Title: Assessment of the effect of autohydrolysis treatment in banana's pseudostem pulp

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ABSTRACT

Banana's pseudostem pulp (BPP) is a potential by-product obtained in the mechanical fiber extraction of banana's pseudostem. Its chemical characterization revealed to have an interesting composition, with a high polysaccharides content and low content in lignin, which makes it particularly relevant for the biorefinery's biochemical platform. Autohydrolysis pretreatment, studied under isothermal (140 ºC) and non-isothermal conditions (140 – 220 ºC), yielded oligosaccharides, mainly gluco-oligosaccharides, as the main soluble products. The highest oligosaccharides production (24 g/100 g raw material) was obtained at a severity factor of 2.3. Autohydrolysis pretreatment effectively disrupted the structure of the material, inducing an improvement of the enzymatic digestibility from 73% for the raw material up to 90% for the most severe conditions. Two stage autohydrolysis, with increasing severity, was also studied, allowing to obtain a higher amount of oligosaccharides (32 g/100 g raw material) and higher digestibility of the remaining solid (up to 97%).

Keywords:

Autohydrolysis; Banana wastes; Biomass pretreatment; Biorefinery; Enzymatic digestibility; Oligosaccharides

1. Introduction

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues. Banana is cultivated over 130 countries, being the second largest produced fruit, after citrus (Mohapatra et al., 2010), and its residues are available around the world (Gabhane et al., 2014), including in Europe. Global production reached a record of 114 million tons in 2017 (FAO). Canary Islands is the largest banana producer region in the European Union, with 0.4 million tons of bananas produced each year (ASPROCAN).

As banana plants only bear fruit once in its lifecycle, once they have been harvested the plant is cut, producing significant amounts of agricultural residues. For each ton of fruit harvested, around four tons of lignocellulosic wastes are generated, among which 75% consists of banana plant pseudostem (Souza et al., 2014). This by-product is sometimes processed into low-grade animal feed by local farmers and has been used to produce various handcrafts, eating utensils, food wrapping, etc. (Santa-Maria et al., 2013); however in most cases it is usually left in the plantation, producing wastes accumulation and having no nutritional value for the soil. An interesting strategy to manage these wastes is the development of new applications, which could also represent an interesting income for banana producers, thus boosting the regional economy (Oliveira et al., 2007), particularly in the Canary Islands, where banana crop is an essential socio-economic pillar.

Mechanical fiber extraction is one of the most relevant alternatives proposed for the valorization of the pseudostem (Saraiva et al., 2012). This material contains 90% of moisture, 0.6% of fiber and 9.4% of pulp (Benítez et al., 2013). Banana fiber has high strength, lightweight, low elongation and shiny appearance, among other textile qualities (Sengupta et al., 2019) and it has been proven in different applications like composite materials (Ortega et al., 2013). Fiber extraction also produces important

amount of a lignocellulosic by-product, banana's pseudostem pulp (BPP), which is the raw material in this study, and whose characterization and exploitation has not been yet explored in the literature. The use of the entire pseudostem and not only the fiber would improve the economic balance of fiber production, making it more attractive and launching its industrial production. On the other hand, these results could be used in abaca (*Musa textilis*) plantations, whose only product is fiber (over 80 000 tons/year) (Ortega et al., 2013).

Liquid hot water (LHW) treatment, or autohydrolysis, is among the most promising fractionation technologies for lignocellulosic biomass-based biorefineries, presenting the attractive that uses just compressed hot water for biomass treatment (Carvalheiro et al., 2016). This process is considered to be the most appropriate choice for the selective separation of hemicelluloses (Moniz et al., 2014), reason why it has been applied primarily to biomass rich in this component. Besides, autohydrolysis is suitable for oligosaccharides (OS) production (Moniz et al., 2014), which are receiving substantial attention due to their functional properties and health benefits as active ingredients in functional foods (Carvalho et al., 2013). The type of oligosaccharide that can be obtained will depend on the raw material and on the operation conditions. On the other hand, autohydrolysis allows the recovery of cellulose and lignin in a solid phase in advantageous conditions (altered surface and improved digestibility) for further processing.

The considerable amount of non-structural glucans in BPP turns it into a suitable candidate for hydrothermal treatments, despite its low hemicellulose content (Guerrero et al., 2017). The effect of the LHW treatment demonstrated for other raw materials encourages the study of this feedstock as a strategy of upgrading BPP in the biorefinery's context. Some references applying hydrothermal treatments to agricultural waste from banana crops have been found. One of them (Santa-Maria et al., 2013)

compared autohydrolysis and steam explosion (hydrothermal treatment using steam instead of liquid water), for the three main lignocellulosic residues of banana plants (leaves, pseudostem and rachis). Other authors (El-Zawawy et al., 2011; Kamdem et al., 2015) evaluated steam explosion treatment on different combined morphological parts of banana plant, while Guerrero et al. (2017) also evaluated steam explosion pretreatment of banana lignocellulosic biomass (rachis and pseudostem) but using an acid as catalyst. However, these studies mainly focused on improving biomass digestibility for bioethanol production, not paying any attention to oligosaccharides production in the liquid fraction. Moreover, no studies dealing with hydrothermal treatment of post-fiber extraction wastes from banana pseudostem have been found.

On the other hand, two-stage autohydrolysis has been proposed in literature for different goals. For example, it can be used as an alternative to a previous ethanol extraction in order to remove easily extractable compounds (as waxes, low molecular weight phenolics and soluble inorganic compounds), facilitating the purification of the liquor obtained in the second stage (Charalampopoulos and Rastall, 2009). Furthermore, the removal of these extractives can improve the oligosaccharide yields attained after hydrothermal processing (Alves-Ferreira et al., 2019). On the other hand, Lee et al.(2010) used a combination of a mild pretreatment to extract hemicelluloses followed by a harsher pretreatment to increase the enzymatic digestibility of the residue as a strategy for the full recovery of sugars from Coastal Bermuda grass. Agricultural waste from banana crops usually presents high contents in extractives, reason why two consecutive autohydrolysis becomes an interesting option.

The goal of this work is to study the production of OS by application of hydrothermal pretreatment on BPP. For this, isothermal and non-isothermal autohydrolysis at different final temperatures (140 – 220 ºC) on BPP were performed.

The effect of the treatment in the recuperation of OS in the liquid fraction, as well as the impact on the enzymatic digestibility and composition of the solid fraction, was evaluated. Moreover, a pretreatment composed of two consecutive autohydrolysis of increasing severity was also evaluated with the objective of obtaining a higher amount of oligosaccharides.

2. Material and methods

2.1. Materials

Pseudostems from *Musa acuminata Dwarf Cavendish* (Gran Enana cultivar) collected from an agro-industrial plantation at Arguineguín, South of Gran Canaria, Spain, were subjected to mechanical fiber extraction using a pilot plant available at Universidad de Las Palmas de Gran Canaria. The produced BPP was used as raw material for this study. It was dried to constant weight, at 40 ºC, and then milled with a knife mill (Fritsh Industriestr, Germany) to particles smaller than 6 mm, after which the sample was homogenized and stored at room temperature. The distribution of particle size was estimated using a vibratory sieve shaker and the 1.0-2.0 mm fraction was collected for isothermal autohydrolysis.

2.2 Hydrothermal processing

2.2.1. Non-Isothermal conditions

Non-isothermal autohydrolysis treatments were performed in a 2 L Stainless steel stirred reactor (Parr Instruments Company, USA). A liquid to solid ratio 11, on weight basis, was used. The raw material was mixed with distilled water in the reactor. The agitation speed was set at 150 rpm and the reactor was heated to reach the desired final temperature and rapidly cooled down. Tested temperatures ranged from 140 to 220 ºC Replicates (carried at selected conditions) present high consistency, always differ by

less than 10% and typically by less than 5%. Temperature, pressure and power consumption were monitored during the process. Upon cooling to room temperature, the liquid and solid phases were separated using a manual hydraulic press. The liquor was filtered and analyzed by HPLC (as described in section 2.3.4. Chemical characterization of liquors)and the solid was washed with twice the amount of water and pressed again. The solid was weighed, and its moisture determined, in order to calculate the solid yield, dry basis (Y_S, total solids after pretreatment/total solids before pretreatment).

Severity factor (log R_0) was estimated, based on the measured temperature profiles data, using the following equation, for non-isothermal conditions (Carvalheiro et al., 2016):

$$R_O = \int_0^t exp\left(\frac{T(t)-T_{ref}}{\omega}\right) \cdot dt \qquad \textbf{(Eq. 1)}$$

where T (t) is the temperature as a function of time, T_{ref} is the temperature up to which the hydrolysis process is considered to be negligible (usually 100 ºC) and ω is a term that can be related to a conventional energy of activation, typically 14.75 (Carvalheiro et al., 2016).

For the two consecutive autohydrolysis, the wet material pretreated (final temperature of 150 ºC) and washed as described was mixed with water with the same liquid to solid ratio of 11, taking into account the moisture content. The treatment was carried out as detailed above, using a final temperature of 180 ºC, after which it was treated also as described above. For severity factor calculation, it was assumed that two stages are dependent and so, the second stage pretreatment effectiveness depends on phenomena occurring in the first stage (Lee et al., 2010); thus, the two-stage process is a combination of reaction ordinate of the first stage $R_{0,1}$ and the second one $R_{0,2}$ and the severity factor was calculated as log (($R_{0,1}$ + $R_{0,2}$)).

2.2.1. Isothermal conditions

Isothermal tests were conducted in mini pressure-reactors (ACE Glass Pressure Tube, 15 mL Capacity, Vineland, NJ) incubated in a thermostatic oil bath. Treatments were performed using 0.5 g of dry material with a particle size of 1.0-2.0 mm at 140 ºC and a liquid to solid ratio of 20, on weight basis. Upper charges of solid were not used because the low density and the hygroscopic behavior of the material hindered the agitation process. Different reaction times (between 120 and 300 min) were tested. After the treatment, the solid and the liquid were recovered separately by filtration. The liquors were analyzed by HPLC (as described in Analytical methods section). The solid was washed and dried to determine the solid yield.

2.3 Analytical methods

2.3.1 Chemical characterization of raw material and pretreated solids

Both the raw material and the pretreated solid obtained after autohydrolysis were chemically characterized, after drying and milling to particles smaller 0.5 mm using a Retsch Ultra Centrifugal Mill ZM200. The moisture was determined by oven-drying at 105 ºC and the ash content at 550 ºC using NREL protocols (Sluiter et al., 2008a). Quantitative acid hydrolysis (QAH) was carried out according to NREL protocol (Sluiter et al., 2008b), allowing the determination of monosaccharides (glucose, xylose, and arabinose), acetyl groups and Klason lignin. The samples were mixed with 72% (w/w) sulfuric acid for 60 min at 30 ºC, diluted to a 4% (w/w), hydrolyzed in an autoclave at 121 ºC for 60 min and rapidly cooled down. A sample of the liquid was filtered to analyze it by HPLC using the system described in Chemical characterization of liquors section and using H_2SO_4 5 mM with a flow rate of 0.4 mL/min as the mobile phase. The rest of the hydrolyzed solution was filtered through a filtering crucible number 3 and the solid was washed with deionized hot water and dried to determine the acid insoluble lignin (Klason lignin) content, after correction for ash.

199 Total extractives of the raw material were determined sequentially with deionized water
200 and ethanol 98% using a Soxhlet apparatus (Sluiter et al., 2008b). QAH of the
201 extractive-free material was also performed. Pectin content of the extractive-free raw
202 material was determined by extraction with hot ammonium citrate (0.1 g/mL) for 1 hour
203 as described in (Fernandes et al., 2015). In addition, total starch content was measured
204 according to AOAC Method 996.11 using the Total Starch Assay Kit (AA/AMG)
205 (Megazyme). Protein content was estimated by the Kjeldajhl method, using a
206 conversion nitrogen to protein factor of 6.25.

207 Holocellulose content was determined according to (Browning, 1967) by selective
208 solubilization of lignin with glacial acetic acid and sodium chlorite in a water bath at
209 70-80 ºC. After that, samples were cooled in a water-ice bath until the temperature
210 dropped to 10 ºC and filtered through a filtering crucible number 2 previously dried to
211 constant weight. The solid was washed with water and dried at 60 ºC to determine the
212 holocellulose content, after corrections for moisture and ash. Cellulose was determined
213 using ANSI/ASTM (American National Standard Institute, 1977b) protocol. Briefly,
214 holocellulose obtained by previously described protocol was treated sequentially with
215 sodium hydroxide 17.5% and 8.3% at room temperature, filtered, and washed with 50
216 mL of NaOH 8.3% and deionized water three times. After that, the suction was
217 interrupted, and the sample was put in contact with 10% glacial acetic acid for 3
218 minutes and washed until neutralization. The solid was dried at 105 ºC to determine the
219 cellulose content, after corrections for ash. Hemicellulose was calculated as the
220 difference between holocellulose and cellulose.

221 2.3.2 Enzymatic digestibility of raw and pretreated materials

Enzymatic digestibility of pretreated solids and untreated material was evaluated according to NREL/TP-510-42629 protocol (Selig et al., 2008). The material was air dried at 40 ºC and milled to <0.5 mm. The reaction mixture contained 0.15 g of biomass (dry weight basis) 5 mL of sodium citrate buffer (0.1 M, pH 4.8), 100 µL of sodium azide solution (2% w/v) as an anti-microbial agent and the amounts of cellulase (Celluclast 1.5L from Novozymes, 51 FPU/mL) and cellobiase (Novozyme 188, 686 IU/mL) necessary to obtain 60 FPU/g and 64 pNPGU/g of dry biomass, respectively. Enzymatic activities were measured according to (Ghose, 1987). Total volume was adjusted to 10 mL with water. Each biomass sample was hydrolyzed in duplicate. A biomass blank (without enzymes) for each biomass and an enzyme blank (without biomass) were also carried out to correct the results. Assays were performed in an orbital shaker incubator (TEQ, Portugal) at 50 ºC and 150 rpm during 72 h and after that, samples were boiled for 5 min, and rapidly cooled in order to inactivate the enzymes. The samples were then centrifuged at 13,000 × g, filtered through 0.45 µm membrane filters and analyzed by HPLC (Aminex HPX-87H column), as described below (section 2.3.4). Enzymatic digestibility was calculated by equation 2.

Digestibility (%) = (glucose obtained / potential glucose in the substrate) x 100 **(Eq 2)**

2.3.3 Scanning electron microscopy

Untreated and pretreated materials were analyzed by scanning electron microscopy (SEM; Hitachi TM 3030 at an acceleration voltage of 15 kV) using different magnification (x100, x600 and x1200). Samples were not subjected to sputtering process before SEM observation.

2.3.4 Chemical characterization of liquors

Liquor from autohydrolysis were directly analyzed by HPLC. For oligosaccharides quantification, an aliquot sample was subjected to post hydrolysis with 4% (w/w) H_2SO_4 at 121 ºC for 60 min, and the increase in sugar monomers measured was used to determine the oligosaccharides concentration. Elution took place at 50 ºC with 5 mmol/L H_2SO_4 at a flow rate of 0.6 mL/min. Glucose, xylose, arabinose and acetic acid were detected with the RI detector; furfural and HMF were detected with the UV detector set at 280 nm. An Aminex HPX-87H column (Bio-Rad, USA) in combination with a cation H^+-guard column (Bio-Rad) was used in an Agilent Technologies Liquid Chromatographer 1100 Series System (Santa Clara CA, USA), equipped with a diode array detector (DAD) and a refractive index detector (RI).

For the characterization of the polymerization degree of oligosaccharides, liquors were injected in Phenomenex Rezex RSO-Oligosaccharide Ag^+ (200 x 10 mm) column at 80 ºC, preceded by 60x10 mm guard column with the same filling. Ultrapure water at 0.3 mL/min was used as eluent. Calibration was carried out using standards indicated in section 2.1.2. A sample of a light syrup sample (mainly composed of malto-oligosaccharides) from Phenomenex was also injected in the column for polymerization degree (PD) comparison.

Liquor obtained under optimal conditions was analyzed for total phenolic compounds by the Folin–Ciocalteu colorimetric method according to (Singleton et al., 1999) and expressed as mg GAE/mL (gallic acid equivalents).

3. Results and discussion

3.1 Biomass characterization

The chemical composition of BPP is shown in Table 1. As observed, the raw material presents a high content of polysaccharides (above 60 %) mainly composed of glucans.

Table 1. **Chemical composition of untreated banana's pseudostem pulp (BPP) (% w/w, dry basis)**

Especially interesting is the low content in lignin, compared with other herbaceous biomasses such as rice straw (14.4%) (Moniz et al., 2014), corn stover (18.5%) (Mittal et al., 2017) or wheat straw (26.5%) (Jaisamut et al., 2013). The measured lignin is in the range obtained by other studies with the entire banana's pseudostem, which varies between 4.70% (Duque et al., 2015) and 12% (Thakur et al., 2013). The low content in lignin could be an advantage to achieve good enzymatic hydrolysis yields without pretreatment or using less severe conditions (Guerrero et al., 2017).

In this work, hemicellulose was estimated by two different procedures, being one of them as the sum of xylan, arabinan, and acetyl groups. Although xylans are the major component of hemicellulose and, concretely in herbaceous crops, glucuronoxylan is the primary hemicellulose (Thomas, 2003), they also contain glucose units. For banana pseudostem, Cordeiro et al.(2004) found a considerable presence of glucose in hemicellulosic fraction. For this reason, a sub-quantification of this component could occur, when characterized according NREL procedure, compared to hemicellulose content determined as the difference between holocellulose and cellulose.

Hemicellulose content found for BPP is relatively low (15-19%) in comparison with other herbaceous biomasses like rice straw (24.3%) (Moniz et al., 2014). Hemicellulose content varies in the different studies found for the entire pseudostem: 9.6% (Guerrero et al., 2017), 29.4% (de Souza et al., 2017), 19.62% (Shimizu et al., 2018); results obtained in this study are within the range found in these studies. The low content in hemicellulose and in acetyl groups could have a negative effect in the autohydrolysis, resulting in a softer effect than working with a typical lignocellulosic biomass, where

294 acetyl groups can account for 1-6% of carbon (Guerrero et al., 2017). The high ash
295 content, which could have a buffer effect on the acetyl groups generated during the
296 autohydrolysis, is also remarkable; other authors (Guerrero et al., 2017, 2016; Li et al.,
297 2016) have also found these levels of ashes, not common for annual plants (Cordeiro et
298 al., 2004), in practically all parts of banana plant; ashes have been related to nutrient
299 transport (Oliveira et al., 2007).

300 The high extractives content for the pseudostem have also been highlighted in literature,
301 when compared with other annual plants (Cordeiro et al., 2004). An important part of
302 these extractives is composed of extractable ashes (9.05 %), as showed the QAH of the
303 raw and extractive free material. Other part of the extractives are free sugars, calculated
304 as the difference of measured sugars in the original and the extractive free material
305 (2.8% of glucan).

306 BPP showed a high starch content, as corroborated by other authors for the pseudostem.
307 Guerrero et al. (2017) reported a content of starch of 20.1% while Bhaskar et al. (2012)
308 obtained a 27.3%. Besides, Oliveira et al.(2007) divided the pseudostem in leaf sheaths
309 and floral stalk and obtained a starch content of 26.3% and 8.4%, respectively. Starch
310 content was also determined to the extractive free BPP, showing that most of this was
311 not solubilized during the extractions with water and ethanol. Due to the considerable
312 presence of starch, cellulose cannot be estimated as glucan content. Cellulose content
313 calculated as the difference between glucan (QAH) content and starch was 22.42%, and
314 according to (ANSI/ASTM, 1977b) was 26.29%, which highly differs from the values
315 of around 40% reported in other studies (Abdullah et al., 2014; de Souza et al., 2017;
316 Oliveira et al., 2007). The closest cellulose content reported by other authors was 20.1%
317 (Guerrero et al., 2017). It is important to remark that the material used in this study was

318 fiber-free pseudostem and that banana fiber presents a high content of cellulose, 64%
319 (Idicula et al., 2006), that is not present in BPP.

320 As shown in Table 1 pectin is found in the BPP, being this amount more than double the
321 amount previously determined in the pseudostem (3.9%) (Jayaprabha et al., 2011).
322 Pectin is a valuable material in the food and pharmaceutical industries (May, 1990),
323 acting as a gelling agent, stabilizing agent in drinks or as a gelatin substitute in baked
324 foods (Xiao and Anderson, 2013). Moreover, the benefits of pectin consumption for
325 human health have been also demonstrated: to reduce blood cholesterol levels in
326 humans (Brouns et al., 2012) or to enhance the immune system (Jun Yan and Katz,
327 2010; Maxwell et al., 2012; Nangia-Makker et al., 2002) are some of its proofed effects.
328 These applications, among others, make pectin and its derived forms a potentially high-
329 value component of BPP (Xiao and Anderson, 2013). Pectins could be obtained by
330 conventional methods, typically comprised with two main steps, acid hydrolysis and
331 subsequently precipitation by ethanol (Sandarani, 2017).

332

333 3.2 Non-isothermal autohydrolysis

334 3.2.1 Composition of the liquors

335 The liquid phases resulting from the non-isothermal autohydrolysis treatments were
336 mainly composed of a mixture of oligomeric compounds (gluco-oligosaccharides
337 (GlcOS), xylo-oligosaccharides (XOS), arabino-oligosaccharides (AOS) and acetyl
338 groups linked to oligosaccharides (AcOS)), monosaccharides (xylose, arabinose and
339 glucose), acetic acid and products resulting from the decomposition of sugars (furfural
340 and HMF), in addition to other compounds that can be solubilized. These compounds
341 may include lignin and products resulting from its decomposition or compounds from

342 pectins solubilization such as galacto-oligosaccharides (included in the quantification of
343 XOS) or galacturonic acid.

344 The liquors composition varied with the severity conditions of the treatment. Contrary
345 to what could be expected, high ash content did not have a neutralizing effect, as it was
346 reflected in pH values of the liquors, which varied between 4.6 and 3.2, for less and
347 more severe conditions respectively. Sugars and oligosaccharides were predominantly
348 found as glucose and GlcOS. GlcOS concentration increased with severity factor
349 achieving a maximum of 24.07 g/100 g of raw material, at $\log R_0$=2.3 (150 ºC), but for
350 higher values of $\log R_0$, concentrations started to decrease. Figure 1 shows the main
351 products yields obtained in the liquor fraction (per 100 g of raw material), as a function
352 of the severity factor.

353

354 **Figure 1. Chemical composition of the liquors obtained for the non-isothermal**
355 **autohydrolysis of banana's pseudostem pulp for different severity factors.**

356

357 Although during autohydrolysis the most common situation corresponds to a
358 majorsolubilization of hemicelluloses (Carvalheiro et al., 2016), a low amount of XOS
359 and AOS was obtained in this occasion, being gluco-oligosaccharides most part of
360 obtained OS, as mentioned. Similar behavior was obtained by Guerrero et al.(2017)
361 using steam explosion with banana plant pseudostem. The separation performed on
362 Rezex RSO-Oligosaccharide column revealed that these GlcOS derived from starch
363 fraction of BPP instead of cellulose, because retention times obtained in the
364 chromatogram (Figure 2) matched with those for a sample of light corn syrup (made of
365 malto-oligosaccharides) instead of matching the ones of injected cello-oligosaccharides.

Figure 2. Molecular weight distribution of the products obtained in the autohydrolysis of banana's pseudostem pulp liquors and cello-oligosaccharides and malto-oligosaccharides patterns.

Different mixtures of oligosaccharides with different molecular weight distribution were obtained depending on the severity of the treatment. Figure 2 shows how high molecular weight oligosaccharides were produced initially (peaks at lower retention times), later being broken into lower molecular weight OS (while peaks at longer retention times started to appear, the ones at lower times disappear) as the severity of the process increases.

No significant increase in monosaccharides and degradation products concentration was observed with GlcOS decrease (Figure 1). The low formation of degradation products and monomeric compounds can be related to a sub-quantification due to different factors. On the one hand, the precipitation and/or condensation of these products with lignin present in the solid phase has been previously described (Xiang et al., 2004). The mass balance of lignin showed a lignin increase, being this more significant for more severe conditions, and so glucose recombination with acid-soluble lignin is expected to have occurred during autohydrolysis. Besides, due to the volatile character of some compounds, they can be eluded in the gas phase (Moniz et al., 2014), being the loss higher as the autohydrolysis temperature increases, as reflected by material balances reported by other authors (Pu et al., 2013). The formation of volatile compounds can be also observed in the pressure and temperature data profiles (supplementary material) which clearly evidences an increase on the system pressure as compared to the blank (water) treatment. These compounds (acetic acid as well as sugar degradation

391 compounds, such as furfural and HMF) are more volatile than water, therefore
392 contributing to an increase in overall system pressure as compared to water in the same
393 conditions (Carvalheiro et al., 2016). On the other hand, other reaction by-products that
394 may arise from autohydrolysis are formic acid and levulinic acid from degradation of
395 furfural and HMF (Ho et al., 2014). Although these compounds were not quantified, its
396 presence was detected in HPLC chromatograms for the assays under the most severe
397 conditions.

398 As explained above, the optimal conditions for OS production corresponded to a
399 severity factor (Log R_O) of 2.3 (maximum operation temperature and pressure of 150 ºC
400 and 200 psi , which are mild operation conditions), obtaining a solid recovery, Y_S, of
401 52.0%. On the other hand, the hydrolysate recovery (recovered liquor/initial water) was
402 74.8%. Considering the liquor recovered, the yield in malto-OS production was 17.8
403 g/100 g raw material. Note that there is a residual amount of OS in the liquor that was
404 not recovered and other part remained adhered to the solid. This fraction might be
405 recovered through a washing stage, but the feasibility of water management should be
406 addressed. Similar yields have been obtained with other feedstock; 15 g
407 oligosaccharides/100 g feedstock (log R_0=3.07) was obtained using *Cistus ladanifer*
408 residues from essential oil distillation (Alves-Ferreira et al., 2019), while 10.5 g of
409 XOS/100 g wheat straw was obtained for a severity of 3.96 (Carvalheiro et al., 2009), or
410 yields of around 17 g oligosaccharides/100 g feedstock were obtained for different
411 lignocellulosic sources with severity factors between 3.62 and 3.75 (Silva-Fernandes et
412 al., 2015). Banana pseudostem studies do not focus on OS but mainly in sugars
413 production; in this sense, steam explosion allows obtaining 22 g of glucose/100 g, for a
414 combined severity factor of 2.3 (Guerrero et al., 2017).

Although XOS and AOS coming from lignocellulosic materials are receiving more attention due to their functional properties and health benefits (Moniz et al., 2014), malto-oligosaccharides are also interesting from the point of view of acting as prebiotics (O. Ibrahim, 2018; Panesar et al., 2013; Tamime and Thomas, 2017).

Finally, degradation products (furfural and HMF) could act as inhibitory of further solid biochemical processes stages, and so a purification stage might be needed. It is interesting to note that, under optimal conditions (severity factor of 2.3), these products were not detected. Moreover, low concentration of phenolic compounds (0.313 mg GA/mL) was observed at these conditions. However, the oligosaccharide-containing hydrolysates also contain other compounds (including monosaccharides, waxes, products from the extractive and acid-soluble lignin fractions, soluble inorganic components of the feedstock, and protein-derived products (Moure et al., 2006)) specially in this case, in which the material has a high content in extractives and thus, purification processes may be required. It can be achieved using several treatments or a combination of them, including precipitation, extraction, adsorption to surface active materials, chromatographic separation techniques and membrane separation technology (Carvalheiro et al., 2016). On the other hand, during the single-stage autohydrolysis a significant part of the dissolved feedstock corresponds to easily extractable compounds; some studies (Moure et al., 2006) have proposed two consecutive aqueous treatments of increasing severity in order to increase the selective solubilization of other fractions during the second step and remove the need of a further purification step. This approach has also been followed in the present study, being the first one in studying the two-steps LHW for BPP.

3.2.2 Composition of the solids

Chemical composition of the solids from non-isothermal autohydrolysis are shown in Figure 3. In general, solid recovery decreased at higher severity due to solubilization of a larger part of the biomass. Solid recovery was also influenced by the losses resulting in pressing stage, due to the presence of fines in the biomass (generated during the milling of it, previous to the autohydrolysis). Experimental results showed that, because of some ashes and extractives removal, a solid enriched in hemicellulose, glucan and lignin is obtained. Due to solubilization of starch fraction, glucan percentage remained practically unchanged. In this case, the majority of glucans will correspond to cellulose fraction that stays practically unaltered. Lignin content increased with the severity factor, indicating that its partial solubilization did not occur in the studied conditions.

Figure 3. Effect of the severity factor on the solid yield and composition of processed solids obtained after autohydrolysis of BPP under non-isothermal conditions

The hemicellulose content in the solid fraction slightly decreased with the severity, increasing at the same time the ratio of lignin. For low severity conditions, xylans do not seem to be solubilized but for values from 3.01, their solubilization starts. Concerning arabinan content in the solid, in general, it decreased with the severity factor and for the most extreme conditions, all arabinans were solubilized. This is in accordance with previous described behavior, acetyl groups are the first to be hydrolyzed, followed by arabinan and xylan (Carvalheiro et al., 2009). With respect to acetyl groups, only a 0.85% was found in the initial solid. High values of acetyl groups determined for pretreated solids can be due to an overestimation. Determination of acetyl groups by sulfuric acid total hydrolysis is believed to give excessive values, due to the formation of acetic acid from sugar degradation (Leschinsky et al., 2009). Also,

during the autohydrolysis pretreatment at high severity extra acetic acid can be formed due to the carbohydrate degradation and lignin oxidation and, as a result, the content of the acetyl group in biomass or its derived solid and/or liquid samples is overestimated (Hu et al., 2018).

Autohydrolysis pretreatment applied to other feedstock allowed to obtain similar results, in general, pretreatment results in an enrichment in cellulose and lignin content (Moniz et al., 2013; Romaní et al., 2011).

3.2.3 Digestibility

Hydrothermal-based processes can be advantageously combined with other processes to achieve the ultimate end of an integral valorization of all biomass components. After hydrothermal processing, enzymatic hydrolysis (EH) is the most promising method for the valorization of the remaining cellulose-enriched solids (Carvalheiro et al., 2016). However, for an efficient EH it is important that the pretreatment can alter the structure of biomass, which can be reflected in the values of digestibility.

A high digestibility was obtained for the raw material: 73.25% using a particle size <0.5 mm (like in pretreated solids analysis) and 45.83% using a particle size <6mm. This result is in accordance with (Guerrero et al., 2017), that presented a digestibility of 42.7% for untreated pseudostem using the same enzyme charge and similar particle size. This same study explains this high yield for the untreated biomass, compared with other untreated materials (21.4% for corn stover (Y. Zhou et al., 2010) or 32.78% for rice straw (Moniz et al., 2015)), because of the starch content of this feedstock. At this point, it is important to remind the BPP has been obtained from a mechanical process (developed for fiber extraction; patent WO2014/174115), which could act as a physical

pretreatment altering the structure of biomass. Besides, the removal of the long fiber (mainly made of crystalline cellulose), could also influence the material's digestibility.

The effect of autohydrolysis was reflected in the values of digestibility (Figure 3). In general, digestibility increased with the severity factor, obtaining the maximum yield (90.73%) for a severity factor of 3.79. These results are in line with values reported for similar pretreatment for other feedstock. Autohydrolysis led to EH yield improvement in rice straw from 32.8 to 88% (Moniz et al., 2015) or from 31.5 to 90% (Moniz et al., 2013) for corn straw. The values of digestibility here achieved are higher than those obtained in other papers for banana wastes; autohydrolysis of banana pseudostem (10 min at 180 ºC and 10% solids (w/v)) led to around 50% of glucan conversion (Santa-Maria et al., 2013). Other pretreatments applied to the entire banana pseudostem include steam explosion, acid and alkali pre-treatments, among others. Digestibility values obtained in the present study are comparable to the ones obtained for those pretreatments; Santa-Maria et al.(2013) obtained a 77 % glucan conversion for steam explosion treated material (76.4 min at 200 ºC), while the pretreatment of the pseudostem with a 25% alkali solution (Shimizu et al., 2018) achieved a saccharification yield of 85%; stem explosion catalyzed by acids resulted in a 97.5% of EH yield (Guerrero et al., 2017), at 187.5 ºC and 2.2% (v/v) of sulfuric acid.

LHW presents the advantage, over other technologies, that no chemicals are added. This is in line with the sustainable principles of biorefineries, namely the utilization of green processing technologies, efficient utilization of feedstock, avoiding waste generation, and the limitation of energy consumption and environmental impact (Carvalheiro et al., 2016). Besides, autohydrolysis allows obtaining oligosaccharides, added-value products with applications in the pharmaceutical and functional food markets.

513 The high values of digestibility obtained for the autohydrolysis pretreated BPP turns it
514 into a good candidate for EH. The sugar solutions thus obtained can be used as
515 fermentation media for the production of bioethanol, lactic acid, acetone-butanol, 2,3-
516 butanediol, 1,3-propanediol, xylitol, or enzymes, among other products in the
517 biorefinery's context (Carvalheiro et al., 2016). Besides, the pre-treated solid can be
518 used in the production of more oligosaccharides (cello-oligosaccharides, in this case)
519 using enzymatic hydrolysis; further assays in this sense have been performed by the
520 authors of this paper (data not shown).

521 On the other hand, the increase of digestibility with the severity factor can be related to
522 the disruption of the morphological structure of the biomass, which include the increase
523 in the accessible surface area, the size of pores and decrease the crystallinity of cellulose
524 (Carvalheiro et al., 2016). This physical changes make the material more susceptible to
525 the action of the enzyme and consequently improve enzymatic hydrolysis.SEM pictures
526 (Figure 4) showed the change in morphology, being this disruption more evident for
527 more severe conditions.

528

529 **Figure 4. SEM images of BPP before (A) and after the autohydrolysis**
530 **pretreatment at 160 ºC (B, log R_O = 2.73) and 200 ºC (C, log R_O = 3.79)**

531

532 3.2.4 Two-stage autohydrolysis

533 Due to the high content in extractives and starch of the raw material, two-stage
534 autohydrolysis becomes an interesting option to extract these components as a first step
535 in the fractionation, followed by a second treatment allowing the recovery of other
536 products and improving the digestibility of the residual solid at the same time.

The study of the first autohydrolysis allowed to conclude that the optimal conditions, in terms of OS production, corresponded to a severity factor of 2.3 (mild/economical operational conditions at industrial level). The material pretreated at these conditions was then submitted to a second autohydrolysis (final temperature of 180 ºC). The composition of the secondary liquor is presented in the Table 2.

Table 2. Composition of the liquor and solid obtained after the second autohydrolysis in the two stage autohydrolysis of BPP, severity factor 3.36 calculated as log ($R_{0,1} + R_{0,2}$)

Liquor contained OS and small amounts of sugars and degradation products. As in the first stage, oligosaccharides obtained were predominantly GlcOS. The analysis of the starch content for material pretreated at 150 ºC (optimal conditions) and 220 ºC (more severe conditions) revealed that the first one had a content in starch of 12.46 ± 1.17 % while the second one did not present starch. It means that during the non-isothermal autohydrolysis treatment, from 150 to 220 ºC, although more starch can be extracted, the degradation of already extracted starch happened and it was not possible to obtain more OS. For this reason, a two-stage autohydrolysis where the first stage autohydrolysis is performed at a lower temperature to recover maximum amount of OS and avoid their degradation, and a second stage at a higher temperature to recover further OS can be used as a strategy for improving the OS production yield. A partial solubilization of hemicellulose occurred during the second autohydrolysis although there is still residual hemicellulose that is not solubilized in the studied condition, as reflected also in the composition of the solid (Table 2). Comparing with the pretreated

561 starting solid (Table 1), the content in glucan and lignin increased and the hemicellulose
562 content decreased.

563 The total amount of GlcOS generated, obtained from the concentration determined by
564 HPLC and considering the amount of water introduced, was 14.78 g/100 g of pre-
565 treated solid, and on the basis of the untreated original BPP was 7.68 g/100 g of raw
566 material. Therefore, the global production of the two-stage autohydrolysis was 31.75
567 g/100 g raw material in comparison with 24 g/100 g in the single-stage autohydrolysis.
568 Moreover, the value of enzymatic digestibility of the remaining solid improved from
569 78.6 ± 0.9 % (pre-treated at 150 ºC) to 96.57 ± 1.71%. This value was higher than
570 values obtained for all one-stage autohydrolysis, avoiding at the same time the breakage
571 of oligosaccharides produced into glucose.

572

573 3.3 Isothermal autohydrolysis

574 Complementarily, isothermal-autohydrolysis was studied as a more viable solution at an
575 industrial level. An operational temperature of 140 ºC is considered as the limit for
576 working under economic conditions, reason why isothermal autohydrolysis at this
577 temperature and different reaction times was evaluated.

578 The findings were similar to the results observed in non-isothermal tests, being sugars
579 and oligosaccharides predominantly found as glucose and GlcOS, although the
580 concentration of oligosaccharides varied more slightly with severity factor in this
581 occasion (Figure 5). A significant increase in monosaccharides and degradation
582 products concentration was neither observed with GlcOS decrease.

Figure 5. Chemical composition of the liquors obtained for the isothermal autohydrolysis of banana's pseudostem pulp for different severity factors.

The yields in GlcOS were similar to those ones obtained in non-isothermal autohydrolysis, with a maximum of 25.5 g GlcOS/100g raw material for $logR_0$=3.43. Similar amount (24 g GlcOS/100g raw material) was obtained for optimal conditions in non-isothermal tests ($logR_0$=2.3).

4. Conclusions

The effect of the autohydrolysis on BPP was evaluated in this study, focusing both on the obtained liquid and solid fractions. A high recovery of oligosaccharides was obtained in the liquor under relatively mild operation conditions together with a low production of degradation compounds. Most oligosaccharides were GlcOS from starch fraction, with potential interest as prebiotics. On the other hand, two-stage autohydrolysis allowed recovering a higher amount of oligosaccharides, avoiding their degradation. Although the raw material had a high digestibility compared with other biomass, pretreatment resulted in its improvement, being for two-stage autohydrolysis close to 100%.

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608

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Table 3. **Chemical composition of untreated banana's pseudostem pulp (BPP) (% w/w, dry basis)**

Component	BPP	Method
Glucan	44.59 ± 1.7	
Hemicellulose	14.98 ± 0.29	
Xylan	7.65 ± 0.29	
Arabinan	6.61 ± 0.01	NREL/TP-510-48087
Acetyl groups	0.79 ± 0.01	
Klason lignin	7.92 ± 0.27	
Ash (total)	15.44 ± 0.05	
Extractable ash	9.05 ± 0.10	
Extractives	21.11 ± 0.79	
Starch (non-resistant)	22.17 ± 0.36	AOAC Method 996.11
Holocellulose	57.48 ± 0.61	Browning, 1967
Cellulose	26.29 ± 2.1	ANSI/ASTM 1977b
Hemicellulose	19.11 ± 2.31	Holocellulose - Cellulose
Pectin	10.50 ± 0.60	Fernandes et al., 2015
Protein	5.88 ± 1.82	Kjeldajhl method

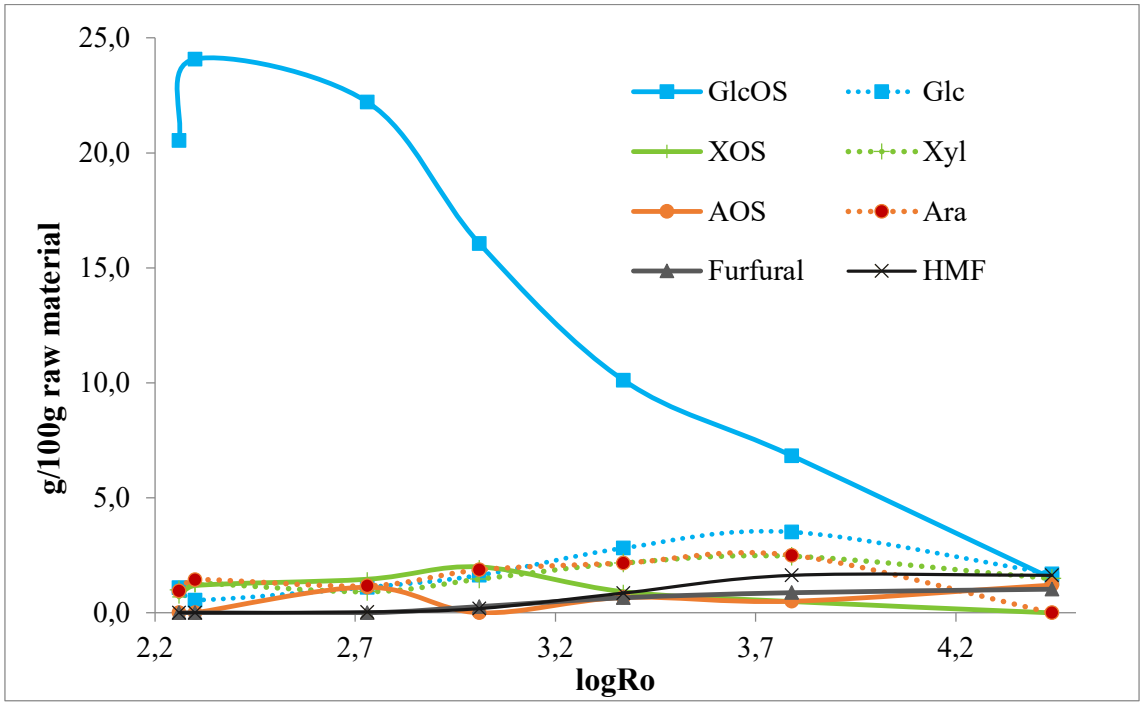
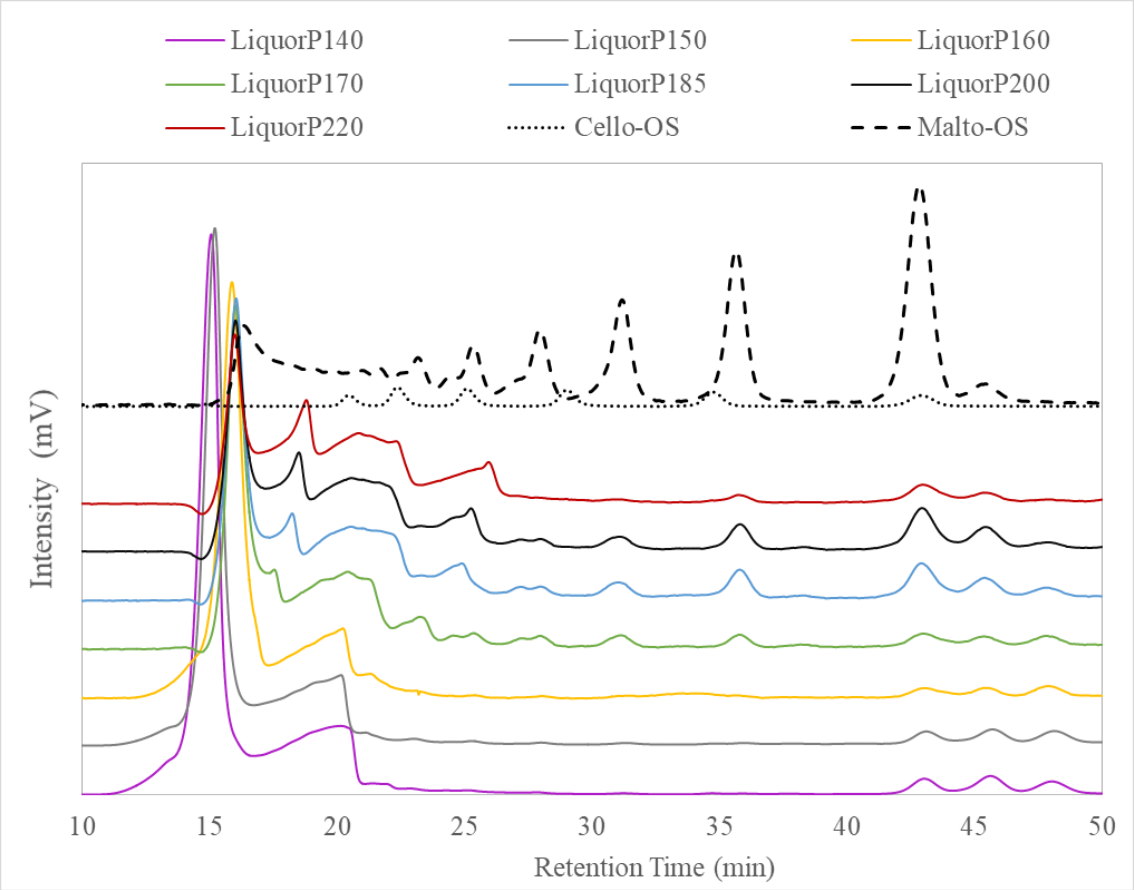


Figure 6. Chemical composition of the liquors obtained for the non-isothermal autohydrolysis of banana's pseudostem pulp for different severity factors.



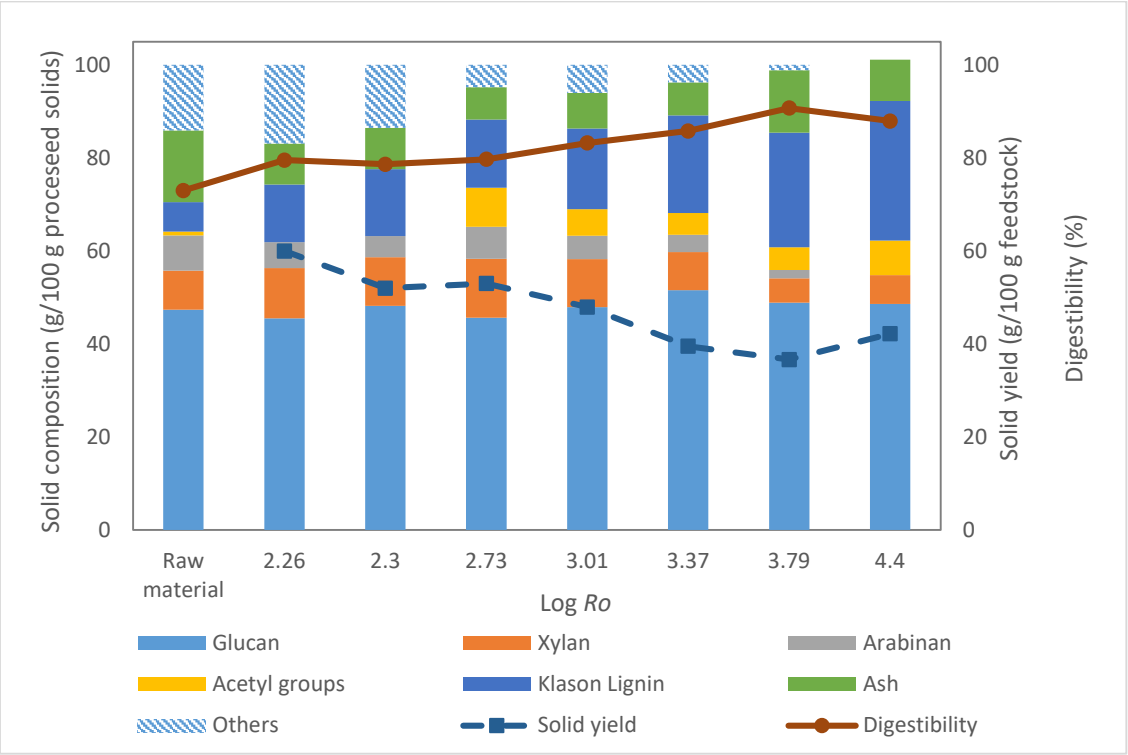
836

837 **Figure 7. Molecular weight distribution of the products obtained in the**

838 **autohydrolysis of banana's pseudostem pulp liquors and cello-oligosaccharides**

839 **and malto-oligosaccharides patterns.**

840



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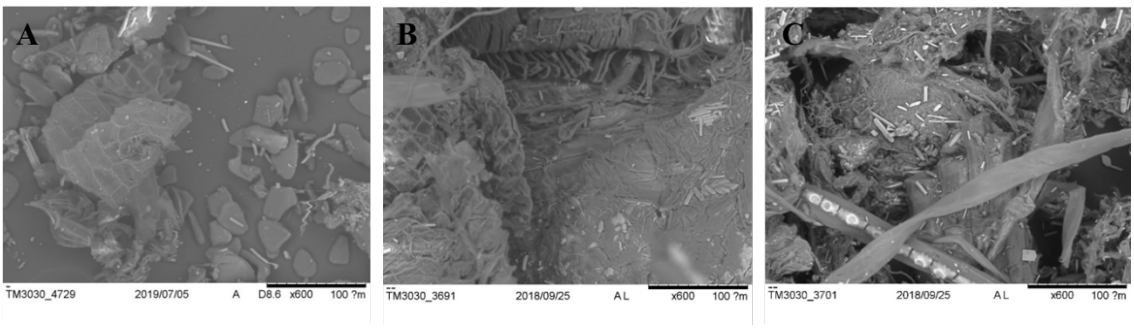
842 **Figure 8. Effect of the severity factor on the solid yield and composition of**

843 **processed solids obtained after autohydrolysis of BPP under non-isothermal**

844 **conditions**

845



846

847 **Figure 9. SEM images of BPP before (A) and after the autohydrolysis**

848 **pretreatment at 160º C (B, log Ro = 2.73) and 200 ºC (C, log Ro = 3.79)**

849

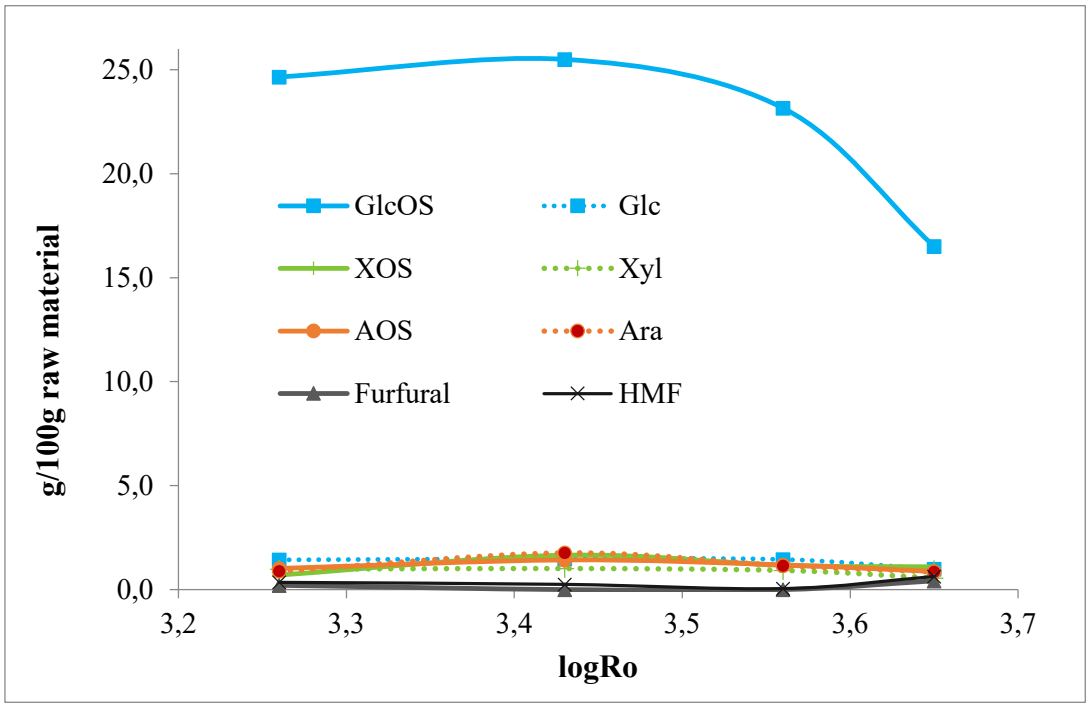
850 **Table 4. Composition of the liquor and solid obtained after the second**
851 **autohydrolysis in the two stage autohydrolysis of BPP, severity factor 3.36**
852 **calculated as log ($R_{0,1}$ + $R_{0,2}$)**

Fraction	Component	P150-180
Liquid (g/L)	Recovered hydrolysate	67.1%
	Gluco-oligosaccharides	14.48 ± 0.44
	Xylo-oligosaccharides	3.89 ± 0.13
	Arabino-oligosaccharides	1.06 ± 0.09
	Acetyl groups linked to oligosaccharides	0.52 ± 0.09
	Glucose	0.974 ± 0.20
	Xylose + Mannose + Galactose	1.06 ± 0.01
	Arabinose	1.62 ± 0.26
	Acetic acid	1.01 ± 0.21
	Furfural	0.80 ± 0.51
	Hydroxymethylfurfural	0.21 ±0.01
Solid	Solid yield	68.4%
(% w/w in dry base)	Glucan	55.97 ± 0.67
	Hemicellulose	8.80 ± 0.80
	Xylan	7.62 ± 0.69
	Arabinan	1.18 ± 0.11

	Acetyl groups	-
	Klason lignin	18.00 ± 0.06
	Ash	9.02 ± 0.08

853

854



855

856 **Figure 10. Chemical composition of the liquors obtained for the isothermal**

857 **autohydrolysis of banana's pseudostem pulp for different severity factors.**

858