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Screening Sweetpotato Breeding Clones [*Ipomoea batatas (L.) Lam*] as a Suitable Potential Source of Feedstock for Bioethanol Production

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Abstract

Experiments were conducted to evaluate white and orange fleshed sweetpotatoes as a feedstock for bioethanol production, based on dry matter (DM), extractable starch, amylose-amylopectin ratio, fermentation times and ethanol yields. Ten sweetpotato cultivars viz., W308, TU0002, WS149 05, BM8342119 TU090W009, DMOI158096, DMOI158204, TIB4008, TIB4085 and, Beauregard, were planted in replicated field variety trials. Plants were established from 30 cm long vine cuttings and N was applied (P and K were sufficient) at 90 kg ha^{-1} based on soil test recommendations. Twenty-four storage roots were collected from each cultivar at harvest, 120 days after planting, and randomly divided into three groups of eight which served as replicates. Samples were collected and contents of dry matter (DM), extractable starch and amylose/amylopectin were determined. Ethanol (EtOH), concentration was determined by HPLC analysis from samples prepared using a solids:liquid ratio 0.13, which were hydrolyzed at 66°C for 90 min and fermented under anaerobic conditions using pure cells of Saccharomyces cerevisiae (yeast) at 30°C for 20 or 40 h. DM ranged from 19.6 to 35.9% and extractable starch from 10.9 to 25.3%, for Beauregard and W308, respectively. Amylose/amylopectin ranged from 19.5% for BM8342119 to 30.8% for TU0002, with ratios of 0.24 and 0.46, respectively. EtOH content ranged from 34.4 g L^{-1} for Beauregard to 66.6 g L^{-1} for TIB085 at 20 h and from 34.0 g L^{-1} for Beauregard to 71.0 g L^{-1} for TIB085 at 40 h of fermentation time. Increases in EtOH content with an additional 20 h fermentation time ranged from marginal to 9.0 g L^{-1}. Significant correlations existed between DM and EtOH, and starch and EtOH. These data suggest that seven of the sweetpotato cultivars can serve as potential feedstocks for bioethanol production, the higher the DM and starch, the greater the yield and a 40 h fermentation time will increase production.

Keywords: *Sweetpotato, Alternative Energy, Fermentation*

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INTRODUCTION

The current higher fuel and transportation costs are among the primary causes for recent higher food prices and cost of other services that are threatening global economic recovery [1]. Higher food prices led to riots in many countries recently, and the need for alternate energy sources cannot be over emphasized.

The U.S. has only 3% of the world's known oil reserves but consumes 25% of the production [2]. The US is the world's largest producer of bioethanol (9.0 billion gallons in 2008), followed by Brazil with 6.4 billion gallons [3].The primary feedstock is corn which is a major staple food and uses a production process that consumes 75–90% as much energy as is available from the fuel [4].

There are concerns about the large quantity of arable land required for production and the impact on grain supply for both humans and animals. One approach to address some of these concerns is to explore alternative sources of biomass feedstocks such as; sweetpotato [*Ipomoea batatas* (*L.*) Lam].

Sweetpotato storage roots, carbohydrate and ethanol (EtOH) yields are about three times that of corn [5] and carbohydrate yields have approached the lower limits of sugarcane, the highest yielding ethanol crop. Ethanol concentrations as high as 67.8 g/L for flour-based fermentation and 34.9 g/L for fresh storage roots have been reported [6, 7]. Sweetpotato yield, dry matter and EtOH concentration are cultivar dependent [8].

Ethanol is obtained through fermentation using mainly yeast from the genus *Saccharomyces,* especially *S. cerevisae* because they have a high alcohol tolerance (9–15%) [8]. Fermentation typically requires 12–72 h depending on the amount of yeast used to start the process and the concentration of sugar in the mash. In previous work [9] samples were fermented for 40 h and post-fermentation analysis showed no detectable amounts of sugars remaining. Our objective was to screen sweetpotato cultivars as a potential source of feedstock for bioethanol production based on dry matter, extractable starch, amylose-amylopectin levels, EtOH concentration and fermentation times to determine the relationship among these parameters.

MATERIALS AND METHODS

Samples Collection

Twenty four (24) US #1 storage roots were collected from each of ten cultivars: W308, TU0002, WS14905, BM8342119, TU090W009, DMOI158096, DMOI-158204, TIB4008, TIB4085 and Beauregard in a replicated field trial at harvest; 120 days after planting. Storage roots were randomly divided into three groups of eight, rinsed twice with tap water, followed by three successive deionized water rinses, and chopped into random sizes, and dry matter determined [10].

Starch Extraction

Three hundred grams (300 g) of storage roots were macerated in 400 ml of deionized water for 2 min and the slurry was successively filtered, twice through 250 and 150 μm screens allowing most of the starch granules to pass. The filtrate was re-suspended in 150 ml of deionized water (1:0.5 w/v), macerated for another 2 min, followed by two successive filtrations and allowed to stand for 3 h [11, 12].

Amylose/Amylopectin Analysis

Amylose/amylopectin content was estimated according to the rapid photometric method [13, 14]. A standard starch solution (2 mg/ml) was prepared from pure amylose by diluting the starch in 2 N NaOH and then transferring it into sodium acetate buffer (pH 5, 0.04 N). This original standard solution was used to prepare starch solutions of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml concentrations, respectively.

Additionally, 2.5 mg/ml starch solutions were prepared from each starch sample similarly as above for the preparation of the standards. Each of the three replicates was sub-sampled twice, after which 0.1 ml of Lugol's solution (2 g KI + 1 g I^2 in 300 ml deionized H_2O) and 0.1 ml of starch solution (both standards and test samples) were mixed directly in microtest plates and absorption of the starch-iodine complex (blue value) estimated at wavelengths of 550 and 620 nm. The amylose content was calculated from the blue value [13]. Based on the assumption that amylose = amylopectin = 100%, amylopectin fraction was calculated by subtracting fraction amylose from 1.

Starch granules were recovered by decantation, followed by three successive deionized water rinses and dried at 20°C for 24 h. Nutrients for the fermentation, and nitric acid used to adjust the pH were all of reagent grade.

Enzymatic Starch Hydrolysis

Starch was hydrolyzed [15] using a solid to liquid ratio of 0.13, with diastatic barley malt (Malt Corp. of America, Saddle Brook NJ.) as the source of hydrolyzing enzymes. Fifteen grams (15 g, 5%) of malt were added to the slurry and autoclaved at 100°C for 30 min, cooled to about 30°C, and pH adjusted to 5.3. Following this, 26.4 g of malt were added to the slurry to supplement enzymes denatured during autoclaving. Temperature was raised to 66 °C over 72 min where it was maintained for another 90 min to complete hydrolysis on an orbital shaker incubator at 200 rpm.

Yeast Propagation and Fermentation

Saccharomyces cerevisiae (ATCC 46534) cells obtained from American Type Culture Collection were propagated according to

ATCC's procedures and a liquid culture of yeast cells was prepared by inoculating 3-4 colonies into 300 ml of broth, followed by incubation overnight at 30 °C. The absorbance of the liquid culture was measured at 660 nm and liquid medium was used to adjust the absorbance to a target of 1.0 [16]. The temperature of the hydrosylate was lowered to 28 °C and pH adjusted to 4.5. A 10% (v/v) inoculum was used for fermentation [15, 16] under anaerobic conditions at 30 °C for 20 h or 40 h with constant agitation at 200 rpm.

HPLC Analysis

Each sample was centrifuged at 26000 rpm for 15 min. Supernatant was poured into 5 ml syringes and filtered through 5 µm filters into sample vials and analyzed with a SHIMADZU Model CBM-20A (Shimadzu Scientific Instruments, Columbia, MD USA) machine with an Aminex HPX-87H ion exclusion column and EtOH content determined.

Statistical Analysis

Experiments were conducted as a completely randomized design with three replications. Data from dry matter analysis, extractable starch, amylose/amylopection content and ethanol concentration and fermentation times were analyzed by the General Linear Models Procedure [3]. The differences among means were determined by the use of Tukeys test at an alpha value of 0.05. The PROC CORR and GLM procedures of SAS were used to determine relationships among percent dry matter, extractable starch and ethanol concentration.

RESULTS

Dry Matter and Starch Content and Ethanol Concentrations

Percent dry matter ranged between 19.6% for Beauregard (orange) to 36% for W308 (white; Table 1). Although W308 had the highest dry matter, it was similar to that of WS14905, TU0002 and BM8342119 but decidedly greater than the other six cultivars. Seven of the white-fleshed cultivars had dry matter in excess of 30%.

Extractable starch also followed a similar trend as dry matter ranging from 10.9% for Beauregard to 25.3% for W308. The highest percent extractable starch was produced by W308, TU0002 and TU090W009, but was also similar to that produced by DMOI158096 and DMOI158204 (Table 1). As with dry matter, TIB4008 and TIB4 085 produced the lowest extractable starch. It is worthy of note that TU090W009 produced extractable starch that was equal to that produced by W308 or TU0002. This occurred in spite of the fact that this cultivar produced a significantly lower dry matter, suggesting that the relationship between storage root dry matter and extractable starch may be cultivar dependent. Extractable starch obtained for Beauregard was the lowest, while that for the white fleshed cultivars ranged between 18.7 and 25.3%, and well within the range of 11.1 to 33.5% [4].

Table 1: *Dry Matter, Extractable Starch, Amylase and Amylopectin Content and Ratio and Ethanol (EtOH) Concentration of Ten Sweetpotato Cultivars*[*].

Cultivars	Dry matter	Extractable Starch	Amylose	Amylopectin	Amylose/Amylopectin ratio	EtOH** (g L^{-1})
WS14905	33.6^{ab}	20.9^{bc}	22.6^{ab}	77.4^{ab}	0.29^{ab}	57.1^{cd}
TU0002	35.1^{a}	24.5^{a}	30.8^{a}	69.2^{ab}	0.46^{a}	53.5^{ef}
W308	35.9^{a}	25.3^{a}	21.4^{ab}	78.6^{ab}	0.27^{ab}	52.3^{f}
TU090w009	31.7^{b}	25.0^{a}	28.3^{ab}	71.7^{ab}	0.41^{ab}	60.2^{bc}
DMOI158096	31.6^{b}	22.5^{ab}	21.0^{ab}	79.0^{ab}	0.27^{ab}	59.7^{ab}
BM8342119	32.8^{ab}	20.7^{bc}	19.5^{b}	80.5^{a}	0.24^{ab}	52.4^{f}
DMOI158204	30.6^{bc}	22.8^{ab}	26.7^{ab}	73.3^{ab}	0.36^{ab}	62.6^{b}
TIB4008	28.2^{cd}	20.2^{ab}	27.6^{ab}	72.4^{ab}	0.38^{ab}	56.3^{de}
TIB4085	26.1^{d}	18.7^{c}	20.9^{ab}	79.1^{ab}	0.27^{ab}	66.6^{a}
Beauregard	19.6^{e}	10.9^{d}	26.9^{ab}	73.1^{ab}	0.37^{ab}	34.1^{g}

[*]*P value < 0.05 for all the parameters in table 1. Also, a, b, c, d, e, f and g denote statistical similarities and differences between data in each column.*
[**]*EtOH = Ethanol concentration.*

Amylose levels ranged from 19.5% for BM 8342119 to 30.8% for TU0002 and amylopectin from 69.2% for TU0002 to 80.5 for BM8342119 (Table 1). The amylose and amylopectin fractions did not differ significantly but TU0002, had the highest amylose fraction hence lowest amylopectin percentage, and BM8342119 with the lowest amylose fraction had the highest amylopectin fraction (Table 1). The amylose/amylopectin ratio ranged from 0.24 for BM8342119 to 0.46 for TU090W009 (Table 1). This means that the higher the ratio, the higher the fraction of starch that is comprised of amylose, and the majority of industrial type starches have about 80% amylopectin [15]. Generally however, there were only marginal differences among all the cultivars except that the ratio for TU0002 of 0.46 was almost twice as high as that obtained for BM8342119, suggesting that the latter had a greater fraction of its starch comprising of amylopectin and was the only cultivar that approached or exceeded the 80% amylopectin industrial threshold. The concentration of EtOH ranged from 34.1 g L^{-1} for Beauregard to 66.6 g L^{-1} for TIB4085 which was significantly greater than all the cultivars (Table 1). All White fleshed cultivars had EtOH concentrations in excess of 52 g L^{-1} and were decidedly greater than that produced by Beauregard, an orange flesh cultivar. This response is commensurate with the low dry matter and extractable starch of this cultivar. These high EtOH concentrations are similar to those reported for sweetpotato flour-based fermentation [7] and for fresh sweetpotato [2]. In fact, the 66.6 g L^{-1} produced by TIB4085 was close to the maximum reported by [7], while the 32.4gL^{-1} obtained for Beauregard was slightly lower than that reported for fresh sweetpotatoes [7]. The difference in EtOH content among cultivars in response to fermentation time was similar (data not shown). When data were assessed across cultivars, results show a significant increase in ethanol content (54.0 vs. 59.0 g L^{-1}) for 20 and 40 h, respectively.

The highest percentage increase in EtOH based on cultivar, after 40 h were 11, 15 and 16.5%, respectively, for TU0904009, TIB4085, and WS14905.

CORRELATIONS

EtOH and Starch

There was a highly significant correlation between extractable starch and EtOH concentration ($r = 0.92$, $P < 0.0002$; Figure 1), and between dry matter and EtOH concentration ($r = 0.93$, $P < 0.0001$). As dry matter or extractable starch increased, a corresponding response was observed in the concentration of EtOH produced (Figures 1 and 2). The coefficients of determination (r^2) were 0.84, and 0.87 for ethanol and starch and dry matter, respectively. This means that EtOH concentration can be predicted for a given dry matter or starch level. Therefore, 84 and 87% of the variation in EtOH yield can be explained by variation in starch, and dry matter, respectively.

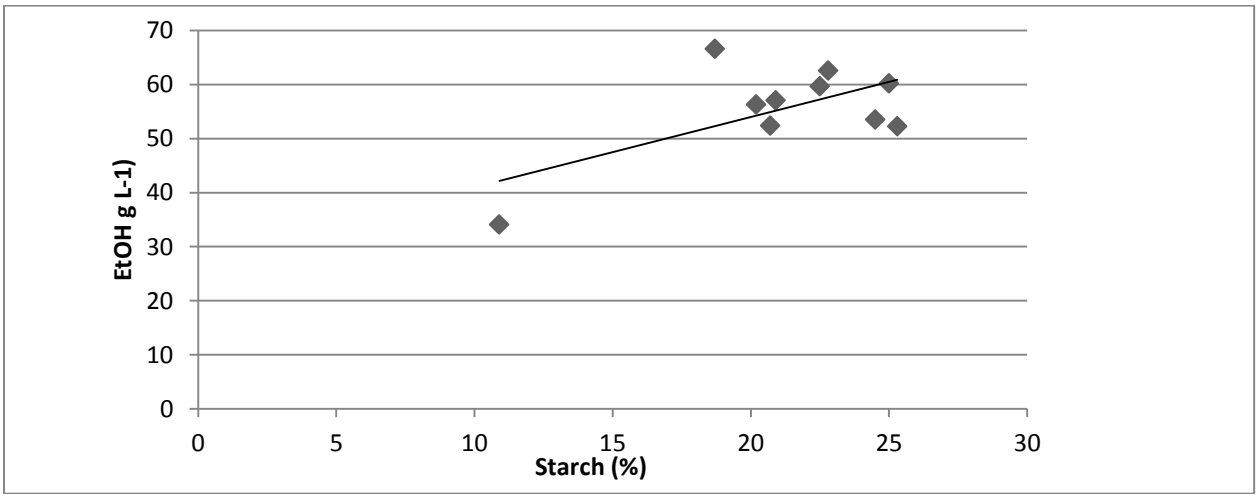

Fig. 1: *Relationship between Ethanol Concentration and Extractable Starch of Ten Sweetpotato Cultivars. The Relationship is Described by Y = 2.1382X + 13.638, $R^2 = 0.8392$, $P < 0.05$.*

DISCUSSION

These results show that seven white flesh cultivars produced dry matter levels above the 30% threshold, and were within the range of 9–45.4% reported for white-fleshed varieties [4]. Dry matter consists of all plant components excluding moisture, including carbohydrates, proteins, oils, and mineral nutrients [17]. Thus, the higher dry matter among the seven white flesh cultivars suggest a greater content of assimilates from photosynthesis, such as starch and sugars, two key constituents for EtOH production. In addition, the processing industry favors cultivars with dry matter of greater than 30–35%.

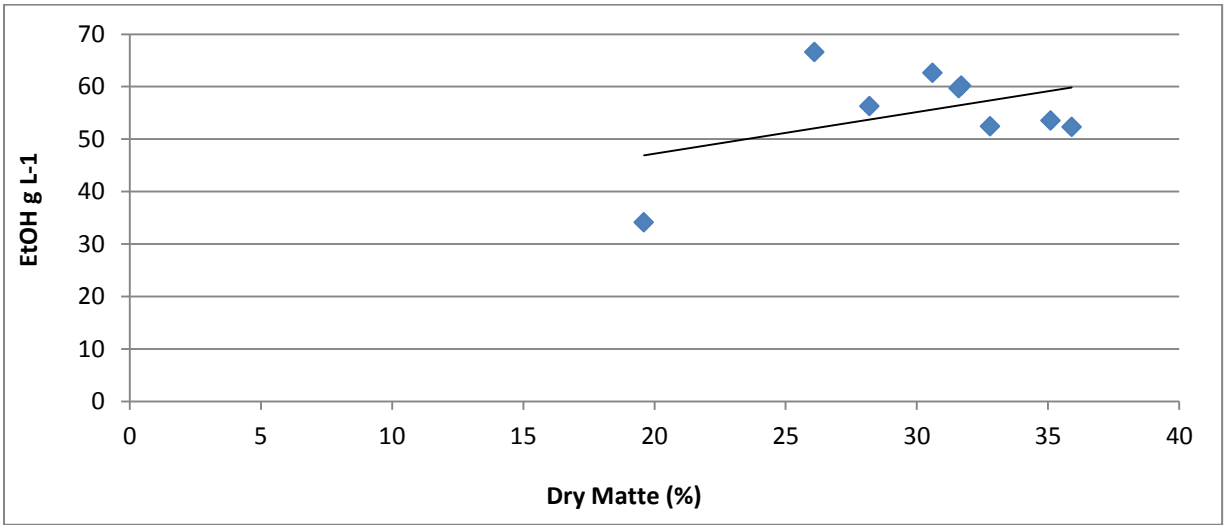

Fig. 2: *Relationship between Ethanol Concentration and Dry Matter of Ten Sweetpotato Cultivars. The relationship is Described by Y = 1.9015X + 0.7779, R^2 = 0.8703, P < 0.05.*

Extractable starch for all cultivars on a fresh weight basis is consistent with the 6.5–25.7% reported for 106 sweetpotato clones from diverse geographical regions and varying flesh color evaluated in a screening study [4], and equaled or exceeded the 20–22% [15] for West Indian sweetpotatoes [15], and the 7–28% for Indian sweetpotatoes [18]. The wide range of extractable starch in our study confirms the findings of others [19, 20] that there are significant differences among cultivars in the content and properties of starch, suggesting considerable room for genetic improvement of this trait [4].

The amylose fraction of 19.5 to 30.8 % was generally greater than the 18.6 to 27.1% reported [4]. The amylose/amylopectin fractions in varying proportions are the major components of starch and vary with different types of starch and species. For example regular corn, potato, wheat and cassava starches have amylose/amylopectin fractions similar to that of sweetpotatoes. However, *Amylomaize* has a much higher amylose/amylopectin ratio while starch from waxy corn has very negligible amount of amylose [15].

The amylose/amylopectin fractions also have implications for hydrolysis in terms of enzyme activities, fermentation and final EtOH yield. Two enzymes, α – and β – amylases only partially degrade amylopectin producing both α – and β – limit dextrins [15]. The addition of glucoamylase, however, results in hydrolysis of the 1, 6 – glycosidic bond producing glucose for fermentation.

Amylose/amylopectin ratio affects EtOH yield, and conversion and fermentation efficiency in corn [21]. They found that conversion efficiency increased as the amylose content decreased, particularly at levels below 35%. Our highest amylose fraction was about 31% suggesting that neither EtOH yield, nor conversion and fermentation efficiencies would be adversely impacted.

The EtOH produced for all the cultivars either equaled or exceeded the 40–63 g L^{-1} of others [2] from three sweetpotato cultivars with dry

matter ranging from 26–48%. Our high EtOH yield is due in part to the fact that total fermentation was achieved after 40 h. This is evident from the fact that HPLC analysis for sugar content in the samples indicated no detectable levels of glucose.

This further suggests that the yeast cells were active throughout the process, with little or no inhibition by ethanol on the sugars. Generally, *Saccharomyces cerevisiae* used in this study is quite tolerant to ethanol, up to 9–15% v/v of ethanol [22, 23]. The highly significant positive correlation between dry matter and ethanol concentration confirm findings of others [4, 24-28].

CONCLUSIONS

Results from this research suggest that seven of the sweetpotato varieties studied could serve as potential feedstocks for bioethanol production; the higher the DM and starch, the greater the yield and a 40 h fermentation time will increase production.

Therefore, storage root dry matter which is not complicated is rapid and inexpensive could be used as a screening tool to select promising cultivars with the potential to produce high ethanol yield. Both extractable starch and ultimately ethanol content could thereafter be measured analytically and confirmed. This would aid in greatly speeding up the whole variety selection process, allowing new/improved varieties to be available sooner.

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REFERENCES

1. Minot, N. *International Food Policy Research Institute*, Washington, DC, 2011.
2. National Resources Defense Council (NRDC). 2006, 6p.
3. SAS Institute. *Version 5 Ed. SAS Inst.*, Cary, NC, 1996.
4. Barbet C., *et al. CIP Program Report 1997-98*. 1999, 279–286p.
5. Ziska Lahr., *et al. Biomass Bioenerg*. 2009; 33: 1503–1508p.
6. Santa-Maria M.C., *et al. J. Amer. Soc. of Plant Biol*. 2006. Available at: http://abstracts.aspb.org/pb2006/public/P46/P46020.html.
7. Duvernay W.H. *MS Thesis, NC State University*. 2008.
8. Monday T. [*MS Thesis*], Aub. Univ. 2009.
9. Nyiawung K. Z. *et al. J. Root Crop.* 2010; 36(2): 242–249p.
10. Goenaga R. *Ann. Bot.* 1995; 76: 337–341p.
11. Collado L.S., *et al. J. Agric. Food Ch.* 1999; 47: 4195–4201p.
12. Noda T. *Food Chemi*. 2004; 86: 119–125p.
13. Hovenkamp-Hermelink J.H., *et al. Potato Res.* 1988; 31: 241–246p.
14. Jansen G., *et al. Potato Res.* 2001; 44:137–146p.
15. Hosein R., Mellowes W.A. *Biol. Waste.* 1989; 29: 263–270p.
16. Davis L., *et al. Biomass Bioenerg*. 2006; 30: 809–814p.
17. Taiz L., Zeiger E. In *Plant Physio*. Sinauer Associates, Inc. Sunderland, MA, 2002, 145–70p.
18. Ray R. C., Ravi V. *Crit. Re. Food Sci. Nut.* 2005; 45: 623–644p.
19. Tian S.J. *J. Sci. Food Agric.* 1991; 57: 459–491p.
20. Woolfe J.A. *Cambridge Univ. Press*, New York, USA, 1992.
21. Wu X., *et al. Cereal Chem.* 2006; 83: 569–575p.
22. Battcock M., Ali S.A. *FAO Agr. Serv. Bull.* 1993; 134: 13–28p.
23. Boulton C, Quain D. *Blackwell Science Ltd. New Jersey, USA,* 2001.
24. Mok I.G., *et al. CIP Program Report 1995-1996*. 1997; 105–109p.
25. Sharma V., *et al. Starch- Starke*. 2007; 59: 549–556p.
26. Marris E. *Proc. Soc. Invitro. Biol.*, 3013p, June 6-10 2009, Charleston SC. *Nature*. 2006; 444: 670–672p.

27. Renewable Fuels Association. 2009. Available at: www.ethanolrfa.org/industry/statistics/

28. Wright L. *Biomass Bioenerg*. 2006; 30: 706–14p.