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Chemical Composition and Characterization of Cellulose for Agave as a Fast Growing, Drought Tolerant Biofuels Feedstock

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Abstract

A major issue raised about development of cellulosic biomass derived fuels technologies is the concern about possible competition for land with agricultural crops and impacts on food and feed supply. However, because agave offers high productivity with low water and nutrient demands, it can thrive on semiarid lands not suitable for conventional agriculture, making it a promising lignocellulosic feedstock for biofuels production. Because agave composition will establish the maximum potential fuel yield that is vital to low cost conversion, detailed chemical composition data and cellulose characteristics were measured by standard biomass analysis procedures and solid-state NMR methods, respectively, for four agave samples: A. *americana* leaves, A. *salmiana* leaves, A. *tequilana* leaves, and A. *americana* heart. For the first time, we report substrate characteristics relevant to biochemical conversion for the tested agave species, specifically cell wall compositional data along with the relative proportions of cellulose ultra-structural components. The experimental results also provide an important baseline for further characterization and conversion of different agave species as biofuels feedstocks for semi-arid lands.

Keywords: Agave, composition, cellulose, biofuels, feedstock.

1. Introduction

Agave, which is well known for tequila production in Mexico, has recently emerged as a potentially attractive lignocellulosic feedstock for conversion to biofuels and chemicals [1]. One reason for this new interest is that agave species have high water use efficiency and drought resistance [2]. Consequently, agaves can be grown on arid and semi-arid lands not suitable for other lignocellulosic feedstocks, such as poplar, switchgrass, miscanthus, and sugarcane. In addition, although agave species are native to the American continent, they have worldwide potential for production, with agave now grown in semi-arid regions in such diverse locations as Brazil, Australia, Southern and Eastern Africa, and areas across the Mediterranean [3-5]. Another vital attribute is the high estimated average annual productivities for agave species of 10-34 Mg ha^{-1} $year^{-1}$ [1, 6] compared to about 15 Mg ha^{-1} $year^{-1}$ for switchgrass [7] and 11 Mg ha^{-1} $year^{-1}$ for poplar wood [8, 9]. Furthermore, with appropriate cultivation, productivities could be as high as 40 Mg ha^{-1} $year^{-1}$ for A. *salmiana* and A. *mapisaga* [10], although lower values will no doubt result with lower water use. Beyond these features, agave offers such environmental attributes as preventing desertification of dry lands [11, 12] and removing heavy metals from water around mines [4, 13]. Such important features as these make agave promising as a means to extend the range of biofuels production to complement that possible with grasses such as switchgrass and woods such as poplar.

Because mass yields from lignocellulosic biomass dominate conversion costs for fuels and any other commodity products, accurate measurements of the chemical composition of biomass are critical to provide a perspective on the maximum fuel yields and ultimate economic merits. In the case of biological conversion to biofuels or chemicals, cellulose and hemicellulose should comprise a substantial portion of the total dry matter. This information is also essential to

assessing how effective pretreatment and enzymatic hydrolysis operations are in deconstructing cellulosic biomass to sugars that can be fermented to fuels [14] or further reacted to furfural, levulinic acid, and other reactive intermediates that may lend themselves to catalytic operations that have recently gained interest for making drop-in fuels [15]. In the case of agave, one of the earlier compositional studies applied a multi-step acid hydrolysis method [16] to determine that A. *lechuguilla* contained 20.7% cellulose, 11.3% hemicellulose, and 12.2 % lignin on a dry basis [17]. These low values would suggest that agave would suffer from low yields of sugars and any products that could be derived from them. However, several more recent studies from diverse fields reported composition results for a few agave species, as summarized in Table 1, that are much more in line with making agave attractive as a biofuels feedstock [3, 18-25]. Unfortunately, these results were based on "fiber" or "bagasse" materials prepared by various extraction/isolation procedures that change the chemical composition of the biomass tested, and the corresponding data may not represent the carbohydrate content of raw agave materials. In addition, the analytical methods applied in the literature to determine cellulose and hemicellulose amounts also varied considerably, making it challenging to meaningfully compare compositions of different agave species. Thus, application of consistent and accurate analytical methods was needed to obtain comparable composition information that would support identification of agave species with the best potential for biofuels production and help select cultivation strategies appropriate to the most promising species. The types of carbohydrates in agave hemicellulose and ultra-structural information about agave cellulose are also important to better understand recalcitrance features of agave and achieve economic agave conversion. Such information, unfortunately, has not been available in previous literature.

Table 1. Composition of different agave species and anatomical fractions reported in the literature (wt %)

Species	Anatomical fraction	Cellulose	Hemicellulose	Lignin	Reference
A. *americana*	leaf fiber	68.4	15.7	4.9	Mylasmy & Rajendran, 2010
A. *salmiana*	bagasse	47.3	12.8	10.1	Garcia-Reyes & Rangel-Mendez, 2009
A. *tequilana*	bagasse	43	19	15	Cedeno-Cruz & Alvares-Jacobs, 1999
A. *lechuguilla*	leaf fiber	79.8	3-6	15.3	Vieira et al., 2002
	leaf fiber	46-48	30	11	Marquez et al., 1996
A. *fourcroudes*	leaf fiber	77.6	5-7	13.1	Vieira et al., 2002
A. *sisalana*	-	43	32	15	McDougall et al., 1993

In this study, a series of laboratory analytical procedures (LAPs) for standard biomass analysis defined by the National Renewable Energy Laboratory (NREL) were applied to determine the chemical compositions of the four agave samples. The measured compositions included extractives, water soluble carbohydrates (WSC), structural carbohydrates, acid-insoluble lignin, crude protein, and ash for agave bagasse, as well as the composition of agave juice. In addition, ^{13}C cross-polarization magic angle spinning (CP/MAS) nuclear magnetic resonance (NMR) was employed to determine the ultra-structural features of cellulose extracted from four agave bagasse samples, including cellulose I_α and I_β, *para*-crystalline cellulose, cellulose associated with accessible and inaccessible fibril surfaces, and the average lateral dimensions of fibril and fibril aggregates. By characterizing such ^{13}C CP/MAS results for isolated agave cellulose, we were able to compare such ultra-structural features of agave to other types of lignocellulosic biomass for the first time. These results should help better understand the potential of agave as a biofuels feedstock suitable for production on semi-arid lands.

2. Materials and Methods

2.1. Chemicals

The following sugars were purchased from Sigma-Aldrich (St. Louis, MO) to serve as standards for determining carbohydrate profiles of agave samples: glucose (Lot No. 089K00601, Sigma), fructose (Lot No. 1253079, Fluka), sucrose (Lot No.1231832, Fluka), and inulin from dahlia tubers (Lot No. 1212695, Fluka). Xylose (Lot No. A0295756, Acros), galactose (Lot No. A0244833, Acros), and arabinose (Lot No.10162224, Alfa Aesar) were purchased from Fisher Scientific (Pittsburgh, PA). Other reagents and chemicals used were of analytical grade and were purchased from Sigma or Fisher Scientific, unless otherwise stated.

2.2. Plant materials preparation

Four samples from three agave species were employed for this study: A. *americana* leaves (AAL), A. *salmiana* leaves (ASL), A. *tequilana* leaves (ATL), and A. *americana* heart (AAH). All samples were freshly collected from the San Jose area (California, USA), wrapped in preservative film, and shipped to the University of California at Riverside (UCR) directly after harvest. Upon receiving the samples, they were frozen at -18°C to avoid sugar degradation.

Figure 1 provides a flowchart of the major steps applied to prepare samples for analysis after their arrival at UCR. Agave samples were first thawed at room temperature and cut into small chips using a knife. The juices were then squeezed from the material by placing it in a 9.5 inch long by 3.5 inch diameter metal pipe followed by forcing a tight fitting metal cylinder into the pipe with a hydraulic press (Model No. 14590, Northern Tool + Equipment, Burnsville, MN). The juices were kept in 50 ml polypropylene centrifuge tubes at -18°C for further analysis. The unwashed agave bagasse solids were then dried at 60°C in an oven (Thermo Scientific Imperial III Incubator, Fisher Scientific Pittsburgh, PA) for 18-24 hours to reduce the sample moisture to about 5%. This drying method was previously optimized for lowest free sugar degradation of

agave materials. Then, a Thomas Wiley® mini mill (Model No. 3383-L20, Thomas Scientific, Swedesboro, NJ) was used to mill the dried agave bagasse through a 40-mesh (425 μm) screen to be sure all tissues were homogeneously milled for further characterization. The moisture content (MC) was measured by an automatic infrared moisture analyzer (Model No. HB43-S, Mettler-Toledo Inc., Columbus, OH).

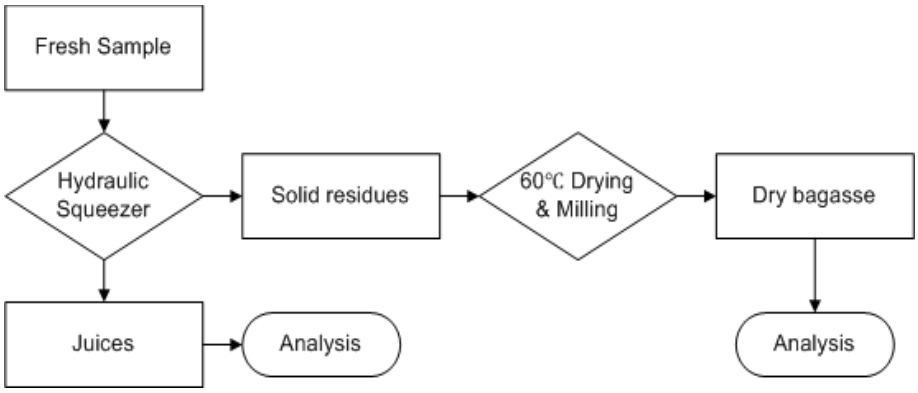

Figure 1. Flowchart of major processing steps for preparation of agave samples for analysis.

2.3. Juice analysis

Free sugars and inulin contained in the agave juice were directly determined with a Waters Alliance e2695 HPLC with a 2414 refractive index (RI) detector (Waters Corporation, Milford, MA). The components were separated on a BioRad Aminex HPX-87P column (Cat No. 125-0098, Bio-Rad Life Science, Hercules, CA), and chromatograms were recorded and quantified by Empower software (Waters Corporation, Milford, MA). The same HPLC method was applied to quantify sugar concentrations in the liquid samples for all subsequent analysis. For oligomers and total sugar content, a modified NREL post hydrolysis method was used [26] in which the total reaction volume was scaled down by a factor of 20 [27]. In addition, hydrolysis was performed at 121°C for 1 hour in 0.5 wt% sulfuric acid instead of the 4wt% acid solution used in the NREL method. Inulin, sucrose, and fructose were used as sugar recovery standards

(SRS) to quantify the corresponding fructose degradation, and average sugar recovery yields from 3 samples run in triplicate were used for subsequent calculations. In addition, concentrations of total soluble solids (TSS) in agave juices was also determined by pipetting 10 mL agave juice that had been passed through a 0.2 μm filter into pre-dried and pre-weighed aluminum weighing dishes and drying them at 60℃ for 48 hours in a conventional oven until a constant weight was reached.

2.4. Bagasse extractives and Water Soluble Carbohydrates (WSC) analysis

The percentages of water and ethanol extractives were determined in sequence by the Soxhlet method described in the NREL LAP "Determination of Extractives in Biomass" [28]. For WSC analysis, 1 g of oven dry unwashed bagasse samples was loaded into 20 mL glass vials. Then, 16 mL of deionized (DI) water and 320 μL of 10 g/L sodium azide solution were pipetted into each vial using Eppendorf pipettes (Eppendorf, Hamburg, Germany). The final slurry contained 0.2 g/L of sodium azide to prevent the growth of organisms. The vials were then sealed and placed in an incubation shaker (Multitron Infors-HT, ATR Biotech, Laurel, MD) for 24 hours at 50℃ and 150 rpm. The amounts of free sugars and total sugar content were measured by the same methods as described in Section 2.3.

2.5. Bagasse structural carbohydrates and lignin content analysis

The percentage of structural carbohydrates and acid insoluble lignin content were measured for the extractive free agave bagasse prepared in Section 2.4 following the NREL LAP "Determination of Structural Carbohydrates and Lignin in Biomass" [29].

2.6. Crude protein and ash analysis

The crude protein content was estimated by the equation:

% Protein = % N × Nitrogen factor (NF) [30]

in which the commonly used NF of 6.25 was applied [31]. About 5 mg of dry, homogenized sample was weighed into tin capsules (Cat No. 240-064-40, CE Elantech, Lakewood, NJ) and sealed. Then the nitrogen content was measured with a Flash EATM 112 N/Protein plus CHNS/O Analyzer (CE Elantech, Lakewood, NJ) with aspartic acid as a standard (CE Elantech, Lakewood, NJ). The ash content was also measured according to the NREL LAP "Determination of Ash in Biomass" [32] and employed to close mass balances.

2.7. Cellulose characterization by ^{13}C CP/MAS NMR

Holocellulose (cellulose + hemicellulose) samples from Agave baggasses were prepared by sodium-chlorite delignification [33]. Isolated cellulose was prepared from the holocellulose samples (1.00 g) by hydrolysis for 4 h in HCl (100.0 mL of 2.5 M) at 100ºC. The isolated cellulose samples were then collected by filtration, rinsed with an excess of DI filtered water, and dried in the fume hood. The NMR samples were prepared from isolated cellulose added into 4-mm cylindrical ceramic MAS rotors. Solid-state NMR measurements were performed on a Bruker Avance-400 spectrometer operating at frequencies of 100.55 MHz for ^{13}C in a Bruker double-resonance MAS probe head at spinning speeds of 10 kHz. CP/MAS experiments utilized a 5 μs (90°) proton pulse, 1.5 ms contact pulse, 4 s recycle delay, and 4 K scans. All spectra were recorded on pre-wet samples (30-40% water content), and line-fitting analysis of spectra was performed using NUTS NMR Data Processing software (Acorn NMR Inc., Livermore, CA).

Error analysis was conducted by performing five individual isolations of NMR acquisitions and line-fit data processing on representative biomass samples to assess typical variations.

3. Results and Discussion

3.1. Characterization of agave raw materials

Leaves and heart are the two main portions from an agave plant that could be utilized as biofuels feedstock. The heart, also called agave piña or head, is a pineapple-like stem base from which the leaves grow. Fresh biomass yields are very close from leaves and heart for some species and close to 50/50 for A. *americana* [34]. Generally, leaves contain more fiber resulting in a higher structural carbohydrate content while the heart is rich in non-structural carbohydrates such as inulin and other water-soluble fructose equivalents.

Table 2 summarizes mass distribution data of these components as determined according to the methods outlined in section 2.2 for the samples received. ATL contained the highest percentage of dry bagasse of the three leaf samples used in this study, and the leaf tissues of *A. americana* were much juicier than its heart. AAH contained twice the amount of total soluble solids (TSS) in the juice portion as in the leaf samples. In total, ATL had a higher solids yield than the other two species based on fresh mass, and AAH contributed more dry-biomass than leaves from the same plant. In addition, because agave heart juice has been reported to be weakly acidic in many papers, the pH of both leaf juices and heart juice were measured in this study. As the average of three measurements, the pH of ATL juice was the lowest (4.58), while the juice pH values of AAL, ASL, and AAH were 5.16, 4.99, and 5.19, respectively.

Table 2. Mass distribution of fresh agave samples (wt %)				
	Dry bagasse	Juice	TSS[a] in juice	Total solids
AAL	5.0	95.0	5.3	9.6
ASL	4.4	95.6	5.1	8.8
ATL	13.0	87.0	5.2	16.9
AAH	12.4	87.6	10.6	20.0

[a]TSS-% total soluble solids

3.2. Sugars in agave juice

As the nutritive storage organ of agave species, agave heart is rich in water-soluble polysaccharides/oligosaccharides, most of which are inulin and its oligomers. In fact, the heart juice is an important sugar source and has been fermented to produce alcoholic beverages for centuries [25, 35]. For example, famous tequila is made from *A. tequilana* Weber, while *A. americana* and *A. salmiana* are used to make mezcal and pulque, respectively. Although making beverages has higher value, juice sugars could become an important contributor to the economic conversion of agave into biofuels if agave is grown at a large scale that would outpace beverage markets. However, detailed analysis of sugars in agave heart juice is still limited and the information on leaf juice composition is scarce. Table 3 shows the sugar composition of the four agave juice samples obtained in this study, with the concentrations of inulin, sucrose, glucose, galactose, and fructose directly measured from fresh juice samples. Fructose and glucose were the major monomeric sugar components in all samples, but AAH juice contained significantly higher inulin and sucrose than the others. To quantify sugar oligomers, the conventional post-hydrolysis acid condition (4 wt% sulfuric acid, 121℃ for 1 hour) could not be directly applied due to the degradation of over 90% of the fructose at these conditions making any sugar recover standard inaccurate for calculating fructose equivalents [36]. Thus, various hydrolytic conditions with acid loadings of 0.1 wt% to 2 wt% were applied, as described in section 2.3, to completely

convert inulin, sucrose and oligomers into monomeric sugars while minimizing fructose degradation. With the modified method, the average fructose equivalent degradation was about 29 %, and was applied to correct for corresponding fructose losses. Glucose, however, was very stable at this condition, with negligible degradation. The percent of oligomers associated with glucose and fructose were calculated by Equation 1, assuming there was one glucose residue for every 80 fructose residues in inulin molecules [37]. The corresponding results in Table 3 show that fructose residues contributed 77.8% to 84.6% of total oligomers, while glucose residues contributed about 14.5% to 17.8% of the total. More than half of the total sugars in AAH juice were oligomers, while monomeric sugars made a major fraction of leaf juices.

$$\%=\frac{C_{\text{after post hydrolysis}}-C_{\text{before post hydrolysis}}-C_{\text{derived from inulin}}-C_{\text{derived from sucrose}}}{C_{\text{oligomers}}} \qquad \text{Equation 1}$$

Table 3. Sugar composition of agave juices (g/L)

	Inulin	Sucrose	Glucose	Galactose	Fructose	Sugar oligomers	Total
AAL	1.4	1.5	12.7	0.3	6.8	4.2 ± 0.1[a] (*15.4*, 84.6)[b]	26.9
ASL	1.4	0.5	9.1	0.1	8.8	4.6 ± 0.1 (*14.5*, 85.5)[b]	24.5
ATL	1.4	1.3	10.0	0.7	7.3	9.3 ± 0.1 (*15.7*, 80.6)[b]	29.9
AAH	8.4	11.7	7.7	0.6	8.0	44.2 ± 0.4 (*17.8*, 77.8)[b]	80.6

a: Values represent the standard deviation of three replicates.
b: The first values in parenthesis represent the percentage of oligomers associated with glucose; the second values in parenthesis represent the percentage of oligomers associated with fructose.

3.3. Composition of agave bagasse

It has been shown that the carbohydrate composition from the same agave species varied according to cultivation regions and climates [3], plant ages, and even the age of leaves when sampled [34]. For example, for the same *A. americana* plant, older leaves (12 years old) were found to have about 8% higher cellulose content than younger leaves (4 years old) [34]. In this study, the source plants were cultivated in the same area and were all between 4 and 5 years old. To eliminate the effects of leaf age, only the biggest leaves which assumingly were also 4 to 5 years

old were collected. The corresponding mass balance of agave bagasse composition is shown in the Table 4.

All four agave samples were successively extracted with water and then by ethanol. The water extractives amount shown in the Table 4 was calculated by subtracting the amount of WSC, determined by the procedures described in section 2.4, from the total water extractive determined by the NREL procedure. In general, the extractives patterns for three leaf bagasse samples were similar, with from 12.6% to 14.2% for water extractives, 1.9% to 3.2% for ethanol extractives, and 4.4% to 7.9% for WSC. However, AAH contained about 6.6% less water extractives and 11.5% higher free sugars than AAL.

Table 4. Mass balance on agave bagasse dry mass composition (%)

	Water extractives[a]	Ethanol extractives[a]	WSC[a]	Structural carbohydrates[b]	K-lignin[b]	Ash[b]	Protein[a]	Total
AAL	12.6	1.9	6.5	45.0 ± 0.3	8.2 ± 0.3	7.4	3.7	85.3
ASL	15.1	2.8	7.9	42.7 ± 1.3	9.8 ± 0.7	6.1	4.9	89.4
ATL	14.2	3.2	4.4	41.7 ± 0.3	11.9 ± 1.2	6.4	5.6	87.5
AAH	6.0	1.3	17.0	39.7 ± 0.9	7.3 ± 0.9	7.2	4.5	83.0

a: Data reported are the mean values of two replicates
b: Data reported are the mean values of three replicates

The breakdown in compositions of structural carbohydrates is shown in Figure 2. These carbohydrate profiles of three leaf samples were very similar, containing about 30% glucan, 7% xylan, and even smaller amounts of galactan and arabinan based on the dry weight of raw materials. Davis et al. also reported that structural carbohydrate profiles were similar among species that were grown in the same region, including *A. angustifolia, A. potatorum,* and *A. cantala* [3]. Both studies, however, indicated that the production region might have important effects on biomass yields and compositions. Compared to leaf bagasse, AAH had a lower glucan content (20.5%) but about twice as high galactan (8.9%). Overall, all agave bagasse samples tested in this study contained more than 50% of dry weight as carbohydrates including free

sugars and structural carbohydrates, but the heart had about 55% or more total structural plus soluble sugars.

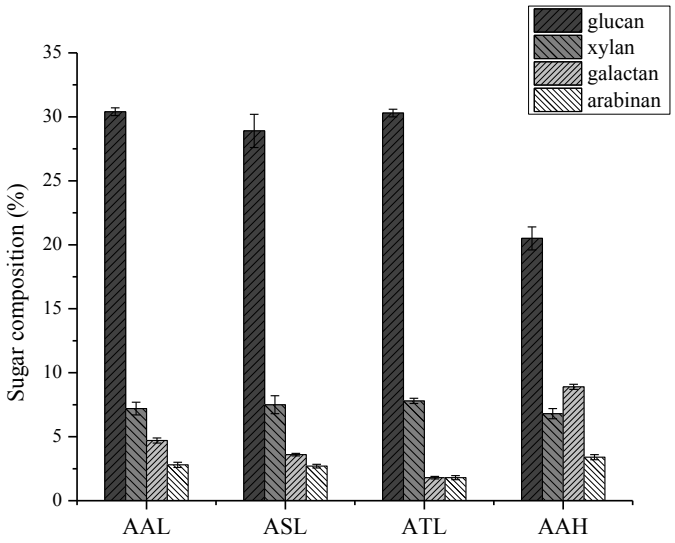

Figure 2. Structural carbohydrates composition of agave bagasse.

As a plant that uses the Crassulacean Acid Metabolism (CAM) pathway, agave species have been recognized as low lignin biofuels feedstocks [3]. As shown in Table 4, the K-lignin contents of the agave bagasse tested were from 7.3% to 11.9%, significantly lower than switchgrass (18.8%) and poplar wood (23.4%) tested by the same method and shown elsewhere [38, 39]. The acid insoluble lignin was not measured in this study due to the lack of reference absorptivity constants. Nonetheless, together with ash and protein contents, the mass balance was about 85 to 90% for all agave leaves tested but about 83% for the one heart sample. The remaining unaccounted for mass could be acid soluble lignin, acetyl and other substituent groups that are often found on the xylan backbone, and pectin, none of which were determined in this study.

3.4. Agave cellulose characterization

A 2-peak integration method [40] was used to analyze the cellulose C_4 region resulting from the acquired ^{13}C CP/MAS NMR spectra of isolated cellulose from various agave samples for crystallinity, with , the calculated results and tabulated in Table 5. The crystallinity index for the agave leaves (AAL, ASL, ATL) tested in this study varied only slightly from 50 to 54% (±2%). However, the crystallinity index of AAH was significantly lower than AAL, indicating cellulose isolated from the heart contained more amorphous cellulose than its leaf regions. In Figure 3 (A) comparing crystallinity data for the agave in this study to values measured by the same methods for other types of cellulosic materials shows that the agave cellulose crystallinity index was similar to that of switchgrass (grass) but lower than that for poplar (hardwood) and pine (softwood). Several studies suggest that a correlation exists between crystallinity and enzymatic digestibility [40-45], with some data demonstrating that the rate of enzymatic hydrolysis is much faster with amorphous cellulose [40]. However, due to the complex interplay of multiple substrate characteristics in native biomass, there has not been a clear consensus on the effect of cellulose ultrastructure on enzymatic digestibility [46]. Although recent work indicated that substrate accessibility may be among the most important rate determining factors for enzymatic hydrolysis [47-49], monitoring agave cellulose ultrastructure should still be valuable in developing a comprehensive representation of the agave cell wall structure and its effects on recalcitrance.

Table 5. Non-linear least-squared spectral fit to the results of the C4 region for ^{13}C CPMAS spectra of isolated cellulose

Sample	% Cr ± 2.0	% I_α ± 3.0	% $I_{\alpha+\beta}$ ± 3.6	% Para ± 6.3	% I_β ± 3.6	% Acc ± 2.1	% Inacc ± 1.0	LFD ± 0.5 (nm)	LFAD ± 2.1 (nm)
AAL	54	2.3	7.6	37.7	6.4	6.4	39.6	4.1	34.0
ASL	50	4.6	11.1	24.8	9.5	5.5	44.5	3.8	39.4
ATL	53	5.7	8.1	32.8	6.4	5.0	42.0	4.0	43.3
AAH	46	3.8	6.5	26.0	9.6	8.9	45.1	3.4	24.1

Cr: crystallinity index; I_α: α-cellulose; I_β: β-cellulose; para: Para-crystalline cellulose; Acc: cellulose at accessible surface; Inacc: cellulose at inaccessible surface; LFD: lateral fibril dimension; LFAD: average lateral fibril aggregate dimension

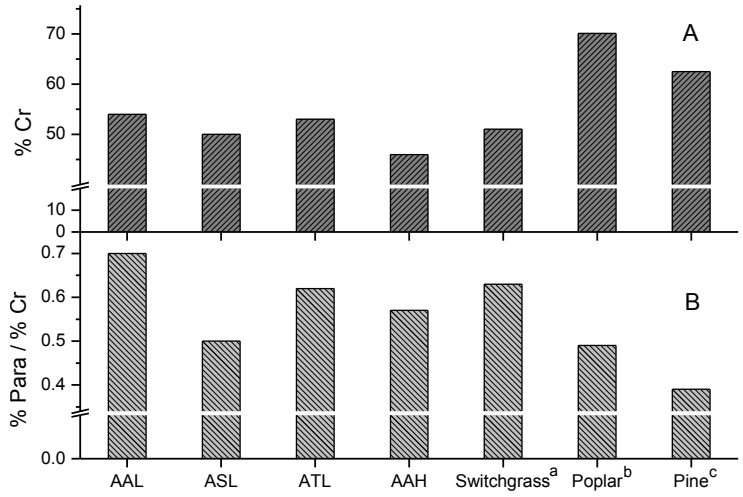

Figure 3. Crystalline index (A) and ratio of *para*-crystalline cellulose to crystallinity index (B). a: Alamo switchgrass leaves [50]; b: Poplar [51]; c: Loblolly pine [52].

A 7-peak non-linear line-fit analysis [53-55] of the cellulose C_4 region was also performed to determine the relative amounts of cellulose crystalline allomorphs and fibril surface for the agave samples employed here, as shown in Table 5. This approach was performed by fitting one Gaussian and three Lorentzian line-shapes to the crystalline cellulose C_4 carbon signals from δ 85–92 ppm that are attributed to domains of cellulose I_α, I_β, and *para*-crystalline cellulose [54, 55]. I_α and I_β are the two natural forms of crystalline cellulose type I, and *Para*-crystalline cellulose is loosely described as a type of cellulose allomorph between amorphous and crystalline cellulose in chain order and mobility [53]. In addition, the non-crystalline cellulose C_4 carbon region δ 80–95 ppm associated with accessible and inaccessible cellulose fibril surfaces was simultaneously fit to three Gaussian line-shapes [54]. To further investigate the crystalline allomorphs of agave

cellulose, the ratio of *para*-crystalline cellulose to crystallinity index was calculated and compared to results for switchgrass, poplar, and pine samples, as shown in Fig 5 (B). All agave samples showed more than a 50% ratio of *para*-crystalline cellulose to crystallinity index, similar to levels for switchgrass but higher than for poplar and pine. These high proportions of *para*-crystalline cellulose suggest that agave could show relatively higher enzymatic digestibility compared to woody materials.

Utilizing a square cross-sectional micro-fibril model, which considers amorphous cellulose as being located only on fibril surfaces, the lateral fibril dimension (LFD) and lateral fibril aggregate dimension (LFAD) can be estimated using the relative intensity of peaks attributed to total fibril surfaces and accessible fibril surfaces [56]. The LFD and LFAD of agave cellulose are displayed in Table 5.

3.5. Implications of these results

These results reveal several important points about the potential use of agave as a biofuels feedstock. First, as shown in Table 6, the range of structural carbohydrate contents based on dry mass of tested raw agave materials from just 21% to 32% is low and only about 30% to at best 55 % of the structural carbohydrate content of energy crops such as switchgrass or poplar. On the other hand, including soluble sugars could increase the total potential sugar content to about 50% of dry mass in the case of agave leaves and nearly 65% for agave heart. Thus, use of soluble sugars from agave will be important to achieve reasonably high mass yields of ethanol or other products through biological or catalytic conversion technologies. However, even if the total sugar content is lower than for some promising energy crops and reduces fuel yields per ton, the sugar yield per land area could be considerably higher when the potentially

high productivity of agave is factored in, as shown in Table 7. In addition, the low lignin content and crystalline structural features suggest that agave bagasse could be more easily deconstructed into sugars than grasses or hardwoods, and given the large cost contribution of overcoming recalcitrance for biological conversion processes [57], ease of conversion could offset the consequences of lower carbohydrate content. Thus, further research is being completed at our laboratory to determine if agave is more easily converted into sugars in pretreatment and enzymatic hydrolysis. Development of information on the relationship between agave structural features and sugar release could also prove invaluable in defining promising features in native plants or attributes to engineer into new varieties of switchgrass, poplar, and other plants to make them more amenable to biological conversion.

Table 6. Mass distribution of carbohydrates in dry raw agave samples[a] (wt %)

	structural	water soluble	total
AAL	23.5	27.2	50.6
ASL	21.4	27.9	49.3
ATL	32.1	16.5	48.6
AAH	24.6	39.4	64.0

[a]Calculation combined bagasse carbohydrates and juice carbohydrates, and based on total dry weight of raw materials.

Table 7. Estimated theoretical maximum ethanol yield

	gallons/ dry ton[a]	gallons/ (hectare · year)[b]
A. americana	96	963-3273
Poplar	115.8	1273
Switchgrass	93.5	1403

[a]Calculation based on 0.51 pounds of ethanol/ pound of sugar and 1 gallon of ethanol/ 6.55 pounds of ethanol, according to the Theoretical Ethanol Yield Calculator of NREL.
[b]Calculation based on average productivity (dry ton ha^{-1} $year^{-1}$) of 10-34 for agave, 11 for poplar and 15 for switchgrass, as introduced in Section 1.

4. Conclusions

For the first time, agave species were characterized by a series of standard biomass analysis procedures to develop detailed information on chemical compositions and cellulose

ultra-structural components. The three agave leaf bagasse samples employed had similar total structural plus soluble carbohydrate contents that contributed about 50% to 55% of the mass of dry bagasse. The xylan content was low in agave species relative to grasses and hardwoods, but galactan was a more important component in agave hemicellulose than for many other plants. Agave heart (AAH) contained lower structural carbohydrates (20.5% of glucan in bagasse) than leaves (AAL) but was rich in inulin, sucrose, and oligosaccharides that were mainly composed of fructose and glucose. Both agave leaves bagasse and heart bagasse had very low lignin contents (7.3%-11.9%). In addition, ^{13}C CP/MAS NMR spectra showed that agave cellulose had a relatively low crystallinity index (around 50%), and *para*-crystalline cellulose contributed over 50% of the total crystalline region. Further research is in progress to determine whether agave offers lower recalcitrance that can offset its lower carbohydrate content and support the use of this plant on semi-arid lands. This research can also suggest features that can be used to identify or improve other plants for conversion to fuels.

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