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Biomass characterization of *Agave* and *Opuntia* as potential biofuel feedstocks

Lisha Yang [a], *Mi Lu* [a], *Sarah Carl* [a], *Jesse A. Mayer* [b,1], *John C. Cushman* [b,1], *Elli Tian* [c], *Hongfei Lin* [a,*]

[a] *Department of Chemical and Materials Engineering, University of Nevada, Reno 1664 N. Virginia St., Reno, NV 89557, USA*

[b] *Department of Biochemistry and Molecular Biology, University of Nevada, Reno 1664 N. Virginia St., Reno, NV 89557, USA*

[c] *Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205, USA*

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ABSTRACT

Sustainable production of lignocellulosic biofuels requires a sufficient supply of biomass feedstocks. *Agave* and *Opuntia* represent highly water-use efficient bioenergy crops that are suitable for expanding feedstock production into semi-arid marginal lands. These feedstocks have garnered interest as dedicated biofuel feedstocks because of their high water- and fertilizer-use efficiency and not competing with major food crops or conventional biofuel feedstocks. To better understand the potential of these feedstocks, the biomass composition of *Agave tequilana* and *Opuntia ficus-indica* was analyzed. Previous extraction procedures and analytical methods have led to variable estimates of the chemical compositions of the biomass of these species. Therefore, National Renewable Energy Laboratory (NREL) standard methods were used in the present study. *A. tequilana* showed higher mass fractions of water-soluble constituents, structural carbohydrates, cellulose, hemicellulose, and lignin than *O. ficus-indica*. In contrast, *O. ficus-indica* had higher protein, water, and ash mass fractions than *A. tequilana*. Both species had lower lignin mass fractions, thus yielding lower heating values, but had higher water and ash mass fractions than most woody biomass feedstocks. The high water mass fractions of these species (85–94%) could prove advantageous for biomass deconstruction and aqueous phase catalytic conversion processes as less exogenous water inputs would be needed. Lastly, solid-state NMR analysis revealed that both *A. tequilana* and *O. ficus-indica* had high amorphous and paracrystalline cellulose mass fractions (>80%), indicating that these biomass feedstocks would be far less recalcitrant to deconstruction than traditional lignocellulosic biomass feedstocks.

* *Corresponding author*. Tel.: +1 775 784 4697.
E-mail address: HongfeiL@unr.edu (H. Lin).
[1] Fax: +1 775 327 5059.

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1. Introduction

With the gradual depletion of fossil fuels, the demand for renewable biomass-based carbon resources to use for lignocellulosic biofuels is expected to increase in the future [1,2]. However, most biomass feedstocks currently used for biofuels production compete with the use of those feedstocks for food [3,4]. Competition for arable land for growing biofuel feedstocks and food can be avoided, in part, by the development of drought-tolerant biomass feedstocks that can be grown in an environmentally responsible manner on semi-arid, marginal, degraded, or abandoned agricultural lands where food crops are typically not cultivated, with an estimated mean productivity of 4.3 Mg ha^{-1} y^{-1} [4–6]. Furthermore water availability is the major factor that constrains the cultivation of bioenergy crops [7]. One potential opportunity for the production of biomass feedstocks on water-limited areas is to use various cultivated crassulacean acid metabolism (CAM) species from the genera *Agave* (Agavaceae) and *Opuntia* (Cactaceae, "prickly pear cactus"), which have growth characteristics that allow these species to thrive in semi-arid regions [8].

CAM plants are typically highly tolerant of high temperatures, UV-B radiation, and drought conditions by virtue of their ability to rapidly take up and store water in above-ground succulent tissues, while tolerating large losses in water mass fraction [8,9]. The high water-use efficiency of CAM species, typically 3- to 10-fold higher than that of C_3 or C_4 species, results in crop water demands that average only 16%–28% of those of C_3 and C_4 crops, respectively, while maintaining comparable above-ground biomass productivities [8,10]. For example, average annual productivity values for various *Agave* species can range from <1 to 34 Mg ha^{-1} y^{-1} under ambient precipitation conditions [4,11–13]. Under cultivated conditions with supplemental irrigation, average annual dry-weight productivities can reach as high as 38–42 Mg ha^{-1} y^{-1} [10,14–16]. Under rain-fed conditions, average annual productivity for *Opuntia* species are about 15 Mg ha^{-1} $year^{-1}$ or more [17]. However, under well-irrigated conditions, average annual dry matter productivities in the range of 40–50 Mg ha^{-1} $year^{-1}$ have been reported [10,14–16,18]. Such biomass production rates are comparable with those of other bioenergy feedstocks such as maize, sugarcane, switchgrass, and poplar [4,8,12].

Although native to the Americas, *Agave* species have been introduced worldwide for commercial production in many countries including Australia, Brazil, Tanzania, Kenya, Madagascar, Mexico, China, and across the Caribbean and Mediterranean regions [11,12]. Although more information is needed from field trials to model the biomass production associated with the water-wise feedstocks [19,20], productivity estimates from immediately available land suggest that an additional 6.1 hm^3 of lignocellulosic ethanol production could be produced with minimal impacts on the environment [11]. Similarly, *Opuntia* originates from the Americas with its center of diversity in Mexico [21], but it has been introduced worldwide with major production occurring in Algeria (and other northern African nations), Brazil, Chile, Mexico, and Italy [22,23]. *Opuntia* species are cultivated primarily for commercial fodder and forage in semi-arid regions worldwide [24,25]. However, tender, young cladodes and fruits from *Opuntia* species are consumed by humans, primarily in Mexico, the southwestern US, and Italy [26–28]. The young cladodes and fruits are also dried and sold as dietary supplements [29,30], in cosmetic formulations [29], and for medicinal use [31–33]. The fruits can be consumed as fresh or made into a variety of jams, jellies, sauces, marmalades, candies, syrups, juices, liquor, and as a natural sweetener due to the high sugar mass fraction (>50%) of the of the fruit syrup [34].

Due to their high mass fraction of water-soluble sugars, many different *Agave* species are currently used for production of alcoholic beverages, such as tequila (*Agave tequilana*) mescal or pulque (*Agave mapisaga*, *Agave salmiana*, *Agave Americana*, *Agave fourcroydes*, and others), aquaamiel (honey water), nectar or syrup sweeteners. Some of these species are also used for fiber production (e.g., *Agave sisalana*, *A. fourcroydes*) [7,8,11,12,35–37]. *Agave* bagasse as a raw material can be used for animal feed, fiberboard production, and other by-products [26,38]. Following extraction of sugars for fermentation to produce mezcal, the bagasse and waste fiber from *A. salmiana* can be used as a renewable energy source for combustion [39]. The cladodes of *Opuntia* cactus are also a potential biomass feedstock for bioethanol production [40]. *Agave* would be comparable to or superior to other ethanol feedstocks, such as maize, switchgrass, and sugarcane, for bioethanol production in terms of life cycle energy and greenhouse gas (GHG) balances [19], while being far more water-use efficient than these crops [41].

The efficient conversion of lignocellulosic biomass into renewable biofuels and value-added chemical compounds is limited by the recalcitrance of plant cell wall material to degradation [42]. Deconstructive pretreatments of raw lignocellulosic biomass account for a majority of the costs associated with lignocellulosic biofuel production [3]. In order to optimize such pretreatments, the composition of lignocellulosic biomass feedstocks must be known. Among plant cell wall biopolymers, lignin is the major cause of recalcitrance to hydrolysis [43,44]; thus, reducing lignin mass fraction or altering its structure are important goals for overcoming this recalcitrance and improving saccharification [45]. For most woody feedstock species, the lignin mass fraction is approximately 9–30% [13,45], whereas for *Agave* and *Opuntia*, the lignin mass fraction are estimated to be lower, in the range of 5–16% depending on the species and technical approaches used [12,13,44,46–48]. However, the readily fermentable, water-soluble carbohydrate (WSC) fraction of *Agave* leaves was comparable to that of conventional lignocellulosic feedstocks, such as sugarcane bagasse and corn stover [38,49]. *Opuntia* stems exhibit similar percentages (3–7% fresh mass fractions) of such carbohydrates [33]. Depending on the study, the compositions of *Agave* and *Opuntia* feedstocks can vary considerably. Such variation might arise from the diverse species, plant or leaf age, growth conditions, and the analytical methods used. In order to obtain more reliable information about the composition and chemical structures of *A. tequilana* and *Opuntia ficus-indica*, standardized National Renewable Energy Laboratory (NREL) analytical methods were used to determine the ash, protein, extractives, lignin, hemicellulose, and cellulose mass fraction, as well as the chemical structures of isolated cellulose, of sampled materials. Such

information is critical for the optimization of strategies for conversion of these feedstocks to renewable biofuels.

2. Materials and methods

2.1. Plant cultivation

O. ficus-indica ((L.) Mill.) and *A. tequilana* [Weber var. *azul*] were grown at the Nevada Agricultural Experiment Station Valley Road Greenhouse Complex in Reno, NV. *Opuntia* and *Agave* were planted in 8-L and 19-L pots, respectively, containing Metromix® 200 soilless mix (Sun Gro Horticulture, Bellevue, WA, USA). Plants were maintained under standard greenhouse conditions with natural light at approx. 1100–1500 μmol m^{-2} s^{-1} and temperature at 28–32 °C day/17–18 °C night. Watering was performed twice a week along with monthly fertilization (Miracle Gro®, Scott's MiracleGro Inc., Marysville, OH, US) and insecticide treatments (Marathon® 1% Granular, OHP, Mainland, PA, US).

2.2. Materials

Mature, fresh *O. ficus-indica* (approximately 1 year old) cladodes and *A. tequilana* (approximately 2 years old) leaves were harvested between 9 and 10 AM from plants maintained in the greenhouse, were snap-frozen and stored in liquid nitrogen, and were transported to the laboratory where they were stored at −80 °C until processing. For chemical analysis and derivatization, the following reagents and products were purchased from Sigma–Aldrich (St. Louis, MO): D-(+)-xylose (99%), xylan from beechwood (90%), glucose (European Pharmacopoeia (EP) Reference Standard), galactose (United States Pharmacopeia (USP) Reference Standard), mannose (United States Pharmacopeia (USP) Reference Standard), D-arabinose (≥99.0%), Bis (trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) (99:1), anhydrous pyridine (99.8%), sulfuric acid (ACS reagent 98%), hydrochloric acid (~36.5–38.0%), ethanol (>99.5%), sodium chlorite (80%), hexane (95%), glacial acetic acid (>99.7%), formic Acid (≥95%) phenol (Tris-buffered, pH = 8.0), and dense SDS buffer (30% sucrose, 2% SDS, 0.1 mol L^{-1} Tris–HCl, pH 8.0, 5% 2-mercaptoethanol).

2.3. Sample preparation

Fig. 1 depicts the flowchart of the major steps used to prepare the samples for analysis. The leaves were first cut into strips, then juice was expelled from the material by placing it in a metal pipe and forcing a tight-fitting metal cylinder through the pipe using a 4350 Carver hydraulic press (Carver Inc., Wabash, IN). The juice was stored in 50 cm^3 polypropylene centrifuge tubes at 4 °C until further analysis. The unwashed bagasse solids were freeze-dried, then milled into particles with an average size of 300 μm in diameter. The water mass fraction and the amount of total solids remaining after 105 °C drying of the biomass sample were determined by the standard National Renewable Energy Laboratory (NREL) laboratory analytical procedure [50]. All the analytical data reported were the mean values of three biological replicates (±standard deviation from the mean).

2.4. Elemental analysis

Elemental analysis was performed using the dried, ground samples by Galbraith Laboratories, Inc. (Knoxville, TN). Carbon, hydrogen, and oxygen mass fractions were determined using the Perkin Elmer 2400 Series II CHNS/O Analyzer. Sulfur determination was performed using a LECO SC-432DR Sulfur Analyzer and determination of Cl^- anions was performed by suppressed ion chromatography. Energy-dispersive X-ray spectroscopy (EDS) analysis was performed using a Hitachi S-4700 II Scanning Electron Microscope (Hitachi High Technologies America, Inc., Schaumburg, IL).

2.5. Extractive, ash, protein, and dry bagasse analysis

The ash mass fraction of the samples from each plant species was measured using the standard NREL analytical procedures for biomass analysis [51]. Each of the samples were extracted with water and ethanol, respectively, for the determination of extractives [52]. The crude protein mass fraction was estimated using a universal phenol-based protein extraction method [53]. Structural carbohydrates and acid insoluble lignin mass fraction for the extractive-free bagasse from *A. tequilana* and *O. ficus-indica* were determined using the standard NREL laboratory analytical procedures [54].

2.6. Juice analysis

The juices were analyzed using a Shimadzu high performance liquid chromatograph (HPLC) (Model LC-10; Shimadzu Scientific Instruments, Columbia, MD) equipped with a UV–VIS Detector (Shimadzu SPD 10-AV), a Refractive Index Detector (Shimadzu RID-10A), and a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Inc., Hercules, CA) using DI water as the mobile phase, at a flow rate of 0.6 cm^3 min^{-1} and a column temperature of 80 °C. For electrospray ionization mass spectrometry (ESI/MS) measurements, the juice samples were analyzed using a Waters e2695 separation module (Waters Corporation, Milford, MA) operated in the negative mode. Free sugar analysis was performed according to the NREL method [55]. The derivatization of the polar components in the juice was performed for qualitative gas chromatography-mass spectrometry (GC-MS) analysis to identify unknown components. The samples were analyzed on an Agilent 6890 series gas chromatograph-mass spectrometer (GC-MS) equipped with an Agilent DB5-MS column (30 m × 0.25 mm ID, 0.25 μm film thickness) and an Agilent 5973 Mass Selective Detector (Agilent Technologies, Inc., Santa Clara, CA). The column temperature was maintained at 80 °C for 5 min then ramped at 10 K min^{-1} to 260 °C and held at 260 °C for 2 min. Additionally, the concentrations of total soluble solids (TSS) in sample juices were determined by pipetting 10 cm^3 of juice that had been passed through a 0.45 μm filter into pre-dried and pre-weighed aluminum weighing dishes, and drying at 100 °C for 24 h in an oven until a constant weight was reached.

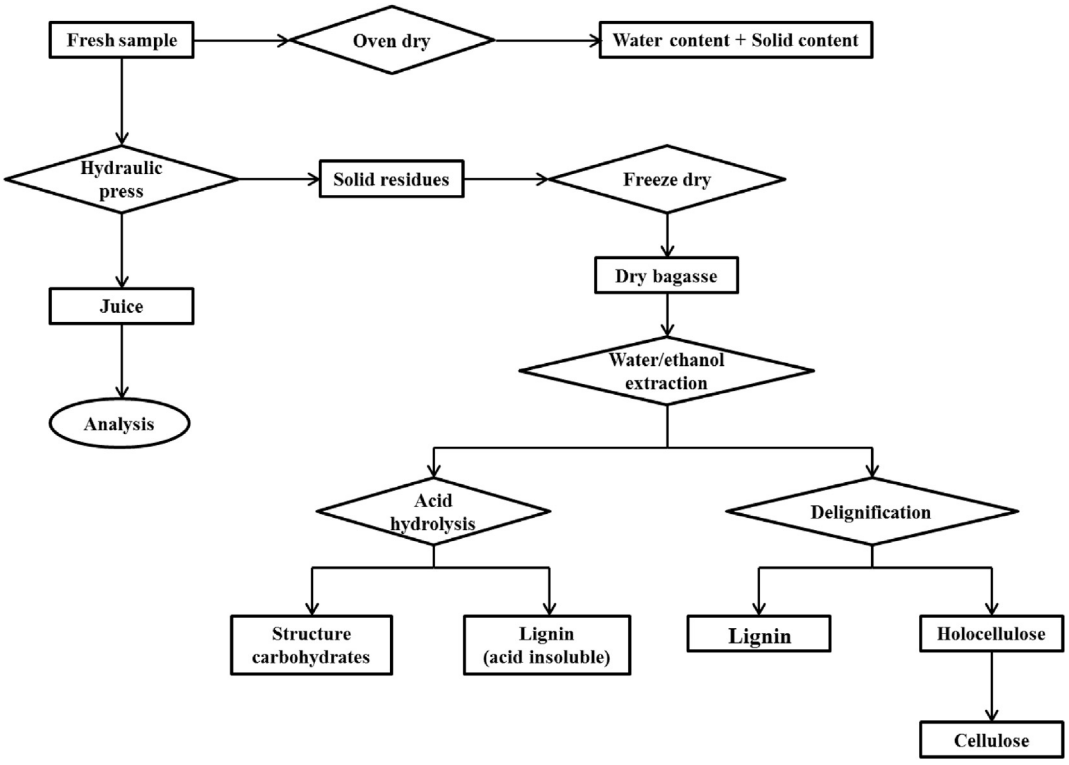

Fig. 1 – Scheme of the processing steps for the compositional analysis of *Agave tequilana* and *Opuntia ficus-indica* samples.

2.7. Higher heating value analysis

The higher heating values (HHV) of *O. ficus-indica* and *A. tequilana* based on dry bagasse was measured by combusting the samples in an adiabatic oxygen bomb calorimeter (Model 6200 Isoperibol Calorimeter, Parr Instrument Company, Moline, IL) according to the ASTM D5865 method.

2.8. Holocellulose fractionation

The holocellulose (cellulose + hemicellulose) samples were conducted according to a modified method in the literature [56].

2.9. Solid state NMR analysis

Cellulose was isolated by refluxing a holocellulose sample (1.0 g dry weight) in the 2.5 mol dm^{-3} HCl (100.00 cm^3) solution according to the method of Hallac et al. [56]. The CP/MAS ^{13}C NMR analysis of the cellulose samples was performed using 2-channel 400 MHz Tecmag Discovery instrument (Tecmag Inc., Houston, TX). A NMR Service GmbH MAS H-X broadband probe was used. The samples were packed in a 4 mm ZrO_2 rotor. The MAS rate was 9 kHz. Acquisition was performed with a proton decupling pulse sequence using a 6 μs carbon 90° pulse. A 10-s delay between pulses was used. After acquisition, the C-4 region of each spectrum was fitted and the quantification of the different cellulose forms was calculated based on the time dependence of the fitted signal intensities [57].

3. Results and discussion

3.1. Elemental analysis

The relative elemental percentages of *A. tequilana* and *O. ficus-indica* were determined based on the oven-dried samples. Elemental mass fractions were similar for both *A. tequilana* and *O. ficus-indica* (Table 1). However, *A. tequilana* displayed a slightly higher C mass fraction than did the sample from *O. ficus-indica*. These values are similar to the C, H, O mass fractions of herbaceous and woody biomass feedstocks, which typically have elemental compositions of 45–50% C, 6–7% H, 40–46% O, and trace amounts of several metal ions [58,59]. Notably, sulfur (S) and chlorine (Cl) mass fractions were higher in *O. ficus-indica* than in *A. tequilana*, which might be a consideration for air pollution impacts. Additionally, such elemental mass fraction data are important information prior to developing procedures for processing biomass into biofuels, because elemental mass fractions might affect subsequent processes. Sulfur (S) and chlorine (Cl), for example, can both cause catalyst poisoning and reactor corrosion [60].

3.2. Compositional analysis

A detailed compositional analysis of the leaves of *A. tequilana* and the modified stems (cladodes) of *O. ficus-indica* was performed according to the processing scheme outlined in Fig. 1. The mass fraction distributions of the components in the samples are summarized in Table 2. *A. tequilana* contained a 4-fold higher mass fraction of dry bagasse (10.7%) than did *O.*

Table 1 – Elemental analysis and higher heating values of *Agave tequilana* and *Opuntia ficus-indica*.

Sample	Dried and ground[a]/atomic mass fractions (%)					Higher heating values (MJ kg^{-1})[b]
	C%	H%	O%	S%	Cl%	
Agave tequilana	42.8 ± 1.1	5.9 ± 0.2	39.9 ± 1.4	0.19 ± 0.007	0.09 ± 0.001	17.50 ± 0.09
Opuntia ficus-indica	35.1 ± 1.1	4.4 ± 0.3	41.3 ± 0.5	0.33 ± 0.006	0.81 ± 0.09	16.95 ± 0.04

[a] Data reported are the mean values of three replicates ± standard deviation from the mean.
[b] Heating values reported are the mean values of three replicates based on water/ethanol extracted bagasse ± standard deviation from the mean.

Table 2 – Mass fraction distribution of fresh *Agave tequilana* and *Opuntia ficus-indica* samples (%, wet biomass basis).

Sample[a]	Dry bagasse	Juice	TSS[b] in juice	Water	Total solids
Agave tequilana	10.7 ± 0.7	89.3 ± 0.7	11.9 ± 2.8	84.9 ± 0.6	15.1 ± 0.6
Opuntia ficus-indica	2.6 ± 0.2	97.4 ± 0.2	3.8 ± 0.3	93.9 ± 0.4	6.1 ± 0.4

[a] Data reported are the mean values of six replicates ± standard deviation from the mean.
[b] TSS: total soluble solids.

ficus-indica (2.6%). In contrast, *O. ficus-indica* contained a slightly higher juice mass fraction (97.4%) compared with *A. tequilana* (89.3%), likely reflecting the higher water content of *O. ficus-indica*. However, *A. tequilana* displayed a higher total soluble solid fraction (11.9%) in the juice and higher total dry matter (15.1%) compared with *O. ficus-indica*. These values are similar to those previously reported for *A. tequilana* leaves by Li et al. [48] and account for the suitability of this species for fermentation to alcoholic beverages and high ethanol conversion efficiency [11].

3.2.1. *Compositional analysis of juice*

The electrospray ionization mass spectrometry (ESI-MS) was used to identify the major constituents of the juice. The sugars and other water-soluble organic compounds in the samples appear in the total ion chromatogram between m/z 40 and 200 (Fig. 2). The identification of these compounds was validated by GC/MS analysis (Fig. 3). In *A. tequilana*, the major soluble monosaccharides were glucose and fructose (Table 3), which is consistent with earlier observations [48]. The water-soluble monosaccharide mass fraction of *O. ficus-indica* was much lower than that of *A. tequilana*, indicating that to be useful as a biofuel feedstock, additional physiochemical pretreatments or microbial digestion of this material would be needed to improve its conversion efficiency. The juice from both species displayed weakly acidic pH values (Table 3) consistent with the performance of crassulacean acid metabolism (CAM) and the associated nocturnal carbon fixation into malate, which is stored in the vacuole as malic acid [8]. As shown in Table 3, both *A. tequilana* and *O. ficus-indica* have relatively high malic

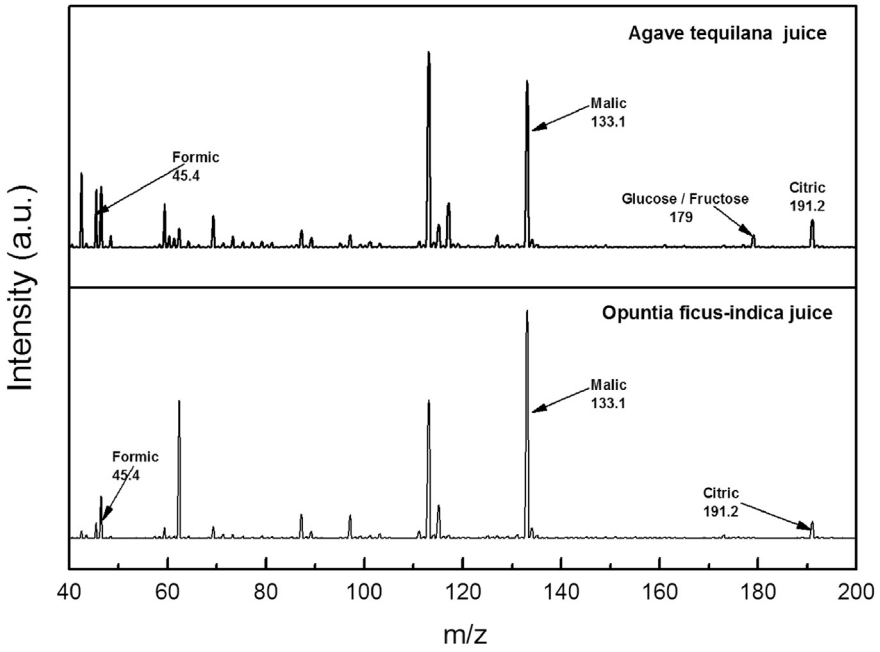

Fig. 2 – ESI/MS spectra of *Agave tequilana* (upper panel) and *Opuntia ficus-indica* (lower panel) juice.

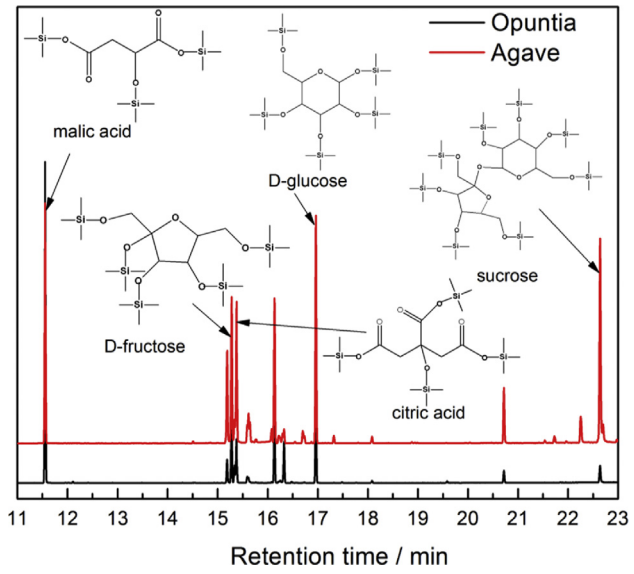

Fig. 3 – GC/MS spectra of *Agave tequilana* (red trace) and *Opuntia ficus-indica* (black trace) juices. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

acid when compared with other species. A relatively high citric acid was also found in *A. tequilana* and *O. ficus-indica* juice. In CAM plants, accumulation and storage of these organic acids occur at night as the products of primary CO_2 uptake and fixation when the leaf-to-air vapor pressure difference is lower than that during the day, which improves the water-use efficiency of these plants [61]. The stored malic acid is decarboxylated and used as a substrate for daytime photosynthesis *via* the Calvin–Benson cycle to generate starch or other storage glucans behind closed stomata.

3.2.2. *Compositional analysis of bagasse*

The solid, freeze-dried residue (bagasse) remaining after hydraulic extraction of the juice was milled and mixed thoroughly to ensure representative samples before being subjected to further mass balance analysis following sequential water and ethanol extractions, acid hydrolysis, and delignification (Fig. 1). The previous reports of the compositions of *A. tequilana* and *O. ficus-indica* varied widely due to different plants or leaf ages, growth conditions, and the analytical methods used [12]. Thus in our study, a comprehensive compositional analysis of the residual dry matters was performed (Table 4). *A. tequilana* showed a 4-fold higher mass fraction of dry bagasse than *O. ficus-indica* (Table 2). The mass fractions of combined water and ethanol extractives of the dried material were higher than published reports for both *A. tequilana* leaves and *O. ficus-indica* cladodes, whereas the structural carbohydrate mass fractions were similar for *A. tequilana* leaves [48], but more variable for *O. ficus-indica* cladodes [40,62] (Table 4). The amount of extractives shown in Table 4 was calculated by subtracting the amount of soluble ash.

The lignin mass fraction of *A. tequilana* leaves (13.8%) was similar to that of *O. ficus-indica* cladodes (12.3%) (Table 4). The lignin mass fraction of *A. tequilana* leaves was *on par* with values reported previously by Iñiguez-Covarrubias et al. and Li et al. [26,48]. Likewise, the lignin mass fraction of *O. ficus-indica* cladodes was similar to the value reported by Mciteka [63], lower than the value of the field-grown plants reported by Ginestra et al. [62], yet higher than that reported by Kuloyo [40]. Overall, the lignin mass fractions in *A. tequilana* and *O. ficus-indica* dry bagasses were generally comparable to the values reported for herbaceous feedstocks (9–18%; e.g., *Miscanthus*, switchgrass, and corn stover) and substantially lower than those for woody feedstocks (21–32%; e.g., poplar, eucalyptus, and pine) [45]. Feedstocks with reduced lignin mass fractions might display reduced recalcitrance during pulp and paper production and lignocellulosic biofuel production [45]. Structural carbohydrate mass fractions were found to be 43.8% and 36.6% for dry *A. tequilana* leaves and *O. ficus-indica* cladodes, respectively.

In order to evaluate the mass fractions of cellulose and hemicellulose, which comprise the principal structural carbohydrates in the dry matter, holocellulose (cellulose + hemicellulose) was first separated from lignin. The cellulose mass fraction of *A. tequilana* (26.0%) was twice than that of *O. ficus-indica* (13.1%), whereas the hemicellulose mass fractions were 22.8% and 18.5%, respectively (Table 4). The lower holocellulose mass fraction of *O. ficus-indica* can likely be explained in part by the higher ash and protein mass fractions of this species. The high water mass fractions of *A. tequilana* (84.9%) and *O. ficus-indica* (93.9%) (Table 2) suggest that these feedstocks might be suitable for our recently reported catalytic aqueous phase process for direct conversion of cellulose and hemicellulose into levulinic acid and lactic acid, respectively [64,65]. This process converts lignocellulosic biomass into organic acids using water as an environmentally benign reaction media, with heterogeneous catalysts such as ZrO_2. The high endogenous water mass fractions of these species would reduce water consumption during the lignocellulosic processing steps. Aside from the production of biofuels, the production of high-value chemical precursors, such as levulinic and lactic acids, which can be used as bio-based chemical intermediates in the production of other useful compounds [66,67], indicates that these arid-land feedstocks might serve as useful biomass sources for a variety of biosynthetic reactions.

O. ficus-indica had a 3.9-fold higher ash mass fraction (23.7%) than *A. tequilana* (6.0%) (Table 4), due primarily to the

Table 3 – pH and composition of sample juices (g dm^{-3}).

Samples[a]	pH	Malic	Citric	Formic	Glucose	Galactose	Fructose
Agave tequilana	4.74 ± 0.002	35.3 ± 0.3	9.5 ± 0.1	39.0 ± 0.2	18.1 ± 0.1	0.7 ± 0.2	16.8 ± 0.3
Opuntia ficus-indica	4.17 ± 0.001	10.9 ± 0.7	3.9 ± 0.1	/	1.0 ± 0.01	0.1 ± 0.005	1.6 ± 0.01

[a] Data reported are the mean values of three replicates ± standard deviation from the mean.

Table 4 – Composition of *Agave* leaf and *Opuntia* cladode bagasse reported in literature and the present study – mass fraction of dry material (%).

Species	Extractives	Structure carbohydrate	Lignin	Cellulose	Hemi-cellulose	Ash	Protein
Agave tequilana (Li et al., 2012) [48]	21.8	41.7	11.9	n/a	n/a	6.4	5.6
Agave tequilana (Iñiguez-Covarrubias et al., 2001) [26]	14	n/a	15.9	64.8	5.1	1.0	n/a
Agave tequilana [a] **(present study)**	**29.0 ± 1.2**	**43.8 ± 1.3**	**13.8 ± 1.3**	**26.0 ± 1.2**	**22.8 ± 1.2**	**6.0 ± 0.1**	**2.4 ± 0.1**
Opuntia ficus-indica [b] (Mciteka, 2008) [63]	n/a	n/a	11.8	6.8	9.1	22.5	5.5
Opuntia ficus-indica [c] (Ginestra et al., 2009) [62]	17.7	26	16	n/a	n/a	n/a	6.42
Opuntia ficus-indica (Kuloyo, 2012) [40]	24.3	42	7.9	13.5	n/a	16.8	7.5
Opuntia ficus-indica [a] **(present study)**	**25 ± 0.9**	**36.3 ± 1.1**	**12.3 ± 1.1**	**13.1 ± 0.7**	**18.5 ± 0.7**	**23.7 ± 0.1**	**7.4 ± 0.3**

The data in bold are our original datacompared to the data inliterature.
[a] Data reported are the mean values of three replicates ± standard deviation from the mean.
[b] Mean of six different varieties.
[c] Mean of three different varieties.

accumulation of inorganic ions and salts, such as Ca^{2+}-oxalate crystals [68]. The ash mass fraction of *A. tequilana* and *O. ficus-indica* was relatively high compared to that of woody biomass feedstocks, which are in the 1–3% range [59]. However, these ash values are similar to those for herbaceous feedstocks, such as *Miscanthus*, reed canary grass, wheat or rice straw, or hay, which can range from 2% to 21% [58,59]. EDS analysis demonstrated that pre-extraction ash was comprised mainly of the monovalent cations potassium (K^+) and sodium (Na^+), and the divalent cations calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the following order of relative abundance in both species (Ca^{2+} >> K^+ > Mg^{2+} > Na^+) (Table 5). Notably, *A. tequilana* had more than 6-fold higher Na^+ mass fraction than *O. ficus-indica*. However, after water and ethanol extraction, most of the K^+, Na^+, sulfur (S), and phosphorus (P) were removed, whereas only Ca^{2+}, Mg^{2+}, and manganese (Mn^{2+} or Mn^{3+}) were retained (Table 5). The high quantitative estimation of ash mass fraction and their relative alkalization potential from alkali chloride deposits is a critical concern because ash can potentially cause corrosion and slagging problems during bioprocessing [69]. However, for subsequent bioprocessing of *A. tequilana* and *O. ficus-indica* into a biofuel, these inorganic elements represent a waste stream that could be recycled and reused as fertilizers.

The protein mass fraction of *A. tequilana* (2.4%) was 3-fold lower than that of *O. ficus-indica* (7.4%) (Table 4) and was lower than the value previously reported [48]. For *O. ficus-indica*, the protein mass fraction estimate was similar to the value reported by Kuloyo [40] and higher than values reported earlier [62,63]. The removal of proteins might be necessary to avoid possible deactivation of catalysts during the biofuel production, which, however, would likely increase processing costs.

3.3. Higher heating value analysis of dry bagasse

The higher heating values (HHVs) of the dry bagasse constituents were measured to assess total energy values. *A. tequilana* leaves and *O. ficus-indica* cladodes had the HHVs of 17.50 MJ kg^{-1} and 16.95 MJ kg^{-1}, respectively (Table 1). These values were lower than those typical for most softwood or hardwood species, which tend to be close to 20 MJ kg^{-1} [70]. The heating value of a plant biomass increases with higher lignin and extractive mass fractions [71]. Thus, the lower heating values observed here were consistent with the relatively low lignin mass fractions measured for both species.

3.4. Cellulose characterization

One of the major barriers to biofuels production from lignocellulosic feedstocks is the recalcitrance of crystalline cellulose to hydrolysis [72]. Thus, pretreatment of lignocellulosic feedstocks is often required to alter the biomass structure in order to make the cellulose more accessible [73]. One of the primary goals of pretreating biomass is to reduce its recalcitrance by degrading the crystalline structure of cellulose. In order to investigate the relative amounts of various crystalline cellulose allomorphs and fibril surface characteristics, CP/MAS ^{13}C NMR analysis was performed on isolated cellulose from *A. tequilana* and *O. ficus-indica*. This analysis was performed by fitting one Gaussian and three Lorentzian line curves to the C_4 signals at δ 75–100 ppm [74], resulting in a 7-peak non-linear line-fit spectrum of the cellulose C_4 region (Fig. 4). The signal assignments for cellulose I_α, I_β, and *para*-crystalline cellulose domains are summarized in Table 6. Additionally, the non-crystalline cellulose C_4 region signals at around δ 80–95 ppm, which is associated with accessible and inaccessible cellulose fibril surfaces, was simultaneously fitted to three Gaussian line-curves. Celluloses I_α and I_β are two natural forms of highly ordered crystalline cellulose (Type I) that are believed to be the most difficult to deconstruct [75]. In contrast, *para*-crystalline cellulose, which has a disordered crystalline structure, is described as a cellulose allomorph with features intermediate to those of crystalline and amorphous cellulose in both chain order and mobility [76]. *Para*-crystalline cellulose is much more readily hydrolyzed than crystalline cellulose [77]. Based on the CP/MAS ^{13}C NMR

Table 5 – Ash-forming elements of *Agave tequilana* and *Opuntia ficus-indica* before and after extraction with water and ethanol measured by EDX spectroscopy.

	Ash of *Agave tequilana*						Ash of *Opuntia ficus-indica*					
	Before extraction			After extraction			Before extraction			After extraction		
	Element	Mass fraction, %	Atomic mass fraction, %	Element	Mass fraction, %	Atomic mass fraction, %	Element	Mass fraction, %	Atomic mass fraction, %	Element	Mass fraction, %	Atomic mass fraction, %
a		9	13	Mg	14	21	Na	1	2	Mg	20	29
Mg		11	16	Ca	86	79	Mg	12	18	Ca	78	70
Ca		57	50	Totals	100	100	Ca	45	40	Mn	2	1
P		3	3				Mn	1	1	Totals	100	100
S		1	1				Cl	1	1			
K		19	17				P	3	4			
Totals		100	100				S	2	2			
							K	34	32			
							Totals	100	100			

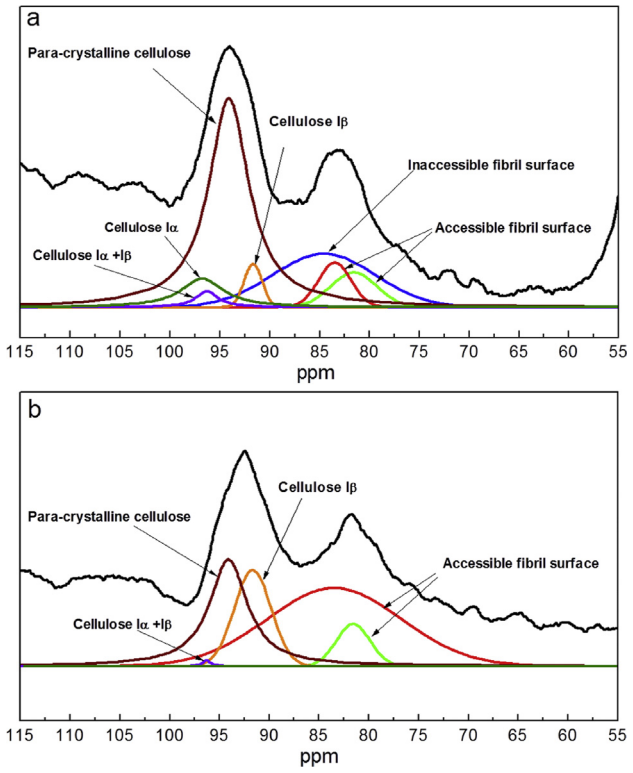

Fig. 4 – Spectral fitting for the C-4 region of CP/MAS ^{13}C NMR spectra of isolated cellulose of *Agave tequilana* (a) and *Opuntia ficus-indica* (b).

analysis (Table 6), *A. tequilana* displayed 50.8% *para*-crystalline cellulose mass fraction. This value is much higher than those found in woody biomass, such as loblolly pine (24.8%) and poplar (31.1%) [72,78], and switchgrass (32.7%) [79]. Additionally, ordered crystalline celluloses in *A. tequilana* were relatively low in mass fractions, with I_α of 7.6% and I_β of 3.3%. The mass fraction of amorphous cellulose in the total cellulose of *A. tequilana* was 36.2%. For *O. ficus-indica*, the mass fraction of *para*-crystalline cellulose was 27.2%, lower than that in *A. tequilana*. The I_β of *O. ficus-indica* is a little higher at 16.6%; however, the amorphous cellulose mass fraction was as high as 55.9%. The resultant compositional analysis indicates that cellulose from *A. tequilana* and *O. ficus-indica* is likely to be far less recalcitrant to deconstruction than cellulose derived from herbaceous or woody biomass feedstocks. However, further investigation of the physiochemical pretreatments and enzymatic hydrolysis of these feedstocks is needed to determine the optimal conditions for deconstructing them prior to saccharification or other biofuel conversion processes.

4. Conclusion

The arid-land adapted, highly water-use efficient CAM species *A. tequilana* and *O. ficus-indica*, which are unique biomass species compared to other C_3 and C_4 plants, were characterized by series of standard biomass analytical procedures. Both *Agave* and *Opuntia* contained a high amount of water at 84.9% and 93.9%, respectively. In addition, carboxylic acids and simple

Table 6 – Assignments of signals in the C_4 region of CP/MAS ^{13}C NMR spectra obtained from isolated cellulose of *Agave tequilana* and *Opuntia ficus-indica*.

Assignment	Chemical shift (ppm)	FWHH[c,a] (ppm)	Intensity[a]	FWHH[c,b] (ppm)	Intensity[b]	Line type
Cellulose I_α	96.8	5.25	7.6%	2.16E+144	0.0%	Lorentzian
Cellulose $I_{\alpha+\beta}$	96.3	2.73	2.2%	0.87	0.3%	Lorentzian
Cellulose I_β	91.7	2.14	3.3%	4.35	16.6%	Lorentzian
Para-crystalline cellulose	94.1	4.90	50.8%	4.59	27.2%	Gaussian
Accessible fibril surface	83.5	3.89	6.2%	15.83	49.2%	Gaussian
Inaccessible fibril surface	84.6	12.17	23.2%	0.20	0.0%	Gaussian
Accessible fibril surface	81.6	5.47	6.8%	4.01	6.7%	Gaussian

[a] *Agave tequilana*.
[b] *Opuntia ficus-indica*.
[c] Full width at half-height.

sugars were found to be the major constituents of freshly expelled juice. *A. tequilana* and *O. ficus-indica* dry bagasses possessed structural carbohydrate mass fractions of 43.8% and 36.3%, respectively, with low relative lignin mass fractions of 13.1% and 12.3%, respectively. The higher heating values observed here for both species were lower than those for woody biomass due to the relatively low lignin mass fractions. Moreover, the amorphous and para-crystalline cellulose fractions accounted for over 80% of the total cellulose in both species. The low lignin mass fraction and low cellulose crystallinity indicated that bagasse from both *Agave* and *Opuntia* might be more readily deconstructed into fermentable sugars than biomass from traditional herbaceous or woody feedstocks. Although, the relatively high protein and ash mass fractions of these feedstocks present chemical engineering challenges to effect optimal strategies for their handling and processing into biofuels, their high water mass fractions might make them especially attractive for aqueous phase processes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2015.03.004.

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