011; Chohnan et al., 2011). This range is optimum for yeast growth and ethanol production (Mountney and Gould, 1988) with optimum ethanol production at about pH 5 (Raikar, 2012). Sufficient nitrogen and zinc are needed in the fermentation medium for sufficient growth and reproduction of the yeasts (Bisson, 1999; De Nicola and Walker, 2009). Clarity of the juice is diminished by cellulose fiber which can have a negative effect on ethanol yield (Han et al., 2012). How these parameters vary among sweet sorghum lines and how they corporately affect ethanol yields are important to understand.

The objectives of this study were to compare available estimation methods for ethanol yields of sweet sorghum with those obtained directly in the laboratory, and to determine relationships between characteristics of the juice and ethanol concentration from fermentation. The information obtained from this study will be useful for choosing the best methods to estimate ethanol yields from field production of soluble sugars and juice characteristics responsible for conversion efficiency through fermentation of sweet sorghum.

2. Materials and methods

2.1. Plant materials

Four genotypes of sweet sorghums (KKU40, Theis, BJ248 and SPV1411) were used in this study. KKU40 is a pure line variety developed at Khon Kaen University, Thailand. Theis (Broadhead et al., 1978) and BJ248 are also pure line varieties introduced from United States and China, respectively. SPV1411 was a variety donated from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. The four genotypes of sweet sorghum were planted during early rainy season on 8 May 2013 at the Field Crops Research Station (fine loamy, siliceous, Oxic Paleustult, Korat soil series), Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand (latitude 16.26° N, longitude 102.50° E).

2.2. Experimental design

A randomized complete block design (RCBD) with four replications was used in this study. The plot size was a 4-row plot with 4 m in length and spacing of 75 cm between rows and 10 cm between plants. Manual planting was carried out for all plots at the seed rate higher than normal planting, and the seedlings were later thinned to obtain one plant per hill 15 days after planting. Fertilizer grade 15–15–15 (N–P–K) at the rate of 156 kg ha^{-1} was applied to the plots at planting and then again as top dressing at 30 days after planting.

2.3. Data collection

Data were recorded for days to 50% flowering when 50% of total plants shed pollen. Total soluble solids (Brix) was recorded at the days to 50% flowering, soft dough (10 days after flowering), hard dough (20 days after flowering), and at harvest (30 days after flowering) from 10 randomly chosen intact plants in each plot using a hand refractometer. Three internode positions (top, middle and bottom) along the stems were tapped to extract juice, and total soluble solids were averaged from three positions (Makanda et al., 2009).

At harvest (30 days after flowering for each sweet sorghum cultivar starting in mid-September 2013), a 3 m long section of plants in each plot was harvested manually, and the stems were cut at ground surface. Plant height and stalk diameter were recorded from 10 randomly chosen plants in each plot. Plant height was measured from the ground to panicle tip (IBPGR and ICRISAT, 1993), and stalk diameter (SD) in mm was measured using a digital vernier caliper and averaged from three positions along the stalks. The plants were cut at the top to remove panicles, and all leaves were also removed. All stalks in the harvest area in each plot were measured, and stripped stalk weight was recorded.

The stalks of sweet sorghum were crushed to extract juice using a sugarcane three-roller crusher, and juice weight and juice volume were determined. Extracted juice was then immediately stored from each harvest at −20 °C for subsequent analysis and use. Specific gravity was then calculated as juice weight divided by juice volume. Total juice yield was calculated by subtracting stalk dry weight from fresh stripped stalk weight. The pH, total soluble solid, total sugar, reducing sugar, fructose, glucose and sucrose of the juice were recorded. The pH of each replicate was measured with a pH meter. Total soluble solids of the juice were recorded using a handheld refractometer (Ade Advanced Otpic®) to measure Brix immediately after squeezing from the stalks. Reducing sugars, fructose, glucose and sucrose of the juice was determined using high-performance liquid chromatography (HPLC) (LC-10AD, Shimadzu, Japan).

2.4. Fermentation

Two hundred and fifty milliliters of juice from each replication of the four sweet sorghum varieties were immediately placed in an ice bath. The juice was autoclaved at 110 °C for 28 min then cooled overnight in flasks prior to fermentation. Yeast (*S. cerevisiae*) TISTR 5048 provided by the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand, was grown in malt extract medium and placed on a rotating shaker