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ARTICLE

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Sugar yield and composition of tubers from Jerusalem Artichoke (*Helianthus tuberosus*) irrigated with saline waters

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Abstract

Currently, major biofuel crops are also food crops that demand fertile soils and good-quality water. Jerusalem artichoke (*Helianthus tuberosus*, Asteraceae) produces high tonnage of tubers that are rich in sugars, mainly in the form of inulin. In this study, plants of the cultivar "White Fuseau" grown under five salinity levels were evaluated for tuber yield. Results indicated that this cultivar is moderately salt-tolerant if the goal is tuber production. Hydraulic pressings of the tubers produced juice that contained 15% (wet weight) or 55% (dry weight) free sugars, with 70% of these in the form of inulin and the rest as fructose, sucrose, and glucose. Importantly, salinity did not affect the total free sugar or inulin content of the tubers. Tubers were composed of about 12% dry washed bagasse (wet weight) or 44% (dry matter basis) and bagasse retained such high quantities of free sugars after pressing that washing was required for complete sugar recovery. Chemical composition analysis of tuber bagasse suggested that it had low lignin content (11–13 wt%), and its structural sugar composition was similar to chicory root bagasse. Because of the high hemicellulose and pectin content of the bagasse, adding xylanase and pectinase to cellulase substantially improved sugar yields from enzymatic hydrolysis compared to at the same protein loading as cellulase alone. In addition to the high total sugar yield of tuber, these first findings on the sugar and lignin content and enzymatic hydrolysis of tuber bagasse can lead to low-cost production of ethanol for transportation fuels.

KEYWORDS
enzymatic hydrolysis, inulin, Jerusalem artichoke, salinity, sugar yield, tuber composition

1 | INTRODUCTION

Jerusalem Artichoke (*Helianthus tuberosus*, L.) is a plant that belongs to the sunflower family (Asteraceae) and is native to North America (Kays & Nottingham, 2007). Its tubers are a rich source of inulin sugar made of β-D-fructosyl units linked by (2 → 1) glycosidic bonds and

Abbreviations: BCA, bicinchoninic acid; dS/m, deciSiemens per meter; EC, electrical conductivity; FPU, filter paper unit; Mg/ha/yr, megagram per hectare per year.

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terminating in α-D-glucosyl unit linked by (1 → 2) bond (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015; Niness, 1999). Jerusalem Artichoke can have a very high sugar productivity that rivals potato and sugar-beet (non-including bagasse structural sugars) (Fuchs, 1987), can grow on marginal lands, and is classified as moderately salt-tolerant (Newton, Myers, & West, 1991). Saline land that is unfit for growth of agricultural crops can be used to grow it for low cost sugar production (Pitman & Läuchli, 2002). Inulins contain fructose and glucose that microorganisms can convert into ethanol for use as a renewable transportation fuel with high yields (Bhagia, Akinosho, Ferreira, & Ragauskas, 2017). Alternatively, designer microbes can convert these six carbon sugars into specialty chemicals such as succinic acid (Cok, Tsiropoulos, Roes, & Patel, 2014) and butanediol (Yim et al., 2011). Fructose can be dehydrated more easily than glucose to 5-hydroxymethylfurfural (5-HMF) (Kabyemela, Adschiri, Malaluan, & Arai, 1999) for catalytic conversion to renewable aviation fuels, plastics, and resins (Rosatella, Simeonov, Frade, & Afonso, 2011).

The US Salinity Laboratory (USDA-ARS) in Riverside, California grew three cultivars of this plant, "Stampede," "White Fuseau," and "Red Fuseau" at salinity levels from 1.2 to 12 dS/m in 2014. One of the cultivars, "Stampede," was investigated for the effects of managed blended and sequential irrigation using low-salinity waters followed by high-salinity waters (Dias, Ferreira, Liu, & Suarez, 2016). Their recent work on "Stampede" concluded that tuber yield was moderately-tolerant to salt stress and that sequential irrigation management of adding high-salinity water after 75% of the crop cycle avoided the negative effects of salt stress on tuber yield (Dias et al., 2016). The tuber inulin content was not affected by salt stress and ranged between 50% and 60% of tuber dry weight. These results demonstrated the bioenergy potential of this plant to offer a low-cost source of free sugars from a crop that accumulates high tuber and aerial biomass and can grow on marginal, alkaline, or saline soils.

In this study, a "White Fuseau" cultivar only irrigated by blended salinity management, with five salinity levels in a US Salinity Laboratory project was evaluated for sugar and tuber bagasse yields, and composition and enzymatic hydrolysis of bagasse. This is the first study to provide a detailed analysis of yield of sugars, juice, bagasse, and water from tubers of Jerusalem Artichoke plants. Previously, only the aerial biomass composition had been reported (Gunnarsson, Svensson, Johansson, Karakashev, & Angelidaki, 2014). Furthermore, this report is the first of sugar and lignin composition of tuber bagasse and its enzymatic conversion of structural sugars by commercial enzyme preparations.

Although high total sugar yields are important to distribute costs over more product in commercial applications, sugar concentrations are important as low concentrations make product recovery from water energy-intensive and costly. Therefore, the material was pressed in this study to increase sugar concentrations to levels that would facilitate product recovery. Unwashed bagasse was dried, milled, and incubated with water to simulate a commercial washing step for production of the second stream of sugars. Compositional analyses of washed dry bagasse were carried out. Since our HPLC was only able to measure monomeric and dimeric sugars, streams that contained mostly inulins and some sucrose, glucose, and fructose were hydrolyzed in acid at 121 °C to convert them into monomeric sugars. Then enzymatic hydrolyses of washed mixed bagasse at different enzyme loadings were performed. Since farmers would be paid based on wet tuber weight, sugar yields based on the mass of both wet tubers and dry bagasse are presented to better estimate the cost of sugar.

2 | MATERIALS AND METHODS

2.1 | Jerusalem Artichoke tubers

Whole Jerusalem Artichoke (*Helianthus tuberosus*, L., cv. "White Fuseau") tubers grown at five salinity levels with blended waters with electrical conductivities (EC_w) of 1.2, 3.4, 6.3, 9.1, and 12 dS/m (deci-Siemens per meter) were obtained from the USDA Salinity Laboratory, Riverside, CA. The experimental design was the same as for the "Stampede" cultivar reported elsewhere (Dias et al., 2016). Wet tubers from two to three plants from each salinity level were kept at −20 °C for about 2 months before processing (Table S1).

2.2 | Component mass balances of tubers

Table S1 lists the tubers and Figure 2 describes the experimental workflow for this study. In most cases, two sets of wet tubers each weighing 100–120 g from each of the plants (total of 25 sets) grown in a five salinity levels were cut into thin cross-sections (Figures S1 and S2) that were hydraulically pressed (Model No. 14 590, Northern Tool + Equipment, Burnsville, MN) until no more juice could be collected. During this process, some juice was lost in the metal cylinder, and some bagasse was lodged in the sieve plate. Through calculation of before and after weights, the percent combined loss of juice and bagasse in the press was quantified. Before every run, the press was thoroughly washed with room temperature DI water and dried. Because tubers from some of the plants were available in smaller quantities, only one set of these samples was pressed but at the same weight charge to avoid bias. Juice recovered from this operation was diluted and analyzed by HPLC. The unwashed wet bagasse was dried at 60 °C in a convection oven (Thermo Scientific Oven Model 6520, Thermo Fisher Scientific Inc., Waltham, MA) overnight, and the remaining solids were weighed to calculate bagasse moisture content. The unwashed dry bagasse (flour) samples were knife-milled in a Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ) fitted with a 1 mm screen. The milled unwashed bagasse was then incubated with water and shaken to simulate an industrial water washing step. This method assured complete recovery of free sugars in the unwashed bagasse. To do this, approximately 0.2 g of milled unwashed dry bagasse was weighed on an analytical balance (Mettler-Toledo AB54-S, Columbus OH) and added to 20-ml glass serum vials (Wheaton cat no. 223687, Duran Wheaton Kimble Industries, Millville, NJ) along with 3.8 ml DI water and 0.2 ml of 1% sodium azide (Fisher Chemical S227I-100), the latter to prevent microbial growth. The vials were then sealed and

shaken overnight at room temperature (66–74 °F) in an orbital shaker (Multitron Standard Infors® 340 HT Biotech, Laurel, MD) at 150 rpm. The supernatant was centrifuged at 15,000 rpm for 5 min (Mettler Toledo, Model no. 5424, Columbus, OH) and diluted for HPLC analysis. The juice density of 1.04 ± 0.016 g/ml was used to convert sugar concentrations determined by HPLC into weight percent.

2.3 | Compositional analysis of washed tuber bagasse

Approximately 2 g of unwashed dry milled bagasse and 45 ml of DI water were added to a 50 ml centrifuge tube and centrifuged at 3,000 g for 5 min (Allegra X-15R, Beckman Coulter, Fullerton, CA). The supernatant was discarded, and this procedure was repeated ten times to remove all free sugars from the bagasse. Washed wet bagasse recovered after repeated centrifugation was dried at 40 °C for 72 hr in the convection oven mentioned above, followed by solvent extraction with ethanol in a pressurized extraction system (Dionex ASE 350, Bannockburn, IL) followed by air-drying in a fume hood. The bagasse structural carbohydrates, Klason lignin, and ash were then analyzed following NREL's standard biomass analytical procedure of strong sulfuric acid hydrolysis (Sluiter et al., 2008).

Equal proportions of biomass grown at all salinity levels were mixed, and the nitrogen content of the washed dry mixed bagasse sample was determined by elemental analyzer (Pyro-cube, Elementar Corp. Mt. Laurel, NJ).

2.4 | Enzymatic hydrolysis of washed mixed tuber bagasse

Due to the large number of samples, enzymatic hydrolyses were carried out on mixed bagasse from all salinities in equal proportions. Enzymatic hydrolyses of washed wet bagasse were carried out according to the NREL's standard procedure (Selig, Weiss, & Ji, 2008) at tuber bagasse solids loadings (dry basis) of 1% (w/v) total carbohydrate in 50 mM sodium citrate buffer at pH 5.0 with 0.02% sodium azide antibiotic added to a 10 ml reaction volume in 25 ml borosilicate Erlenmeyer flasks (Kimble® Kimax® Cat. No. 5650025EMD, Rockwood, TN). The flask contents were then hydrolyzed in the same orbital shaker mentioned above at 150 rpm for 120 hr with sampling at 24 and 120 hr. Enzyme loadings of 5, 15, and 100 mg protein per gram carbohydrate in bagasse were employed of either the commercial Accellerase 1500 cellulase preparation or a mixture of Accellerase 1500:Multifect Xylanse:Multifect Pectinase (cocktail) in the ratio 5:1:4. The Accellerase® 1500 (Batch No. 1662334068), Multifect® Xylanase (Lot no. 301-04021-015), and Multifect® Pectinase FE (Batch No. 4011150255) were all kind gifts by DuPont Industrial Biosciences in Palo Alto, CA and had a protein content of 82 mg/ml, 42, and 82 respectively, as determined by the bicinchoninic acid (BCA) assay (Smith et al., 1985). The specific activity of the Accellerase® 1500 preparation was 0.5 FPU/mg, as reported elsewhere (Alvira, Negro, & Ballesteros, 2011; Kumar et al., 2012).

2.5 | Analysis of juice and soluble sugars in bagasse

Sugars in the juice and soluble sugars in the bagasse wash (free sugars) were analyzed in two ways. First, diluted juices (20-times in DI water) and soluble sugars were analyzed directly by HPLC for free sucrose, glucose, fructose, and any other mono- or dimeric sugars. Second, to break down sugar oligomers into monomers that could be measured via our HPLC, diluted juices (50-times in DI water) and diluted soluble sugar liquids (10-time in DI water) were hydrolyzed in 0.5% sulfuric acid at 121 °C for 30 min in an autoclave (Model HA-300MII, Hirayama Manufacturing Corp., Tokyo, Japan) (Li et al., 2012; Nguyen, Sophonputtanaphoca, Kim, & Penner, 2009) followed by centrifuging at 15,000 rpm for 5 min, neutralizing with calcium carbonate to pH 7, and centrifuging again at 15,000 rpm for 5 min, followed by HPLC quantification for total glucose and fructose in the supernatant. Degradation of sugars due to this post hydrolysis at 121 °C was calculated as percentage sugar recovery through inclusion of sugar recovery standards along with unknown samples. Juices were diluted twenty times, and soluble sugar solutions from bagasse were not diluted for direct analysis by HPLC. Juices were diluted by a factor of fifty and soluble sugar solutions by a factor of ten prior to acid hydrolysis to bring the sugar concentrations into the range for HPLC analysis. Inulin content of tubers was calculated as the difference between total glucose plus fructose after acid hydrolysis corrected for sugar degradation, and sucrose, monomeric glucose, and fructose in unhydrolyzed liquids as determined by HPLC. Starch content was analyzed as previously described (Dias et al., 2016; Hendrix, 1993) by alkaline gelatinization, neutralization, and digestion with amyloglucosidase, followed by quantification of glucose.

2.6 | Sugars analysis

Sugars were analyzed on a Waters® e2695 HPLC equipped with Separations Module and RI detector (model 2414; Waters Corp., Milford MA). The separations were carried out through two Bio-Rad Aminex® HPX-87P columns connected in series at 85 °C with Milli-Q water as the mobile phase at a flow rate of 0.3 ml/min to keep the pressure below 1,000 psi.

2.7 | Statistical analysis

For statistical analysis, a one-way ANOVA with salinity as the factor and several response variables (Table S2) was applied at an alpha level of 0.05 using R software (R-Core-Team, 2015). Bonferroni post-hoc method was used for comparison of means. Tukey box plots were designed in OriginPro OriginLab v. 6.4.

2.8 | Calculations

$$\text{Wet tuber weight} = \text{Juice} + \text{Unwashed wet bagasse} + \text{Loss in hydraulic press}$$

$$\text{Juice} = \text{Inulin} + \text{Fructose} + \text{Sucrose} + \text{Glucose} + \text{Water}$$

$$\text{Unwashed wet bagasse} = \text{Inulin} + \text{Fructose} + \text{Sucrose} + \text{Glucose} + \text{Dry washed bagasse} + \text{Water}$$

$$\text{Glucose as inulin} = [(\text{Glucose after acid hydrolysis corrected for loss}) - (\text{Glucose} + (1.053^{*}(\text{Sucrose}/2)))]^{*}0.995$$

$$\text{Fructose as inulin} = [(\text{Fructose after acid hydrolysis corrected for loss}) - (\text{Fructose} + (1.053^{*}(\text{Sucrose}/2)))]^{*}0.995$$

$$\text{Inulin} = [((\text{Total glucose} + \text{fructose from HPLC after acid hydrolysis}) / (\text{acid hydrolysis degradation correction})) - (\text{Glucose} + \text{Fructose} + 1.053^{*}\text{Sucrose})]^{*}0.995$$

where 0.995 is the recommended correction factor for unknown degree of polymerization of inulin (Steegmans, Iliaens, & Hoebregs, 2004), the 1.053 value accounts for the mass of water added when converting sucrose was converted into monomeric glucose and fructose, and 0.99 and 0.89 are correction factors for degradation caused by acid hydrolysis of glucose and fructose, respectively.

$$\text{DP of inulin} = 1 + (\text{Conc. of fructose as inulin} / \text{Conc. of glucose as inulin}) \text{ (Gunnarsson et al., 2014)}$$

$$\text{Enzymatic hydrolysis individual sugar yield, \%} = \text{Individual sugar concentration(g/L)}^{*} \text{ Reaction volume(L)} {}^{*}\text{anhydrous factor}^{*}100/\text{Initial dry mass of bagasse(g)} {}^{*}\text{individual sugar fraction(Bhagia, Li, Gao, Kumar,\& Wyman, 2016)}$$

$$\text{Enzymatic hydrolysis total sugar yield, \%} = (\sum (\text{Individual sugar concentration(g/L)}^{*}\text{anhydrous factors})) {}^{*}\text{Reactionvolume(L)}^{*}100/\text{Initail dry mass of bagasse (g)} {}^{*} \text{ total sugar fraction}$$

$$\text{Total sugar in bagasse} = \text{Glucan} + \text{xylan} + \text{galactan} + \text{rhamnan} + \text{arabinan} + \text{mannan}$$

Anhydrous factor for glucose, xylose, galactose, rhamnose, arabinose, and mannose are 0.9, 0.88, 0.9, 0.89, 0.88 and 0.9, respectively.

3 | RESULTS

The highest tuber yield of 82 Mg/ha was achieved from plants irrigated with saline water of EC_w = 3.9 dS/m (Figure 1). However, analysis of variance suggested no difference in tuber yield for salinity levels of 3.9, 6.6, and 9.3 dS/m but showed a major drop in tuber yield when salinity was increased to EC_w = 12 dS/m (25 Mg/ha). These results agree with those reported previously with "Stampede" under the same growing conditions and irrigation water salinity, with a drop in tuber yield of 11% at EC_w = 6.0 dS/m and 47% at EC_w = 12 dS/m (Dias et al., 2016). The probability values in Table S2 from one-way analysis of variance indicated that only tuber productivity was affected by salinity, while other inherent tubers parameters remained unchanged for plants irrigated at different salinity levels. Therefore, data other than tuber productivity were random variations and are presented in the form of box plots that allow assessment of variation in yield expected from this plant.

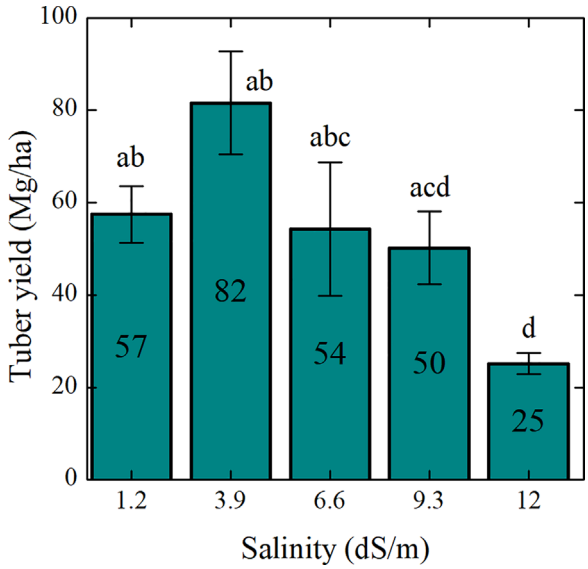

FIGURE 1 Yield of fresh (wet) tubers according to salinity of irrigation water

As shown in Figure 2, a hydraulic press was utilized to separate tuber juice from bagasse as envisioned to simulate a low-cost industrial scale sugar recovery operation. Figure 3 shows the average weights of components after hydraulic pressing of tubers from fourteen plants, with two replicates from each plant in most cases to exclude variability introduced by the press (Table S1). Roughly 65% of tuber mass was water, and bagasse contained more water than juice. The mass of all free sugars (including inulin) from juice and unwashed bagasse combined was more than the mass of dry washed bagasse. Most of the mass of juice ranged between 30 and 38 g out of 100 g of wet tubers (Figure S3). This work did not consider minor components such as micronutrients and protein that may be present in juice but were beyond the scope of this study that focused on sugar yields. Juice contained inulins, fructose, sucrose, and glucose, in that order of amounts (Figures 4a and 4b). The starch content was too low in JA tubers (~0.7 mg/g tuber dry matter) to measure. No other free sugars were detected by HPLC. Sucrose, fructose, and glucose, combined, constituted around 1% of wet whole tuber mass and around 3% of tuber mass on dry basis. Most of the inulin in juice ranged between 3% and 5% of wet tubers and 10–17% of dry matter. The total sugar content and its statistical distribution in juice (inulin + sucrose + glucose + fructose) were governed by inulin content and its statistical distribution as the other three sugars were much lower in quantity that inulin. Figures 5a and 5b shows that there was more sugar of every type in in the stream obtained by washing dried unwashed bagasse than in juice on an absolute mass basis. The difference in trends between juice and unwashed bagasse resulted from the presence of higher quantities of monomeric glucose and fructose in unwashed bagasse. Sucrose yields from juice and bagasse were roughly the same. In the case of unwashed bagasse, the total sugar distribution was

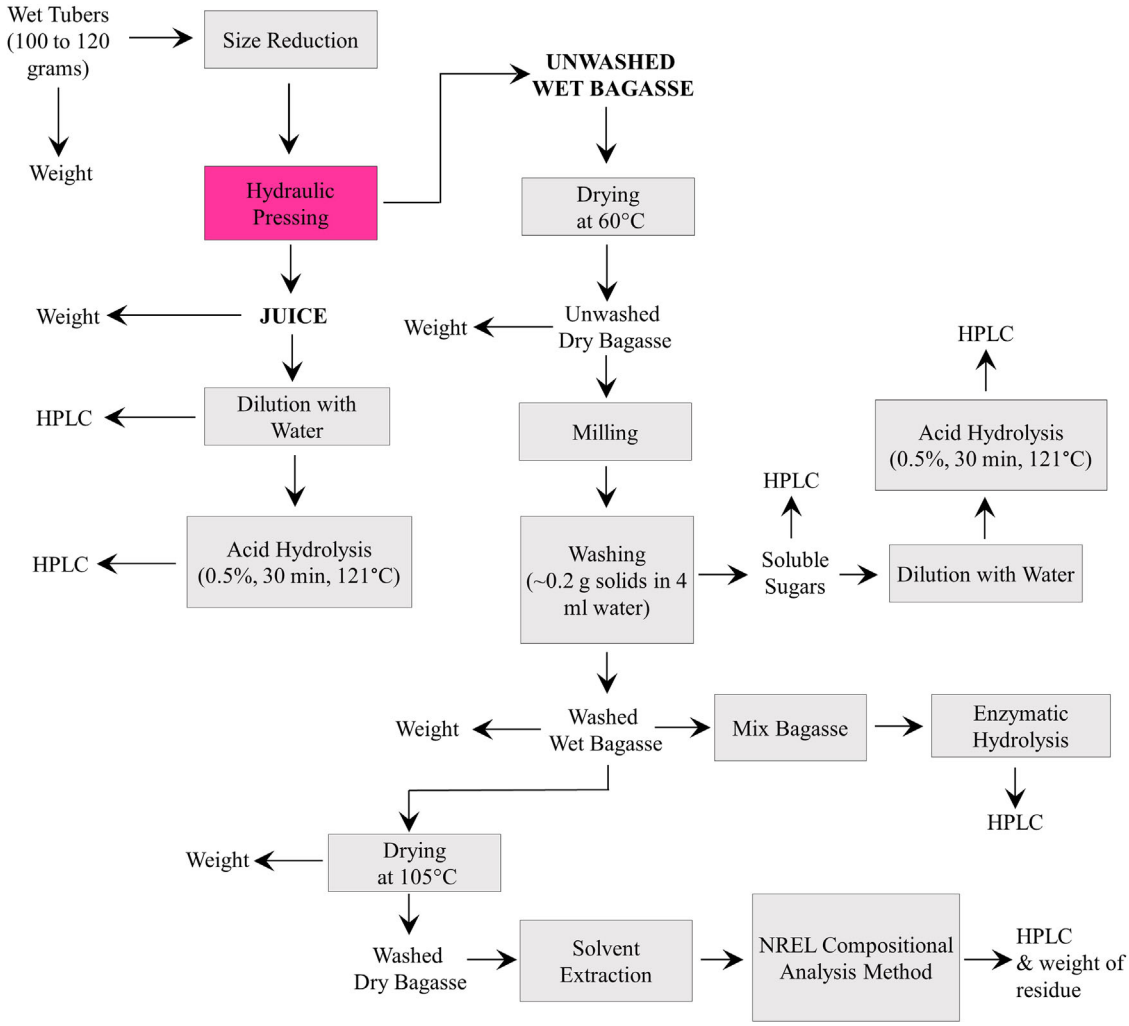

FIGURE 2 Experimental workflow for this study

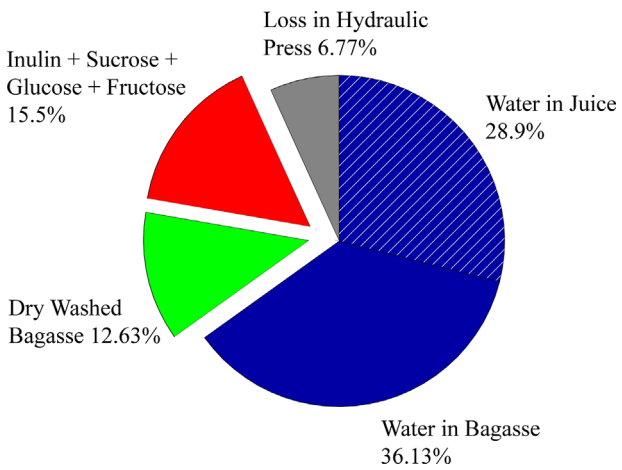

FIGURE 3 Distribution of average mass of major components in both bagasse and juice

governed not only by inulin but also monomeric fructose. Bagasse retained high amounts of total sugar after hydraulic pressing, as the minimum and median of total sugar contained in unwashed bagasse were 32% and 37% of dry matter, respectively.

Expressing all sugars in terms of glucose and fructose, around 2 g of glucose, 12–14 g of fructose, and 11–15 g of dry washed bagasse were recoverable from 100 g of wet tubers (Figures 6a and 6b). On a dry matter basis, these represented roughly 9 g of glucose and 43–51 g of fructose. Out of 51% to 60% total sugars, 43–51% was inulin as calculated on dry matter basis. These sugars were obtained in high concentrations after acid hydrolysis of juice (Figure S4), with most of the glucose and fructose concentrations varying between 22–31 g/L and 114–136 g/L, respectively. However, they could reach as high as 37 and 156 g/L, respectively. The degree of polymerization of inulin was between 6 and 8.

As shown in Figures 7a and 7b, bagasse structural carbohydrate polymers consisted mainly of three sugars: glucose, arabinose, and galactose. Xylan, rhamnan, and mannan combined were roughly 5% of the weight of dry washed bagasse, and the median glucan content,

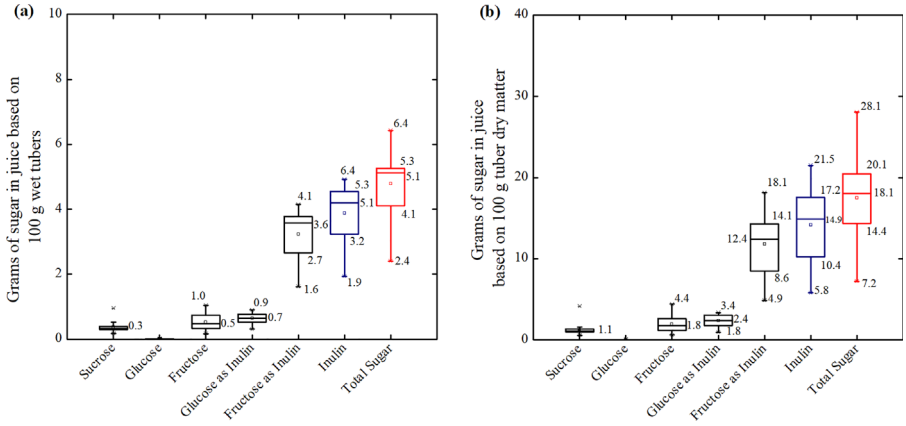

FIGURE 4 Variation in sugar content of juice from tubers based on wet tuber mass (a) and tuber dry matter (b)

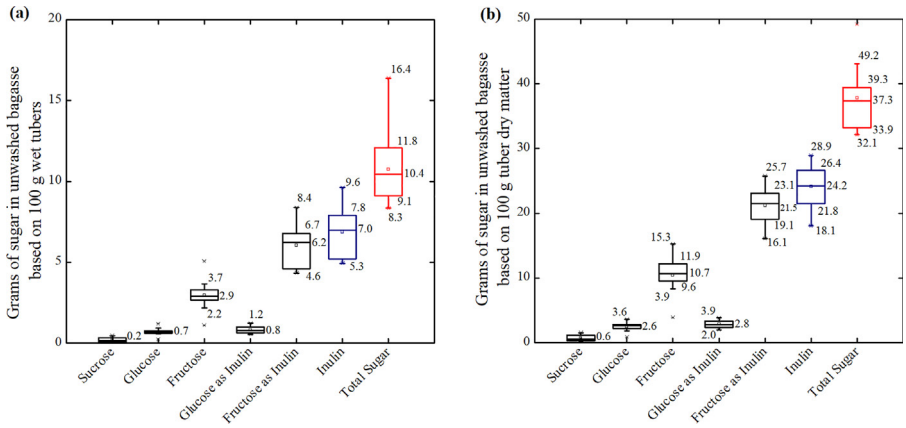

FIGURE 5 Variation in free sugar contents in unwashed bagasse from tubers based on wet tuber mass (a) and tuber dry matter (b)

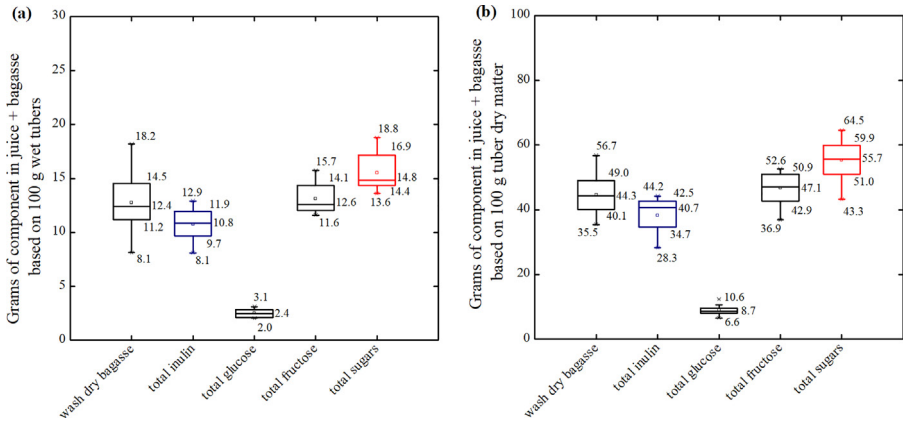

FIGURE 6 Variation in free sugar content in juice + bagasse and mass of dry washed bagasse from tubers based on (a) wet tuber mass (b) tuber dry matter

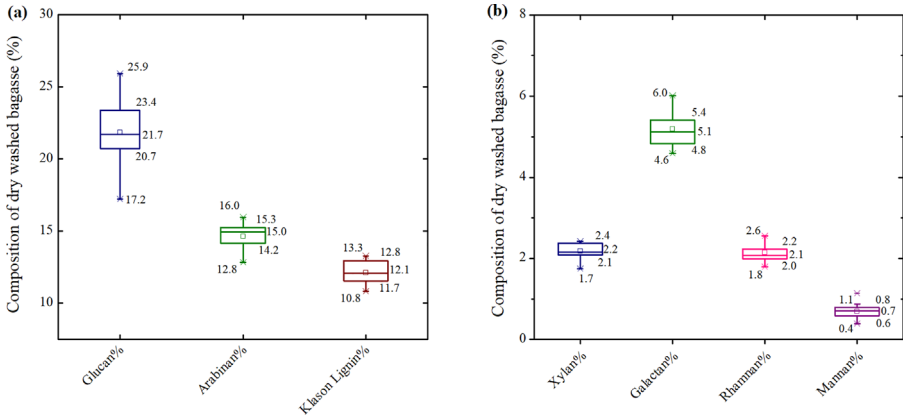

FIGURE 7 Composition of structural sugars and Klason lignin in dry washed bagasse

mostly in cellulose but little in hemicellulose, was only 22%. The bagasse Klason lignin content was between 11% and 13%, and the nitrogen content of the mixed sample from all salinity levels was 1.39%. Assuming a factor of 6.25, the protein content of bagasse amounts to 8.69% of dry washed bagasse, whereas the total ash content was low (1%). These components added up to roughly 70%, indicating that the remaining 30% was likely mostly galacturonan in pectic substances, as reported elsewhere (Liu, Shi, Xu, & Yi, 2016), along with acid soluble lignin.

Washed bagasse from all tubers was combined in equal proportions as mixed bagasse substrate for enzymatic hydrolysis. Figure 8 shows that individual and total sugar yields except glucan yields were significantly higher for an enzyme cocktail containing cellulase:xylanase:pectinase in a 5:1:4 ratio instead of just cellulase at the same total protein loadings of 5 mg (low) and 15 mg (medium) protein per gram total carbohydrate in JA washed dry bagasse after 120 hr. There was little release of galactose, rhamnose, and mannose in enzymatic hydrolysis of tuber bagasse with cellulase alone. The total sugar yield after 120 hr increased from 27% to 49% when 100 mg cellulase was replaced by 100 mg of cellulase + xylanase + pectinase. Overall, these results show that at low and medium protein loadings that are more relevant for commercial applications, a considerable fraction of the structural sugar was not hydrolyzed. Even at very high enzyme loadings of the cocktail, nearly half of the total of biomass polysaccharides remained unhydrolyzed after 5 days of reaction.

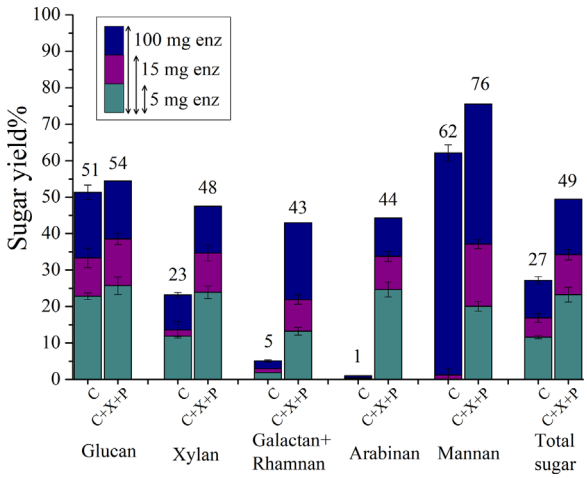

FIGURE 8 Individual and total sugar yields from enzymatic hydrolysis of tuber bagasse at loadings of 5, 15, and 100 mg enzyme protein per gram carbohydrate in the original dry bagasse with either cellulase alone (C) or a cellulase:xylanase:pectinase (C + X + P) cocktail in a 5:4:1 ratio after 120 hr of reaction

4 | DISCUSSION

The large drop in tuber production at the highest salinity level (12 dS/m) is consistent with results reported by Newton (Newton et al., 1991) and Dias (Dias et al., 2016). Irrigation water with EC_w = 9.3 and 12 dS/m corresponds to an EC_w of 3.97 and 5.7 dS/m, respectively, in the soil water extract (Cornacchione and Suarez, 2015; Dias et al., 2016). Soils with electrical conductivity of 4 dS/m are considered saline (Ali Harivandi, Butler, & Wu, 1992; Richards, 1954). As it has been recently shown, irritation of Jerusalem artichoke with low salinity water followed by high salinity water (sequential strategy) relieved salt stress on tuber productivity when tested for salinities up to that for an irrigation water EC of 9.3 dS/m. Thus, high tuber yields from this plant could be sustained on saline lands that cost less than crop land (Qadir & Oster, 2004). Also, the tuber yield drop for "Stampede" was lower than for "White Fuseau" at the same EC_w tested. This variation in tuber yield with salinity suggests that different cultivars will have different salinity tolerances and should be screened for optimum biomass and tuber production under salinity.

Roughly 15 kg of non-structural sugars from 100 kg of wet tuber or 55 kg per 100 kg dry matter with 70% as inulin could be recovered from "White Fuseau" Jerusalem Artichoke tubers. Considering a tuber yield of 82, 12.3 Mg/ha sugars could be produced from this cultivar. The wide variation in sugar yields indicates potential for optimization of growth-related factors to realize much higher sugar yields, as evidenced by the maximum total sugar yields of 65 kg per 100 kg dry

matter (19 kg based on 100 kg wet tuber mass) in this study. Dias et al.'s report (Dias et al., 2016) from US Salinity Laboratory on "Stampede" cultivar found that the free sugar (inulin + sucrose + glucose + fructose) content ranged from 56% to 72%, with an inulin content between 50% and 60% of dry matter. Thus, the lower total sugar and inulin yields from "White Fuseou" compared to "Stampede" cultivar indicate that there can be large differences in sugar yields between cultivars of this plant. These cultivars were randomly selected by US Salinity Laboratory to test for salinity tolerance and no systematic study exists to identify the best cultivars vis-à-vis total sugar yields. With over 300 existing clones and accessions of this plant (Kays & Nottingham, 2007), even higher sugar yields may be possible that would have tremendous value for renewable energy production worldwide.

Hydraulic pressing recovered juice containing high concentrations of inulin, sucrose, fructose, and glucose, with the resulting 27 g/L of glucose and 128 g/L of fructose released by acid hydrolysis at mild conditions having the potential to keep costs low by avoiding the need to concentrate sugars prior to fermentation. On the other hand, microbes such as *Kluveromyces* produce inulinase naturally that avoids the need for an acid hydrolysis and neutralization step as well avoids production of inhibitors such as 5-hydroxymethylfurfural formed by sugar degradation in acid (Bajpai & Bajpai, 1991). For example, Duvnjak, Kosaric, Kliza, and Hayes (1982) showed that 92% of the theoretical ethanol yield could be obtained in 50 hr from unhydrolyzed juice by *Kluveromyces fragilis*. In addition, 94% of the theoretical ethanol yield was achieved by continuous cultures of *Zymomonas mobilis* on enzymatically hydrolyzed inulin (100 g/L sugar substrate) (Allias, Torres, & Baratti, 1987). For yields of 12.3 metric tons of free sugar/ha (not including bagasse structural sugar), 7,951 L/ha or 850 gal/acre of ethanol (theoretical productivity) could be produced from this cultivar. For a comparison, the theoretical limit to ethanol productivity from corn in the USA and cane sugar in Brazil can be around 4,182 and 6,471 L/ha, respectively (Goldemberg & Guardabassi, 2010).

It was discovered that in spite squeezing the juice out of tubers, large quantity of sugar was retained in the bagasse that could be easily recovered with water at room temperature. In our laboratory, incubation of milled unwashed bagasse with ample water was carried out overnight at room temperature to be sure all sugars were accounted for. However, the amount of water can be optimized and added to bagasse after extraction of juice and squeezed again with a press to produce a second stream of sugars in high concentration. This method was not chosen in the laboratory as every pressing operation resulted in loss of material. The 7% mass of wet tubers lost in the hydraulic press was unavoidable as some juice stuck to its cylinder and small quantity of bagasse got lodged in the sieve plate. This loss could be very low in industrial operations as the press may not have to be cleaned between pressings unlike laboratory setting where cross-contamination between tubers was a concern. Because tubers oozed juice when they were severed, they were cut in transverse sections so their sugar content could be more fully accounted for. However, for large scale operations, the tubers could be chopped in smaller sections and a higher quantity of juice recovered, which translate into less free sugars remaining in the unwashed bagasse.

The composition of Jerusalem Artichoke tuber bagasse bore a striking similarity with another plant that is used to produce inulins commercially, chicory. However, chicory stores inulins in its roots, for which Panouillè (Panouillé, Thibault, & Bonnin, 2006) reported bagasse composition. The 21.9% glucan content in tuber bagasse was close to 23.2% from chicory root bagasse, and the xylan, mannan, galactan, rhamnan, and protein contents were only 1–2% different from that in chicory root bagasse. The 15% arabinan content in Jerusalem artichoke tubers was double that of chicory roots (7.2%). The relatively low Klason lignin content of tubers should benefit cellulosic ethanol production as high lignin contents can be detrimental to solubilization of structural sugars (Bhagia, Muchero, Kumar, Tuskan, & Wyman, 2016). It also provides an explanation for its successful use as an animal feed (Vhile, Kjos, Sørum, & Øverland, 2012) as well as dietary fiber for human consumption (Bach et al., 1995).

King and Bayley's study (King & Bayley, 1963) utilized Jerusalem artichoke tubers to understand cell wall plasticity related to pectin by inducing growth using plant hormones. Their compositional analysis of cell wall extracts excluded lignin, and artificial hormone-induced rapid tissue growth in the laboratory does not provide a direct comparison to our findings. Nevertheless, looking at the trends, both studies showed that the relative sugar content followed the order glucan > arabinan > galactan > xylan = rhamnan. However, the average mannan content of 0.7% of dry bagasse was lower than 5% mannan of cell wall extract of "water control" tissue in their work. Unlike sugar and lignin composition, pectin content has been previously quantified to be 18.52% (Liu et al., 2016).

Despite a low lignin content of roughly 12% dry weight of bagasse, total sugar yields from enzymatic hydrolysis indicated that hemicellulose and pectin contributed to recalcitrance that could be reduced by supplementation of cellulase with xylanase and pectinase. Insufficient breakdown of structural sugars in JA bagasse at low (5 mg) and medium (15 mg) enzyme loadings even after adding xylanase and pectinase suggests that a mild pretreatment is necessary to increase recovery of fermentable sugars. Liquid hot water and dilute acid pretreatments that remove much of the hemicellulose and pectin and change lignin structure increase the surface area of cellulose accessible to cellulases and enhance sugar yields (Bhagia, Li, et al., 2016). Since pectin is a valuable food product, it may be beneficial to recover it in pure form prior to breaking down structural sugars in bagasse. The bagasse can then be pretreated for hemicellulose recovery followed by enzymatic hydrolysis to produce soluble sugars that can be blended with free sugars pressed out first for high productivity ethanol production.

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CONFLICT OF INTEREST

CEW is the founding Editor-in-Chief of Biotechnology for Biofuels. The other authors declare that they have no competing interests.

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SUPPORTING INFORMATION

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