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Enhancing saccharification of cassava stems by starch hydrolysis prior to pretreatment

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ABSTRACT

Chemical characterization of cassava stems from different origin revealed that glucans accounted for 54–63% of the dry weight, whereas 35–67% of these glucans consisted of starch. The cassava stems were subjected to a saccharification study including starch hydrolysis, pretreatment with either sulfuric acid or 1-ethyl-3-methylimidazolium acetate ([Emim]OAc), and enzymatic hydrolysis of cellulose. Starch hydrolysis prior to pretreatment decreased sugar degradation, improved enzymatic convertibility of cellulose, and increased overall glucan conversion. Glucan recovery after pretreatment of starch-free cassava stems (SFCS) was around 85%, but below 52% when the stems were pretreated under the same conditions without preparatory starch hydrolysis. The total amount of hydrolyzed glucan after cellulose hydrolysis was two-fold higher for pretreated SFCS than for directly pretreated stems. Pretreatment with [Emim]OAc resulted in 20% higher glucan conversion than pretreatment with acid. Pyrolysis-GC/MS, X-ray diffraction, CP/MAS ^{13}C NMR and FTIR analyses revealed major differences between H_2SO_4- and [Emim]OAc-pretreated material.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a starchy crop belonging to the *Euphorbiaceae* family. It is cultivated in many countries across Africa, Asia and South America, where it is an important nutrition source and a crucial food security factor for more than 500 million people (Uchechukwu-Agua et al., 2015). Due to its high carbohydrate content the cassava root can be processed into human food, animal feed, and alcoholic drinks (Falade and Akingbala, 2011), as well as into first-generation ethanol (Sriroth et al., 2010). The annual global production of cassava root is estimated to be over 250 millions of tons (Uchechukwu-Agua et al., 2015; Zhu et al., 2015).

During the harvest, which is usually done between eight and twelve months after planting, the woody stem, which represents as much as half of the weight of the root, is separated from the cassava roots. It is estimated that around 116 millions of tons of fresh cassava stems are produced worldwide each year, and that 32–35 millions of tons are available on a dry mass base (Wei et al., 2014; Zhu et al., 2015). Approximately 10–20% of the stem is used for propagation, and small amounts are used as fuel or animal feed, while most of it has to be cleared from the fields and is abandoned or burned, causing emissions and environmental problems (Zhu et al., 2015). Although collection and transportation of cassava stems remain a logistical challenge for industrial use, its high carbohydrate content and availability, which has been overlooked before, make it a potential feedstock for producing fuel ethanol without directly affecting the food sector (Martín et al., 2006). Ethanol yields above 300 L/ton of stems could be achieved provided that efficient hydrolysis and fermentation are ensured. However, in spite of its high potential, the reports focusing cassava stems as raw materials for ethanol production are rather scarce in the literature.

In order to produce ethanol from cassava stems, it is necessary to enhance the cellulose reactivity towards cellulolytic enzymes, and that can be achieved by pretreatment. Among the different methods described in the literature (Kumar et al., 2009), dilute-acid pretreatment, which is an effective method with high potential for industrial application, has been the focus of a few studies on cassava stems (Han et al., 2011; Martín et al., 2007), although the results have not always been as good as expected. The enzymatic convertibility of dilute-acid pretreated cassava stems was unexpectedly found to be lower than that of the untreated material (Martín et al., 2007). That result was attributed to the possible presence of easily-hydrolysable glucans. In line with that, it was recently reported that cassava stems have a high starch content, for some varieties up to 42% of the dry weight, which can vary with both biotic and abiotic parameters such as genotype (variety), and growth environment (Wei et al., 2015).

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The high content of starch and woody nature of cassava stems distinguish this material from other raw materials for producing ethanol through either the conventional starch-based route (*i.e.*, 1G ethanol) or emerging lignocellulose-based technologies (2G ethanol). In order to produce ethanol from cassava stems, the optimal utilization of both starch and cellulose has to be considered. The presence of starch, which has different hydrolysis conditions optimum compared to cellulose, is a challenge to classic hydrolytical processes in general and to dilute-acid pretreatment in particular. Since starch can be hydrolysed already under relatively mild conditions, and as the released glucose is susceptible to degradation under the conditions used for acid pretreatment of cellulose, an especially designed tailor-made pretreatment approach should be considered for cassava stems. Designing such an approach is an obvious challenge taking into account that processes combining the 1G and 2G routes for this unique feedstock have, to our knowledge, not been reported. We hypothesized that disconnecting the starch hydrolysis from the acid pretreatment would improve the sugar yield and optimize the cassava-stem-to-ethanol process chain.

The pretreatment of lignocellulosic materials with ionic liquids (IL), which are molten salts having high dissolving power towards lignocellulosic components, has been the focus of considerable research efforts in recent years (Gräsvik et al., 2014). The effectiveness of the IL 1-ethyl-3-methylimidazolium acetate ([Emim]OAc) as a pretreatment agent has been proved for different biomass materials (Ebner et al., 2014; Karatzos et al., 2012; Li et al., 2010; Mood et al., 2014).

This work was aimed to investigate the effect of a preparatory hydrolysis of starch on the effectiveness of pretreatment of cassava stems. For that purpose, starch was removed from cassava stems by enzymatic hydrolysis, and the starch-free material was then subjected to pretreatment by using either dilute sulfuric acid or the IL [Emim]OAc. In parallel, a sample was acid-pretreated directly without being subjected to starch hydrolysis, as shown in the simplified scheme displayed in Fig. 1. Additionally, the composition of cassava stem samples from different varieties was thoroughly investigated.

2. Materials and methods

2.1. Raw material

Cassava stems of the varieties South China 205 (SC205), Xinxuan 048 (XX048), and South China 5 (SC5) were collected from three locations (Longan, Heng, and Wuming) in the Guangxi Zhuang Autonomous Region, China. The stems, which were collected 290 days after planting, were chopped, dried at 75 °C, milled to pass a 0.5 mm-screen, and stored in sealed plastic bags at room temperature until analysis. Based on the higher starch content of the SC205 samples, some material from that variety was pooled for the saccharification study.

2.2. Starch hydrolysis

Six batches of SC205 cassava stems were treated in a two-step enzymatic hydrolysis process consisting of liquefaction by thermostable α-amylase (Liquozyme SC4X, Novozymes, Bagsværd, Denmark) and saccharification by amyloglucosidase (Spirizyme Excel XHS, Novozymes) to remove the starch according to the method described by Palmarola-Adrados et al. (2005). Each batch was prepared by suspending 40 g (dry mass, DM) of milled stems in water at a 9:1 liquid-to-solid ratio in a 1-L blue-cap flask. The pH of the suspension was adjusted to 5.8 with 0.5 M H_2SO_4, and the flask was heated to 85 °C in a water bath. Then, 0.4 mL of α-amylase was added, and the reaction mixture was incubated for four h with magnetic stirring and periodical hand mixing each every ten to fifteen minutes without removing the flasks from the bath. When the liquefaction time had elapsed, the flasks were held at room temperature for around 10 min, and then the pH was adjusted to 5.0. Then 0.6 mL of amyloglucosidase was added, and the reaction mixtures were incubated at 65 °C and 250 rpm for 48 h in an Ecotron orbital incubator (INFORS HT, Bottmingen, Switzerland). By the end of the hydrolysis, the slurries were vacuum filtered, and the solid residues, hereafter referred to as starch-free cassava stems (SFCS), were washed with water, dried at room temperature, thoroughly mixed to form a single large batch, and stored in a plastic bag at room temperature. The liquid fractions, i.e. the hydrolysates, were stored frozen in plastic containers until analysis.

2.3. Dilute-acid pretreatment

The pretreatment method and conditions are based on a previous work by this group (Martín et al., 2007) and on our pilot tests. Ten grams (DM) of SFCS were mixed with diluted H_2SO_4 (1%), at a liquid-to-solid ratio of 9:1, in a 250-mL stainless steel cylindrical vessel placed on a heating plate with magnetic stirring and temperature control (MR Hei-Standard, Heidolph Instruments, Schwabach, Germany). The reaction mixture was heated (heating time, 20–30 min) to either 150, 170 or 190 °C, and held at that temperature during 20 min. Then, the reactor was cooled for around 15–20 min by immersion in a water bath. After that, the slurry was vacuum-filtered, and the solids were collected. The pretreated solids were washed with several portions of water until a neutral pH of the washing was reached, and then dried at room temperature until the dry matter content was around 90%. The pretreatment liquors were stored frozen, whereas the water from the washes was discarded. Direct dilute-acid pretreatment of a sample of raw cassava stems at 170 °C was also performed (see Fig. 1). All experiments were performed in duplicates.

2.4. Ionic liquid (IL) pretreatment

[Emim]OAc was acquired from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and was used as received. Its water content, determined by Karl-Fischer titration (Metrohm 756, Herisau, Switzerland), was 0.4%. Five hundred milligrams (DM) of SFCS were suspended in 9.5 g of [Emim]OAc in glass flasks with screw caps, and pretreated in duplicates at 110 °C with magnetic stirring for 3 h in an oil bath following a modification of the method described by Karatzos et al. (2012). After pretreatment, the content of each flask was transferred to a 50-mL Falcon tube, and 30 mL of deionized water was added to re-precipitate the dissolved biomass. The mixture was mixed vigorously and then centrifuged at 15 000*g* for 20 min. The supernatant was decanted to a 15-mL Falcon tube, and the precipitate was treated with a new portion of deionized water, and then centrifuged. All the supernatants were stored frozen. The pretreated solids were air-dried at room temperature for five days, and then stored in microcentrifuge tubes until further use.

2.5. Cellulose hydrolysis

Approximately 50 mg (DM) of pretreated material was suspended in 900 μL of 50 mM sodium citrate buffer (pH 5.2), and 50 μL of a 50:50 mixture of *Trichoderma reesei* cellulases and the β-glycosidase preparation Novozym 188 (both supplied by Sigma-Aldrich) were added. The reaction mixture was incubated for 72 h in the Ecotron orbital incubator at 45 °C and 170 rpm. At the end of hydrolysis, the hydrolysate was separated by centrifugation, diluted, filtered and subjected to HPLC analysis for glucose quantification. The glucose values were used for calculating the enzymatic

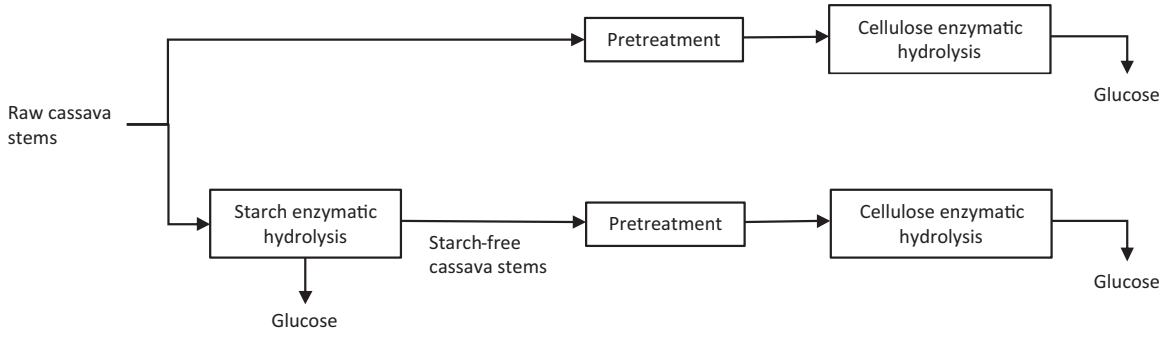

Fig. 1. Schematic layout of the experimental procedure. The upper branch shows the direct acid pretreatment, and the lower one shows the combined process of preparatory hydrolysis of starch and pretreatment.

conversion of glucan. All experiments were performed at least in duplicate.

2.6. Chemical analysis

The carbohydrate and lignin contents of raw and pretreated stems were determined by analytical acid hydrolysis (Sluiter et al., 2008a), followed by quantification of the sugars by high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). A Dionex ICS-3000 system (Sunnyvale, CA, USA) equipped with a 3 × 30 mm guard column and a 3 × 150 mm separation column (CarboPac PA20, Dionex) were used. Prior to analysis all samples were diluted with ultra-pure water and filtered through a 0.2 μm nylon membrane (Millipore). Elution was performed with a 2 mM solution of sodium hydroxide (NaOH) for 25 min, followed by regeneration with a solution of 200 mM NaOH and 68 mM sodium acetate for 5 min, addition of a solution of 200 mM NaOH for 5 min, and equilibration with a 2 mM solution of NaOH for 25 min. The flow rate was always 0.4 mL/min. PAD was performed on Gold Standard PAD waveform with Ag/AgCl as reference electrode. The sugar concentrations were used for calculating the content of polysaccharides. Acid-insoluble lignin (Klason lignin) was determined gravimetrically, whereas soluble lignin was determined spectrophotometrically at 240 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The contents of extractives were determined by sequential extraction with water and 95% ethanol in a Büchi B-811 Soxhlet system (Flawil, Switzerland) following the NREL protocol (Sluiter et al., 2008b). Ash content was determined by incineration at 550 °C in a Carbolite muffle oven (Sheffield, UK). Nitrogen content was determined by the Kjeldahl method (Kirk, 1950), and it was multiplied by 6.25 for obtaining the protein content. The dry matter content was measured with a halogen moisture analyser (Mettler-Toledo HG63, Greifensee, Switzerland).

Starch content was determined according to a protocol based on enzymatic hydrolysis (Sluiter and Sluiter, 2008). Glucose in the hydrolysates, and acetic, formic and levulinic acids in the pretreatment liquors were analyzed with an Agilent Technologies 1200 Series HPLC system (Santa Clara, CA, USA) fitted with an RI detector. The separation was performed with an Aminex HPX-87H column (Bio-Rad Laboratories AB, Solna, Sweden) operating at 60 °C with an isocratic gradient of 0.01 N H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min. The sugar concentration in the pretreatment liquors was determined by HPAEC both by direct injection and after post-hydrolysis with a 4% solution of sulfuric acid, which allowed calculation of the content of oligosaccharides. Furfural and 5-hydroxymethylfurfural (HMF) were analyzed with the HPLC system (Agilent Technologies 1200) equipped with a diode-array detector and a 3 × 50 mm, 1.8 μm Zorbax RRHT SB-C18 column (Agilent). Aqueous 0.1% (v/v) formic acid (A), and acetonitrile with 0.1% (v/v) formic acid (B) were used as eluents and with a flow rate of 0.5 mL/min. The absorption was followed at 282 nm, and the temperature of the column oven was 40 °C. All the analyses were performed at least in duplicates.

2.7. X-ray diffraction (XRD)

The XRD patterns of the samples were collected with a Bruker d8 Advance instrument (Bruker Biospin, Germany) in θ-θ mode, with an optical configuration including Cu $K_{\alpha 1,2}$ radiation, and a Vântec detector. The samples were mounted on a Si single crystal low-background sample holder and set in rotation mode during data collection. Continuous scans were applied within the range of 10–40 in 2Θ. The data collection time for each sample was at least 5 h. The crystallinity index (CrI) was calculated by the peak height method using the expression:

$$\mathrm{CrI} = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

Here, I_{002} is the intensity of the diffraction for the crystalline portion of cellulose at about $2\theta = 22.5^{\circ}$, and I_{am} represents the intensity of the non-crystalline material, which is taken at an angle of about 18.0–18.4° 2θ in the valley between the peaks (Kumar et al., 2009; Terinte et al., 2011).

2.8. Cross polarization magic-angel spinning (CP/MAS) ^{13}C NMR

The CP/MAS ^{13}C NMR spectra were acquired on a 500 MHz Bruker Advance III spectrometer equipped with a 4 mm magic angle sampling (MAS) probe. Approximately 80 mg of sample, moisturized with 50% deionized water were added to the 4 mm ZrO_2 rotor. The contact time was 1 ms, and 4 096 scans were collected for each sample. A Gaussian window function was used in the spectral processing performed in Topspin 3.2 (Bruker Biospin). Samples were analyzed at ambient temperature. For calculating the crystallinity index, the area of the crystalline peak of the C4 region (93–87 ppm) was divided by the total area assigned to the region (93–80 ppm) (Park et al., 2010).

2.9. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

Approximately 50 μg of ball-milled (MM400, Retsch, Germany) cassava stem powder was applied to a pyrolyzer equipped with an auto sampler (PY-2020iD and AS-1020E, Frontier Lab, Japan) connected to a GC/MS (7890A/5975C, Agilent Technologies AB, Sweden). The pyrolysate was separated and analyzed as described by Gerber et al. (2012). Multivariate data analysis by principal component analysis was performed with SIMCA 13.0 (Umetrics AB, Sweden) on integrated peaks.

2.10. Fourier transform infrared spectroscopy (FTIR)

Finely divided samples (10 mg) were ground and dispersed in 390 mg KBr (Merck, Darmstadt, Germany) in an agate mortar before measurements. The spectra were recorded under vacuum (4 mbar), using a Bruker IFS 66 v/S spectrometer (Ettlingen, Germany) equipped with a diffuse reflectance 16-sample holder carousel accessory (Harrick Scientific Products, Pleasantville, NY, USA). In each carousel run, a background (pure KBr) was recorded. The spectra were collected with 128 scans at a resolution of 4 cm^{-1} in the region 400–5200 cm^{-1}, and were converted to data point tables using the OPUS 5.5 software (Bruker). The spectra were normalized to the intensity of the band at 2916 cm^{-1}.

3. Results and discussion

3.1. Chemical composition of different cassava stem varieties

The investigation of the composition of stems of three cassava varieties harvested in three different locations showed that cassava stems have a very high carbohydrate content, which reaches around 70% of the dry weight in most of the samples (Table 1). The glucan content was above 50% for all five samples, and it surpassed 60% in two of them. The high glucan content was due to a high starch contribution, especially in the variety SC205, which is in good agreement with previous results (Wei et al., 2015; Zhu et al., 2015). Starch represented 18.5–42.4% of the dry weight of the stems and 35.2–67.0% of the quantified glucan. The contents of hemicelluloses and lignin were relatively low ranging between 10 and 14% and 12–17%, respectively (Table 1). The main hemicellulose component was xylan, followed by minor amounts of mannan, arabinan and galactan. The Py-GC/MS analysis revealed that cassava stem lignin has a guaiacyl-rich *p*-hydroxyphenyl:guaiacyl:syringyl composition.

Water extractives represented between one fifth and one fourth of the stem dry weight, and their content seemed to be more dependent on the growing location than on the variety (Table 1). The stems from Wuming contained less extractives than those from Longan and Heng. Protein and carbohydrates are the main water extractive components, but mineral compounds were also important for the Wuming samples. It is worth mentioning that a part of the carbohydrates and mineral compounds contained in the water extractives is accounted for also in the glucan and ash fractions, which explains why the sum of the components presented in the table exceeds 100%. The ash content, which was above 5% in the Wuming samples and below 3% in those from Longan and Heng, also seemed to be more related to the location than to the variety. This result is in line with a previous report showing that the content of ash and ash-forming elements varies significantly with location, and correlates well with the composition of the soil where the cassava was grown (Wei et al., 2014).

In general, according to their glucan profile and contents of hemicelluloses and lignin, cassava stems are rather similar to the residues from the production of starch from cassava roots, which can contain around 26% cellulose, 32% starch, 17% hemicelluloses and 16% lignin (Xu et al., 2014). The high amount of glucans in general, and starch in particular, is a positive feature of cassava stems for ethanol production, and its similarity with the residues of the cassava-to-starch industry is an important issue to be considered for the integrated utilization of both materials.

3.2. Starch hydrolysis

Since the variety SC205 displayed the highest starch content within the investigated samples, it was used for the saccharification study, which started with the enzymatic starch hydrolysis (Fig. 1). The hydrolysis led to a decrease of the starch content from 35.7% in the original material to 2.5% in the solid residue (Table 2), and the material balance showed that around 96% of the initial starch was hydrolyzed. The yield of hydrolysis residue revealed that 47% of the initial material was solubilized. Since that value is higher than the amount of hydrolyzed starch it is evident that solubilisation of other compounds, presumably water extractives, also occurred. As a result of the starch hydrolysis the content of cellulose, xylan and lignin increased from around 31, 8.2 and 14.4% to 49, 15.4 and 24.4%, respectively. The ash content decreased, apparently due to the solubilisation of some mineral components during the liquefaction stage, which was held at a relatively high temperature.

As expected, glucose was the main sugar found in the hydrolysate, and its concentration was rather high (Table 2). Minor amounts of *gluco*-oligosaccharides, resulting from an incomplete amyloglucosidase reaction, were detected. Other carbohydrates, mainly oligosaccharides, were also found in low concentrations. Their presence can be attributed to the partial hydrolysis of hemicelluloses that could have occurred during the high-temperature liquefaction stage.

Since the obtained starch hydrolysates have relatively high glucose concentration they can be used as substrate, either alone or combined with cellulose hydrolysates, for ethanolic fermentation. Higher glucose concentrations could be achieved provided that a lower liquid-to-solid ratio would be used.

3.3. Pretreatment

Parallel acid pretreatments of raw and starch-free stems were run at 170 °C for evaluating the effect of starch hydrolysis on glucan recovery and enzymatic hydrolysis (Fig. 1). Alternatively, acid pretreatments at 150 and 190 °C, and the use of the IL [Emim]OAc were also evaluated for the SFCS.

3.3.1. Yield and carbohydrate content of the pretreated solids

As indicated by the yields of pretreated solids (Table 3), the pretreatment led to a moderate solubilisation of the SFCS and to a massive solubilisation of the stems that had not previously been submitted to a preparatory starch hydrolysis. The yield of solids after acid pretreatment of starch-free material at 170 °C was 76.7% (Table 3), which was almost twice the yield achieved after the direct acid pretreatment of raw stems under the same conditions (39.7%). If the yield during the starch hydrolysis is taken into account (Table 2), the overall yield of solids for the SFCS was only slightly higher than that achieved for the direct acid pretreatment of the raw material. Decreasing the temperature of the acid pretreatment of SFCS to 150 °C increased the yield of solids, whereas increasing it to 190 °C led to a considerable decrease (Table 3). For the IL pretreatment of the SFCS both the pretreatment yield (83.7%) and the overall yield of solids (44.4%) were comparable with those achieved by acid pretreatment at the lowest temperature.

Independently of the method used, the pretreatment of the SFCS led to an increase of the glucan content from 49% (Table 2) to 57–61% (Table 3). The material balance revealed that glucan recoveries in the pretreated solids decreased from 95.3% at 150 °C to 74.1% at 190 °C, which might be due to the partial hydrolysis of the amorphous sections of cellulose and the starch remains. Glucan recovery in the IL pretreatment was close to the value achieved with the lowest acid pretreatment temperature. For the pretreatment at 170 °C the raw material-based glucan recovery was comparable for both the direct process and the one with preparatory starch hydrolysis.

The content of xylan and other carbohydrates decreased under all the acid pretreatment conditions as a result of the hydrolysis of hemicelluloses (Table 3). The decrease was proportional with the

Table 1
Main components of raw cassava stem samples, %.

	LSC205	HSC205	WSC205	WXX048	WSC5
Total glucan	54.0 (4.2)	63.0 (1.1)	61.4 (0.5)	54.4 (1.0)	52.6 (3.4)
Cellulose	*24.6*	*20.6*	*29.3*	*33.3*	*34.1*
Starch	*29.4 (0.7)*	*42.4 (1.1)*	*32.1 (0.5)*	*21.1 (1.0)*	*18.5 (0.3)*
Hemicelluloses	13.0	10.3	12.4	13.9	13.1
Xylan	*7.5 (1.1)*	*5.9 (1.2)*	*7.8 (1.3)*	*8.9 (2.5)*	*8.3 (1.9)*
Mannan	*2.0 (0.3)*	*2.1 (0.2)*	*2.2 (0.1)*	*2.5 (0.4)*	*2.5 (0.4)*
Arabinan	*1.9 (0.1)*	*1.2 (0.1)*	*1.0 (0.1)*	*1.1 (0.2)*	*1.1 (0.1)*
Galactan	*1.6 (0.1)*	*1.2 (0.2)*	*1.4 (0.2)*	*1.4 (0.4)*	*1.2 (0.1)*
Lignin	15.4 (0.8)	12.2 (0.4)	14.9 (1.0)	16.7 (1.0)	15.2 (0.2)
Guaiacyl units, %	55.2	52.2	49.1	51.9	56.0
Syringyl units, %	42.3	46.0	49.2	46.1	41.8
p-Hydroxyphenyl units, %	2.5	1.8	2.7	2.0	2.2
Water extractives	26.9	23.7	19.9	19.2	19.4
Glucose	*2.7 (0.0)*	*1.0 (0.0)*	*0.9 (0.1)*	*0.9 (0.0)*	*0.6 (0.2)*
Gluco-oligosaccharides	*4.7 (0.2)*	*6.6 (0.1)*	*4.2 (0.0)*	*3.2 (0.1)*	*3.2 (0.1)*
Other carbohydrates	*0.6 (0.0)*	*1.4 (0.0)*	*1.3 (0.0)*	*0.9 (0.0)*	*0.4 (0.0)*
Protein	*11.9*	*9.1*	*10.8*	*9.3*	*9.9*
Minerals	*1.5 (0.3)*	*1.6 (0.2)*	*3.0 (0.1)*	*3.3 (0.8)*	*3.3 (0.4)*
Ethanol extractives	1.7	1.7	2.3	1.3	1.6
Total ash	2.8 (0.1)	2.7 (0.0)	5.5 (0.1)	5.2 (0.1)	5.4 (0.0)

Mean of at least two replicates. The standard deviations are shown in parentheses.

Table 2
Yield of solids after starch hydrolysis, content of starch-free (SFCS) and raw (RCS) cassava stems, and concentration of sugars in the starch hydrolysate.

	SFCS	RCS
Yield of solids, %	53.0 (0.8)	–
Content of the main components in the solid materials, %		
Cellulose	49.0 (1.3)	31.4 (0.3)
Starch	2.5 (0.7)	35.7 (2.8)
Xylan	15.4 (0.4)	8.2 (0.1)
Other carbohydrates[a,b]	5.5 (0.1)	3.6 (0.1)
Lignin	24.4 (1.1)	14.4 (0.1)
Ash	2.3 (0.1)	3.8 (0.3)
Sugar concentration in the starch hydrolysate, g/L		
Glucose	37.6 (1.7)	–
Gluco-oligosaccharides[c]	1.0 (0.1)	–
Other carbohydrates[b,d]	1.4 (0.2)	–

Mean of at least two replicates. The standard deviations are shown in parentheses.
[a] Sum of mannan, arabinan and galactan.
[b] The standard deviations are average.
[c] Difference between glucose concentration before and after hydrolysis.
[d] Sum of xylose, mannose, arabinose and galactose.

Table 3
Yield of solids after pretreatment, and characterization of the solid and liquid fractions.

	Acid pretreatment				IL pretreatment/SFCS
	SFCS 150 °C	SFCS 170 °C	RCS 170 °C	SFCS 190 °C	
Yield of solids in the pretreatment and in the whole process including starch hydrolysis, %					
Pretreatment yield	86.6 (1.1)	76.7 (4.4)	39.7 (0.5)	64.2 (3.6)	83.7 (5.7)
Overall yield	45.9	40.7	–	34.0	44.4
Content of carbohydrates and ash in the solid materials, %					
Glucan	57.2 (0.9)	61.3 (0.7)	58.2 (1.9)	56.8 (2.0)	57.3 (0.4)
Xylan	4.5 (0.5)	2.2 (0.9)	0.8 (0.2)	0.1 (0.0)	15.1 (0.4)
Other carbohydrates[a,b]	2.5 (0.1)	1.7 (0.2)	1.5 (0.1)	0.4 (0.1)	3.4 (0.1)
Ash	0.5 (0.2)	0.5 (0.1)	0.5 (0.1)	0.7 (0.1)	ND
Glucan recovery based on the starch-free (SFCS) and on the raw (RCS) cassava stems, %					
Based on the SFCS	95.3	87.7	–	74.1	93.9
Based on the RCS	38.8	34.7	34.4	28.6	37.2
Concentration of carbohydrates and furan aldehydes, g/L					
Glucose	1.6 (0.1)	1.3 (0.0)	29.9 (1.4)	1.2 (0.0)	0.2 (0.0)
Xylose	3.6 (0.1)	2.5 (0.2)	5.8 (0.1)	1.0 (0.0)	1.3 (0.1)
Other carbohydrates[b,c]	2.5 (0.0)	1.4 (0.1)	3.4 (0.1)	0.4 (0.0)	1.4 (0.1)
HMF	0.3 (0.0)	0.5 (0.0)	4.1 (0.1)	1.1 (0.0)	0.0 (0.0)
Furfural	1.3 (0.0)	1.9 (0.2)	1.9 (0.1)	2.4 (0.1)	0.0 (0.0)

Mean of at least two replicates. The standard deviations are shown in parentheses.
[a] Sum of mannan, arabinan and galactan.
[b] The standard deviations are averaged.
[c] Sum of mannose, arabinose and galactose.

temperature increase, and the solids resulting from the pretreatment at 190 °C contained almost no xylan. The overall content of carbohydrates, considering both glucan and hemicelluloses, was higher for the material submitted to a preparatory starch hydrolysis (65.2%) than for the one that was directly pretreated (60.5%). It was even higher (75.8%) for the IL pretreatment, where the content of xylan and other carbohydrates remained at approximately the same levels as in the SFCS (Table 2), and no significant evidence of hydrolysis of hemicelluloses was observed.

3.3.2. Carbohydrates in the pretreatment liquid

Analysis of the composition of the liquors from the acid pretreatment of SFCS and raw stems showed very clear differences (Table 3). In the liquors from the pretreatment of SFCS, the sugar concentrations were generally low, and xylose or its dehydration product furfural were predominant, whereas glucose was the main sugar in the liquor from the RCS (Table 3). The glucose concentration in the RCS hydrolysate was high, obviously due to the occurrence of starch hydrolysis during that stage. It should be noted, however, that the glucose concentration in the RCS pretreatment liquor (29.9 g/L; Table 3) was lower than in the starch hydrolysate (37.6 g/L; Table 2). The reason for that is the degradation of glucose rather than a low hydrolytical conversion. The concentration of HMF, a product of glucose degradation, in the pretreatment liquor was 8 times higher for the direct acid pretreatment than for the pretreatment of the SFCS under the same conditions. The formation of formic and levulinic acids was also higher in the liquor of the direct acid pretreatment than in that of the pretreatment of SFCS (data not shown). The formation of furan aldehydes and aliphatic acids increased with the temperature for the acid pretreatment, whereas no sugar degradation was detected during the IL pretreatment.

3.3.3. Overall glucan recovery

The overall glucan recovery was calculated taking into account the glucan recovered in the pretreated solids and the glucose released in the pretreatment liquors and starch hydrolysate. The amount of glucan degraded to HMF, formic acid and levulinic acid was also included in the calculation (Fig. 2). As the graph shows, glucan was better preserved when starch was removed prior to acid pretreatment. For pretreatments at 170 °C with and without preparatory starch hydrolysis around 85% and 53% of the initial glucan was recovered, respectively. In agreement with that, the fraction of glucan degraded to HMF and to acids was estimated to be 2.8% for the pretreatment of SFCS and 9.7% for the direct pretreatment. Glucan degradation in direct pretreatment at 170 °C was even higher than in pretreatment of SFCS at 190 °C. These results demonstrate that a preparatory starch hydrolysis prior to the pretreatment minimizes sugar degradation and maximizes the ethanol potential of the raw material. The non-closure of the glucan balance suggests that, in addition to generation of HMF, formic acid and levulinic acid, a part of glucan was diverted to the formation of unidentified products.

3.3.4. Lignin content in the pretreated solids

The total lignin content increased from 24.4% in the SFCS to 33.5–46.2% in the acid-pretreated solids, and it was higher for the direct acid pretreatment (40.0%) than for the pretreatment of the SFCS at the same temperature (34.9%) (Table 4). The material balances indicated that the amount of lignin recovered in the acid-pretreated solids exceeded the content in the raw material. This apparent increase was proportional to the temperature, and it was higher when no starch hydrolysis was performed. However, the lignin recovery data for the acid-pretreated materials were not supported by the FTIR peak at 1505 cm^{-1} (see Section 3.4), which is considered the best calibration fit for lignin determination (Rodrigues et al., 1998). The divergence can be attributed to formation of pseudo-lignin, which is a Klason-positive aromatic material resulting from partial thermal decomposition of polymeric carbohydrates during acid pretreatment. The two peaks in the CP/MAS ^{13}C NMR spectra of the acid-pretreated materials at 148–142 ppm (Fig. 3A), which have previously been found in isolated pseudo-lignin samples (Hu et al., 2012), support this assumption. The highest pseudo-lignin formation in the direct acid pretreatment might be a consequence of starch degradation, and it might be a major reason of the non-closure of the glucan balance.

The lignin content of the IL-pretreated solids was comparable to the one of SFCS, but the recovery results indicate that the IL pretreatment led to a solubilisation of 15.1% of the lignin, which was supported by a corresponding decrease of the intensity of the 1505 cm^{-1} peak of the FTIR spectrum (Table 4). That delignification degree is lower than what was achieved with [Emim]OAc pretreatment of sugarcane bagasse (Karatzos et al., 2012) and switchgrass (Li et al., 2010), and it can be explained by the lower temperature used in this work, and perhaps also by the properties of the biomass material.

The two pretreatment methods exerted different effects on the subunit composition of the lignin. While the ionic liquid did not have any major effect on the syringyl/guaiacyl (S/G) ratio, the acid pretreatment led to a decrease in S/G ratio that was proportional to the temperature (Table 4). It is apparent that sulfuric acid has promoted changes, such as demethoxylation (Wang et al., 2012) and other reactions, which resulted in a decreased S/G ratio.

3.4. FTIR of pretreated materials

The regions between 3650 and 2550 cm^{-1} and between 1800 and 850 cm^{-1} of the normalized FTIR spectra of pretreated cassava stems are displayed in Fig. 4. No major spectral differences were found among the acid-pretreated samples, but there were clear differences between acid-pretreated and IL-pretreated samples. In the IL-pretreated sample there was a significant reduction of the intensity of the band at 3348 cm^{-1}, which has been assigned to O—H stretching vibrations and related to the hydrogen bonds in cellulose (Kumar et al., 2009). That indicates that cellulosic hydrogen bonds were more disrupted by [Emim]OAc than by the pretreatment with dilute sulfuric acid. This observation is consistent with published results on pretreated switchgrass (Li et al., 2010) as well as with the high hydrogen bond basicity of the acetate anion (Brandt et al., 2010).

The 1730 cm^{-1} band, assigned to C=O stretching vibration in acetyl groups of hemicelluloses (Karatzos et al., 2012), is very weak for the acid-pretreated samples, but it is well defined for the IL-pretreated material (Fig. 4B). This is an indication of release of the acetyl groups by acid pretreatment and their preservation by the ionic liquid, and it matches well with the observed effects of the pretreatments on the xylan content (Table 3). On the other hand, the intensity of the band at 1505 cm^{-1}, corresponding to the aromatic skeletal of lignin (Rodrigues et al., 1998), is significantly lower in the IL-pretreated sample than in the acid-pretreated one. For facilitating the comparison between different spectra, the percentage change was calculated by subtracting the intensity of the relevant peaks in the raw material from that of the respective peaks in the treated samples. The calculation revealed that the intensity of the 1505 cm^{-1} peak decreased by around 6% for the acid pretreatment and by 17% for the pretreatment with the ionic liquid. The latter value fits well with the lignin recovery trend of the IL-pretreated material (Table 4), but the reduction observed for the acid pretreatment contradicts the increased lignin recovery calculated for that material, something that, as previously mentioned, can be attributed to formation of pseudolignin.

The analysis of the signals at 1425, 1103 and 897 cm^{-1} backs the crystallinity trend discussed below (section 3.5). The peak at

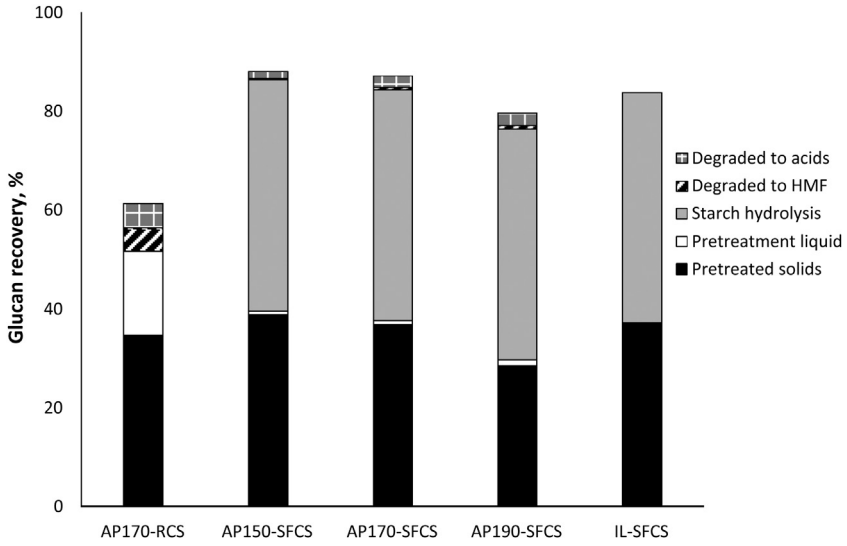

Fig. 2. Overall glucan recovery after pretreatment of raw and starch-free cassava stems (% of initial glucan). Glucan recovered in the pretreated solids (black), in the pretreatment liquids (white) and in the starch hydrolysis (grey). The percentage of glucan degraded to HMF and to formic and levulinic acids is also included in the balance.

Table 4
Lignin content, recovery and composition in the starch-free cassava stems (SFCS) and in the pretreated solids.

	SFCS	Acid pretreatment				IL pretreatment SFCS
		SFCS 150 °C	SFCS 170 °C	RCS 170 °C	SFCS 190 °C	
Content[a], %	24.4 (1.1)	33.5 (1.2)	34.9 (2.8)	40.0 (1.9)	46.2 (2.4)	24.0 (0.1)
Recovery[b], %	–	101.9	105.4	110.3	112.8	84.9
Decrease of 1505 cm^{-1} signal[c], %	–	0.5	6.5	7.3	−3.7	16.6
Guaiacyl units, %	54.9	58.9	59.9	60.7	63.0	55.0
Syringyl units, %	42.3	39.1	37.9	37.2	34.5	43.4
p-hydroxyphenyl units, %	2.7	2.0	2.2	2.1	2.5	1.6

[a] Sum of acid-insoluble (Klason) and acid-soluble lignin.
[b] Percentage of the lignin contained in the SFCS that was recovered in the pretreated solids.
[c] Decrease in percent of the intensity of the 1505 cm^{-1} signal for the pretreated samples in relation to the SFCS.

1425 cm^{-1}, assigned to the symmetric CH_2 bending vibration and related to crystalline cellulose (Ciolacu et al., 2011), was stronger for the acid-pretreated samples than for the IL-pretreated one (Fig. 4B). The 1103 cm^{-1} peak, which also refers to crystalline cellulose (Kumar et al., 2009), is clearly visible for the acid-pretreated materials but it is not present in the spectrum of the IL-pretreated stems. On the other hand, the peak at 897 cm^{-1}, assigned to the C—O—C stretching of the glycosidic linkages in amorphous cellulose (Ciolacu et al., 2011), was more intense for the IL-treated sample than for the acid-pretreated materials, and it was weaker for the directly pretreated sample than for the one pretreated after starch hydrolysis. These observations are in line with previous reports on [Emim]OAc and sulfuric acid pretreatment of other lignocellulosic materials (Li et al., 2010; Mood et al., 2014).

3.5. Biomass crystallinity

Pretreatment can change cellulose crystal structure by disrupting inter- and intra-chain hydrogen bonds, and thus lead to improvement of the enzymatic convertibility. The impact of pretreatment on biomass crystallinity can be evaluated using different techniques, such as XRD, FTIR, Raman spectroscopy and solid state (Karatzos et al., 2012)C nuclear magnetic resonance (NMR). The crystallinity index (CrI) varies depending on the choice of measurement method (Park et al., 2010).

3.5.1. Crystallinity in raw and starch-free cassava stems

The analyses of the crystallinity index using the XRD peak height method and CP/MAS ^{13}C NMR spectra revealed the same trend for most of the samples (Table 5). Both methods show that raw cassava stems are moderately crystalline (CrI 34.1–35.4), and that the CrI increased after starch removal. Although that increase is apparently higher by XRD than by NMR, it is well known that the XRD peak height method provides a less accurate measure of cellulose crystallinity, and its results can only be taken as an approximation (Park et al., 2010). Indeed, the analysis of the signal cluster with a chemical shift distribution between δ 92 and 80 ppm in the NMR spectra showed only minor differences between the raw stems (Fig. 3B) and the SFCS (Fig. 3C). The peak at δ 89 ppm, within the region assigned to the C4 carbons from crystalline forms, was slightly more intense for the SFCS, which could be interpreted as an increase of the crystallinity as a result of starch removal. On the other hand, the broad signal at δ 80–86 ppm, which corresponds to the amorphous domains, was similar for both samples, except that the peak at around 81.5 ppm, assigned to hemicel-

Table 5
Crystallinity index of the treated and untreated cassava stems using the XRD peak height method and CP/MAS (Karatzos et al., 2012)C NMR spectroscopy.

	XRD	NMR
Raw cassava stems (RCS)	35.4	34.1
Starch-free cassava stems (SFCS)	47.4	34.5
Acid-pretreated stems		
SFCS 150 °C	48.0	50.6
SFCS 170 °C	52.9	54.6
RCS 170 °C	56.8	55.7
SFCS 190 °C	48.5	59.6
Ionic liquid-pretreated SFCS	23.5	20.6

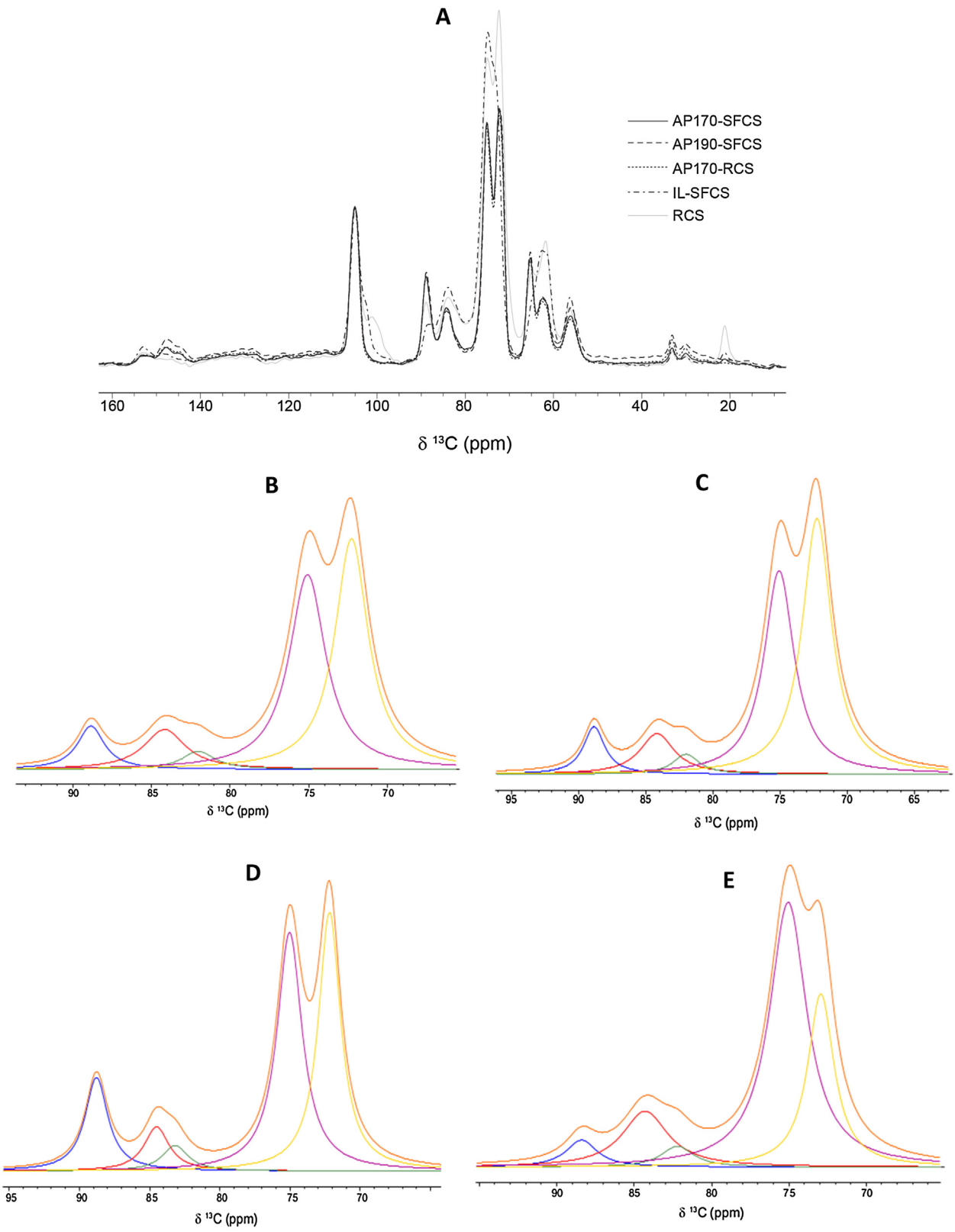

Fig. 3. CP/MAS ^{13}C NMR spectra of raw and treated samples. Full spectrum (A); spectral fitting of the 95–65 ppm region of raw (B), starch-free (C), acid-pretreated (D) and ionic liquid-pretreated (E) samples. The acid-pretreated sample corresponds to the pretreatment at 170 °C.

luloses (Hult et al., 2002), was better defined for the SFCS as a consequence of the increase of hemicellulose content after starch removal (Table 2).

3.5.2. Effects of pretreatments on crystallinity

Acid pretreatment led to an increase of the crystallinity (Table 5), which can be attributed to the hydrolysis of hemicelluloses, remaining starch and amorphous sections of cellulose. The increase

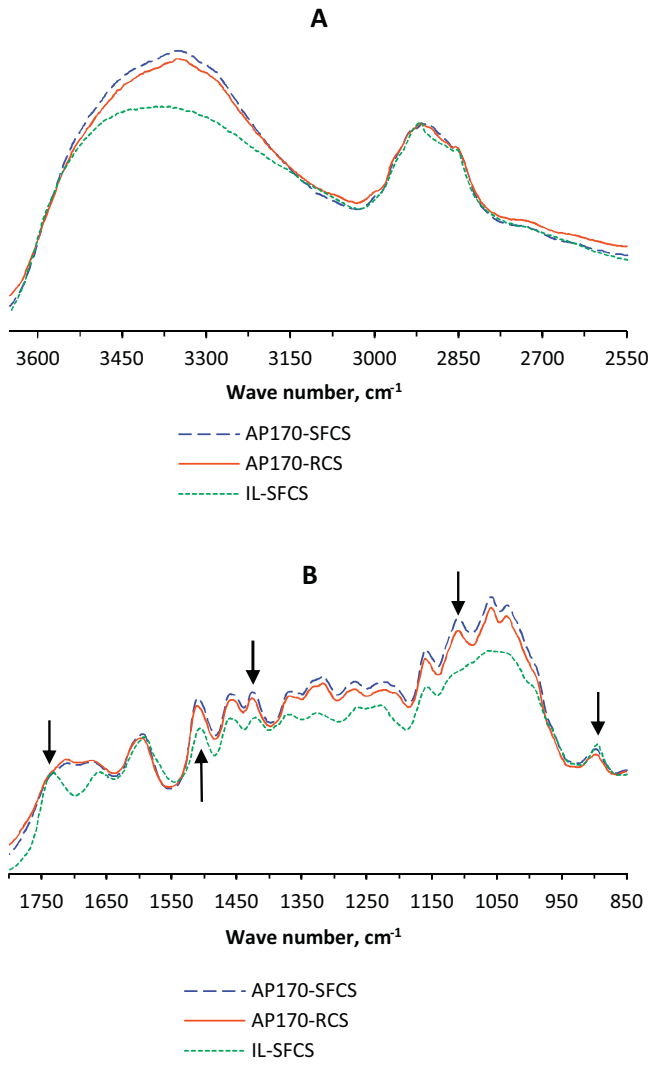

Fig. 4. Normalized FTIR spectra of the 3650–2550 cm^{-1} (A) and 1550–850 cm^{-1} (B) regions of the pretreated materials. Some relevant bands are indicated in B.

of the CrI was proportional to the temperature. Directly-pretreated stems had higher CrI than the material pretreated after starch hydrolysis, which was confirmed by both measurement methods. On the other hand, the IL-pretreatment led to a strong decrease of the crystallinity. The peak assigned to the crystalline portion of biomass in the XR diffractogram of the [Emim]OAc-pretreated sample was weakened and shifted to lower 2θ angle values. Compared to the acid-pretreated sample (Fig. 3D), the IL-pretreated material displayed a lower intensity peak at δ 92–86 ppm, and stronger signals at δ 84 and δ 81.5 ppm (Fig. 3E). The intensity of the peak at δ 81.5 ppm correlates well with the hemicellulose content in the pretreated materials (Table 3). It is also noteworthy that the shape of the two peaks at δ 81–70 ppm, corresponding to the C2, C3 and C5 carbon atoms of anhydroglucose units (Atalla and VanderHart, 1999) is different for the IL-pretreated material than for all the other samples.

It can be summarized that pretreating cassava stems with sulfuric acid leads to an increase of the crystallinity of cellulose, while pretreating them with [Emim]OAc leads to a weakening of the crystalline structure. A similar trend was reported previously for the pretreatment of switchgrass with sulfuric acid and [Emim]OAc (Li et al., 2010).

3.6. Enzymatic hydrolysis of cellulose

The hydrolytic conversion was calculated based on the cellulose contained in either the pretreated solids (hereafter referred to as relative conversion) or the starch-free material (absolute conversion). For the acid pretreatment, the relative conversion increased with the temperature (Fig. 5A). The low value at 150 °C indicates that, under the time and acid concentration used in this study, that temperature is not enough for activating cellulose towards enzymatic hydrolysis. Some improvement was observed as the temperature increased, but, even at 190 °C, the absolute conversion was still not very high due to the rather low glucan recovery during pretreatment (Table 3). Anyway, the removal of starch improved the effectivity of the acid pretreatment of cassava stems. For the material pretreated at 170 °C, the relative cellulose conversion was slightly better for the SFCS than for the directly-pretreated sample (Fig. 5A). The higher lignin, and pseudo-lignin, content (Table 4), as well as the higher crystallinity (Table 5), might have been limiting factors in the hydrolysis of the sample not subjected to preparatory starch hydrolysis. Furthermore, the overall hydrolyzed glucan throughout the whole sequence was considerably higher for the starch-free acid-pretreated material than for the directly pretreated sample (Fig. 5B). Although the fraction of cellulose hydrolyzed during the enzymatic hydrolysis was comparable for both processes (9.9–10.5% of the initial glucan) (Fig. 5B), it is evident that much more sugar from starch was recovered in a separate hydrolysis step (Fig. 5B, third column) than in a harsh pretreatment of starch-containing stems (Fig. 5B, first column). Overall, 58% glucan was hydrolyzed in the process including preparatory starch hydrolysis but only 27% in the process not including it.

The ionic liquid was much more effective for improving cellulose enzymatic digestibility. Around 72% of the cellulose contained in the IL-pretreated stems was hydrolyzed, which was almost 66% higher than the best result achieved by acid pretreatment (Fig. 5A). The absolute cellulose conversion remained almost as high as the relative one, which was due to the high glucan recovery during IL pretreatment (Table 3). This result is in agreement with previous reports showing effective [Emim]OAc pretreatment of switchgrass (Li et al., 2010), sugarcane bagasse (Karatzos et al., 2012), corn stover (Mood et al., 2014) and wood pulp (Ebner et al., 2014). The better digestibility of the IL-pretreated material compared to the acid-pretreated one can be explained by its lower structural order revealed by the crystallinity assessment (see section 3.5), and by its lower lignin content (Table 4).

The enzymatic hydrolysis of unpretreated SFCS led to a glucan conversion of 12.2% (Fig. 5A), which is within the values normally achievable for non-pretreated lignocellulose (Ioelovich and Morag, 2012). However, it was intriguing the high conversion (43.7%) observed for the raw stems. To explain this phenomenon, an additional experiment, aimed to elucidate the ability of the cellulase preparations to hydrolyze different glucan sources, was conducted. The trial resulted in a conversion of 78% of Avicel (Fig. 5C), while the hydrolysis of the raw and starch-free cassava stems gave conversions comparable with those obtained in the main experiment. Apparently the mixture of Celluclast 1.5L (cellulase-rich preparation from *T. reesei*) and Novozym 188 (β-glucosidase-rich enzyme preparation from *A. niger*) had enough α-amylase and amyloglucosidase activity to hydrolyze as much as 30% of a pure starch substrate. The presence of α-amylase and amyloglucosidase activities in crude preparations from *T. reesei* (Farkas, 1983) and *A. niger* (Rehman et al., 2014) has previously been reported.

4. Conclusions

The hypothesis about the need of dephasing starch hydrolysis and acid pretreatment of cassava stems was confirmed. Starch

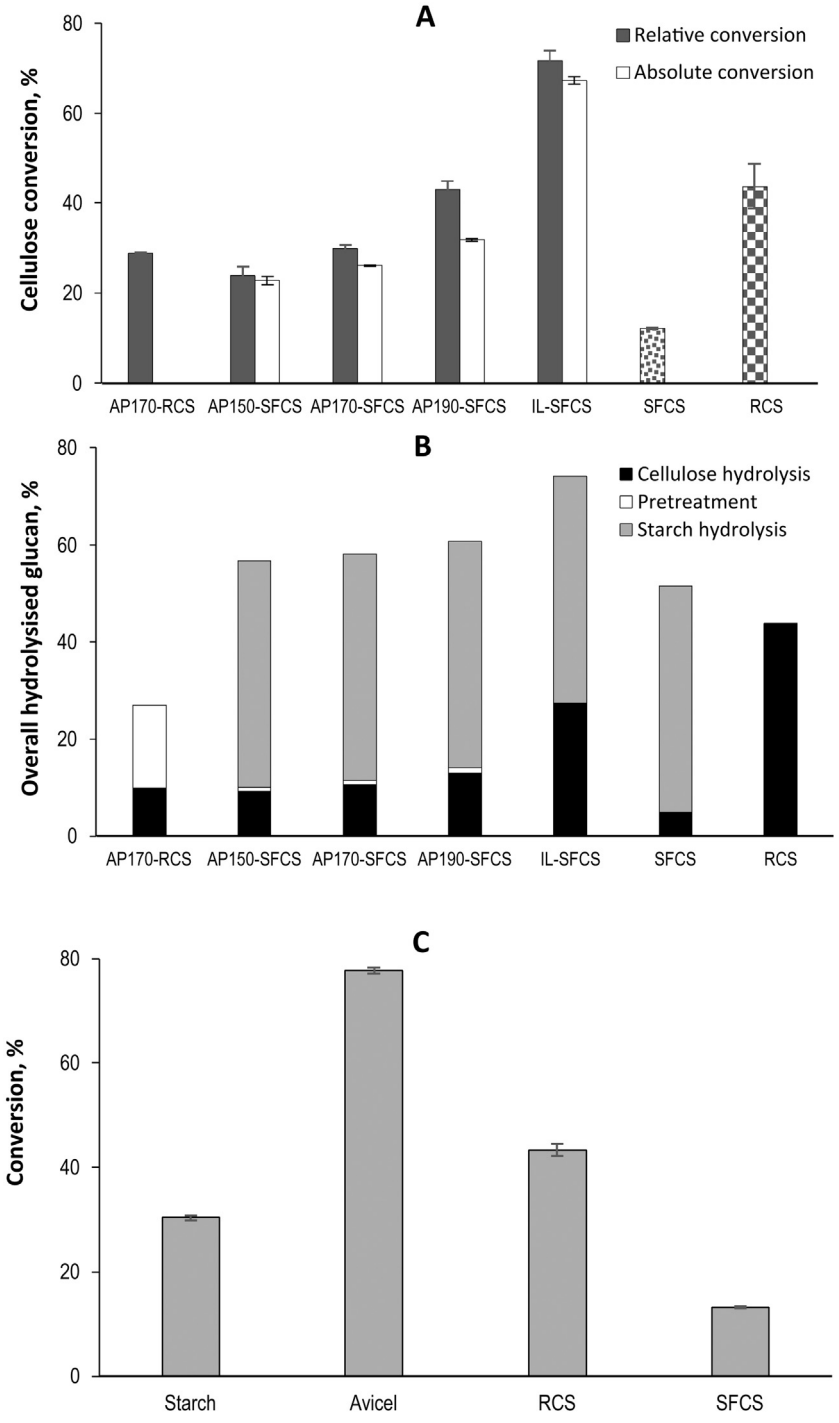

Fig. 5. Enzymatic hydrolysis of cassava stems. A, Conversion of (i) cellulose contained in the pretreated materials, and (ii) glucan contained in the starch-free (SFCS) and raw (RCS) cassava stems; B, Overall hydrolyzed glucan (% of initial content); C, Conversion achieved during enzymatic hydrolysis of different glucan sources. In A and C, the columns represent the mean of two replicates, and the error bars show the standard deviation.

hydrolysis prior to pretreatment minimizes sugar degradation, increases glucan conversion, and maximizes the ethanol production potential of the stems. Pretreating cassava stems with [Emim]OAc reduces cellulose crystallinity and lowers the lignin content, resulting in a stronger enhancement of the enzymatic convertibility of cellulose than that achieved by acid pretreatment.

Cassava stems are a glucan-rich feedstock, with a high proportion of starch, which makes them especially suited for ethanol production or other processes based on the sugar-platform concept.

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References

Atalla, R.H., VanderHart, D.L., 1999. The role of solid state ^{13}C NMR spectroscopy in studies of the nature of native celluloses. Solid State Nucl. Mag. 15, 1–19.

Brandt, A., Hallett, J.P., Leak, D.J., Murphy, R.J., Welton, T., 2010. The effect of the ionic liquid anion in the pretreatment of pine wood chips. Green Chem. 12, 672–679.

Ciolacu, D., Ciolacu, F., Popa, V.I., 2011. Amorphous cellulose – structure and characterization. Cellulose Chem. Technol. 45, 13–21.

Ebner, G., Vejdovszky Ph Wahlström, R., Suurnäkki Schrems, M., Kosma, P., Rosenau, T., Potthast, A., 2014. The effect of 1-ethyl-3-methylimidazolium acetate on the enzymatic degradation of cellulose. J. Mol. Catal. B: Enzym. 99, 121–129.

Falade, K.O., Akingbala, J.O., 2011. Utilization of cassava for food. Food Rev. Int. 27, 51–83.

Farkas, V., 1983. Laminarinases: xylanases and amylases in the crude cellulolytic enzyme complex from *Trichoderma reesei*. Biologia (Bratislava) 38, 721–726.

Gerber, L., Eliasson, M., Moritz, T., Sundberg, B., 2012. Multivariate curve resolution provides a high-throughput data processing pipeline for pyrolysis-gas chromatography/mass spectrometry. J. Anal. Appl. Pyrol. 95, 95–100.

Gräsvik, J., Winestrand, S., Normark, M., Jönsson, L.J., Mikkola, J.-P., 2014. Evaluation of four ionic liquids for pretreatment of lignocellulosic biomass. BMC Biotechnol. 14, 34.

Han, M., Kim, Y., Kim, Y., Chung, B., Choil, G.-W., 2011. Bioethanol production from optimized pretreatment of cassava stem. Korean J. Chem. Eng. 28, 119–125.

Hu, F., Jung, S., Ragauskas, A., 2012. Pseudo-lignin formation and its impact on enzymatic hydrolysis. Bioresour. Technol. 117, 7–12.

Hult, E., Larsson, P., Iversen, T., 2002. A comparative CP/MAS ^{13}C NMR study of the structure of polysaccharides in sulphite and kraft pulps. Holzforschung 56, 179–184.

Ioelovich, M., Morag, E., 2012. Study of enzymatic hydrolysis of mild pretreated lignocellulosics biomasses. BioResources 7, 1040–1052.

Karatzos, S.K., Edye, L., Doherty, W., 2012. Sugarcane bagasse pretreatment using three imidazolium-based ILs; mass balances and enzyme kinetics. Biotechnol. Biofuels 5, 62.

Kirk, P.L., 1950. Kjeldahl method for total nitrogen. Anal. Chem. 22, 354–358.

Kumar, R., Mago, G., Balan, V., Wyman, C.E., 2009. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. Bioresour. Technol. 100, 3948–3962.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H., Auer, M., Vogel, K., Simmons, B., Singh, S., 2010. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. Bioresour. Technol. 101, 4900–4906.

Martín, C., López, Y., Plasencia, Y., Hernández, E., 2006. Characterisation of agricultural and agro-industrial residues as raw materials for ethanol production. Chem. Biochem. Eng. Q. 20, 443–446.

Martín, C., Alriksson, B., Nilvebrant, N.-O., Sjöde, A., Jonson, L.J., 2007. Dilute-sulfuric acid pretreatment of agricultural and agro-industrial residues for ethanol production. Appl. Biochem. Biotechnol. 136/140, 339–352.

Mood, S.H., Golfeshan, A.H., Tabatabaei, M., Abbasalizadeh, S., Ardjmand, M., Jouzani, G.S., 2014. Comparison of different ionic liquids pretreatment for corn stover enzymatic saccharification. Prep. Biochem. Biotechnol. 44, 451–463.

Palmarola-Adrados, B., Chotěborská, P., Galbe, M., Zacchi, G., 2005. Ethanol production from non-starch carbohydrates of wheat bran. Bioresour. Technol. 96, 843–850.

Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A., Johnson, D.K., 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biofuels 3, 10.

Rehman, S., Aslam, H., Ahmad, A., Khan, S.A., Sohail, M., 2014. Production of plant cell wall degrading enzymes by monoculture and co-culture of *Aspergillus niger* and *Aspergillus terreus* under SSF of banana peels. Braz. J. Microbiol. 45, 1485–1492.

Rodrigues, J., Faix, O., Pereira, H., 1998. Determination of lignin content of Eucalyptus globulus wood using FTIR spectroscopy. Holzforschung 52, 46–50.

Sluiter, A., Sluiter, J., 2008. Determination of starch in solid biomass samples by HPLC. Technical Report NREL/TP-510-42624. Golden, Colorado.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008a. Determination of structural carbohydrates and lignin in biomass. Technical Report NREL/TP-510-42618. Golden, Colorado.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008b. Determination of extractives in biomass. Technical Report NREL/TP-510-42619. Golden, Colorado.

Sriroth, K., Piyachomkwan, K., Wanlapatit, S., Nivitchanyong, S., 2010. The promise of a technology revolution in cassava bioethanol: from Thai practice to the world practice. Fuel 89, 1333–1338.

Terinte, N., Ibbett, R., Schuster, K.C., 2011. Overview on native cellulose and microcrystalline cellulose I structure studied by X-ray diffraction (WAXD): comparison between measurement techniques. Lenzinger Berichte 89, 118–131.

Uchechukwu-Agua, A.D., Caleb, O.J., Opara, U.L., 2015. Postharvest handling and storage of fresh cassava root and products: a review. Food Bioprocess Technol. 8, 729–748.

Wang, K., Yang, H., Yao, X., Xu, F., Sun, R.C., 2012. Structural transformation of hemicelluloses and lignin from triploid poplar during acid-pretreatment based biorefinery process. Bioresour. Technol. 116, 99–106.

Wei, M.G., Zhu, W.B., Xie, G.H., Lestander, T.A., Wang, J.S., Xiong, S.J., 2014. Ash composition in cassava stems originating from different locations varieties, and harvest times. Energy Fuels 28, 5086–5094.

Wei, M.G., Zhu, W.B., Xie, G.H., Lestander, T.A., Xiong, S.J., 2015. Cassava stem wastes as potential feedstock for fuel ethanol production: a basic parameter study. Renew. Energy 83, 970–978.

Xu, X.-Q., Wu, X.-B., Cui, Y., Cai, Y.-X., Liu, R.-W., Long, M.-N., Chen, Q.-X., 2014. Enzymatic saccharification of cassava residues and glucose inhibitory kinetics on β-glucosidase from Hypocrea orientalis. J. Agric. Food Chem. 62, 11512–11518.

Zhu, B., Lestander, A., Örberg, H., Wei, G., Hedman, B., Ren, J.W., Xie, G.H., Xiong, S.J., 2015. Cassava stems: a new resource to increase food and fuel production. GCB Bioenergy 7, 72–83.