

## FRACTIONATION OF THE MAIN COMPONENTS OF BARLEY SPENT GRAINS FROM A MICROBREWERY

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The chemical composition of barley spent grains generated in a microbrewery and their fractionation by acid hydrolysis and delignification were investigated. The material contained high amount of carbohydrates (60%), while its lignin content was lower than that reported for other sorts of barley spent grains. Different dilute-acid hydrolysis methods were evaluated for separating the main components of the spent grains, without affecting the sugars generated by starch hydrolysis. The utilization of a two-step dilute-acid hydrolysis approach allowed to hydrolyse starch in a first step, at 100 °C, and hemicelluloses in a second step, at 121 °C. Acetosolv and alkaline delignification were used for solubilising the lignin fraction. A higher lignin solubilisation (95% of the lignin contained in the raw material) was achieved after alkaline delignification, whereas only 34% of the initial lignin was removed by direct acetosolv. When the acetosolv treatment was combined with acid hydrolysis, lignin solubilisation increased to 74%. Lignin was precipitated from the liquors at recovery rates from 40 to 93%, as depending on the hydrolysis/delignification method used.

**Keywords:** barley spent grains, acid hydrolysis, alkaline delignification, acetosolv

### INTRODUCTION

During the last two decades, the interest for fuel ethanol has been continuously increasing, as due to concerns for environmental protection and for the possible depletion of fossil fuels. In more recent years, the increased prices of petroleum-derived fuels in the world market, as well as technological developments, national energy security concerns and governmental motivations have made ethanol fuel even more attractive. In 2009, the global ethanol production was 73.9 billion L, with USA (40.7 billion L), and Brasil (24 billion L), as the main ethanol producers.<sup>1</sup> However, the use of sugar- and starch-containing raw materials, which also constitute important sources of food and feed, restricts the further expansion of ethanol industry. To satisfy the growing demand for ethanol without affecting the food sector, alternative raw materials, such as lignocellulosic bioresources, should be utilized.

Lignocellulosic materials are abundant and cheap, however converting their cellulose, and eventually hemicelluloses, to ethanol is still expensive.<sup>2</sup> Hydrolytic processing of polysaccharides, with either enzymes or chemicals, is required for making the sugar monomers available for bioconversion to ethanol.<sup>3,4</sup> Since cellulose is embedded in a cross-linked matrix of lignin and hemicelluloses, a pretreatment is crucial for separating the main components, and for making the cellulose fibers accessible to the hydrolyzing agents. Delignification of the pretreated material prior to cellulose hydrolysis is also advisable, since lignin can inactivate the enzymes and prevent their access to the cellulose macromolecules, leading to lower hydrolysis rate and yield.<sup>5</sup> Pretreatment and delignification processes are important technological steps for the fractionation of lignocellulosic materials into their main components, in view of their

utilisation according to a biorefinery philosophy.<sup>6</sup>

Different pretreatment and delignification methods have been investigated. Many of them are expensive and destructive, their efficiency often depending on the raw material. Therefore, the selection of the most appropriate method for each raw material is very important. The ultimate goal of the pretreatment is to separate the three main polymeric constituents of the lignocellulosic bioresources, and to enhance the enzymatic hydrolysability of cellulose with minimal alteration of hemicelluloses and lignin.<sup>7</sup>

Different approaches of dilute acid hydrolysis have been shown as effective pretreatment processes.<sup>8</sup> Acidic pretreatments break down hemicelluloses into monomeric sugars and simultaneously remove part of the lignin. The cellulose in the resulting material is better exposed to enzymes. However, acid pretreatments require expensive anticorrosive materials and cannot avoid partial degradation of hemicelluloses. Alkaline solutions can remove lignin and part of hemicellulose, and improve the enzymatic conversion of cellulose. In addition, alkaline treatments are effective in avoiding destruction of hemicelluloses. Pretreatments with organic solvents, such as alcohols and aliphatic acids, also offer good potential for separating the main components of the lignocellulosic materials and for activating the cellulose towards enzymatic hydrolysis. The organosolv methods are environmentally friendly and allow high recovery of lignin, susceptible to further utilization.<sup>9</sup>

Barley spent grains, the main residue of the brewing industry, are lignocellulosic materials which could be of interest for bioethanol production. The spent grains are formed by the husks of the original barley, but they can also contain unconverted kernels. The chemical composition of spent grains depends on the raw materials, harvesting time and process conditions.<sup>10</sup> When spent grains contain kernel remains, the presence of starch makes the hydrolytic processing of the material difficult. Due to the better hydrolysability of starch, the glucose resulting from its hydrolysis can be destroyed under the hydrolysis conditions applied for cellulose and hemicelluloses.

The present work investigates fractionation methods that would allow the integral utilisation of the main components

of a sort of barley spent grains with high starch content.

## EXPERIMENTAL

### Raw material

Barley spent grains were supplied by a microbrewery located at the University of Matanzas. The fresh material was washed with water, air-dried for a week, dried at 50 °C for 48 h and milled to a 2 mm particle size. A 100 g portion was further milled to pass a 1 mm screen, and used for compositional analysis.

### Analysis of raw material

The content of moisture, mineral components, extractives, structural polysaccharides and lignin in the raw material was analyzed according to NREL analytical procedures. Moisture was determined<sup>11</sup> gravimetrically, after drying the material at 105 °C. The mineral components were determined<sup>12</sup> as ash, after incineration of an aliquot of the material at 550 °C. The extractives were determined by ethanol extraction on a Soxhlet apparatus.<sup>13</sup> Cellulose, hemicelluloses and lignin were determined by analytical acid hydrolysis of the extractive-free material, followed by chromatographic quantification of the sugars contained in the hydrolysate, and by gravimetric determination of the acid-insoluble lignin.<sup>14</sup> The sugars from the analytical acid hydrolysates, determined by a HPLC system (Waters, Milford, USA), equipped with UV-vis and RI detectors (Waters, 2414), were separated with a Pb-based column (Aminex HPX-87P, Bio-Rad, Richmond, CA, USA) at 85 °C, using deionised water as eluent, at a flow rate of 0.6 mL/min. Starch was determined as the glucan fraction hydrolysed at 100 °C.

### Dilute-acid prehydrolysis

20 g of spent grains were mixed with a 1.25% sulfuric acid solution in 500 mL glass flasks, giving a 1:8 solid-to-liquid ratio. The reaction mixture was held at 121 °C in an autoclave for 17 min, as described by Mussatto and Roberto.<sup>15</sup> When the hydrolysis time had elapsed and temperature dropped to 70 °C, the flasks were taken out and cooled in a water bath, and the treated material was vacuum-filtered; the filter cakes were washed with water, dried at 105 °C overnight and weighed for yield determination.

For two-step acid hydrolysis, the reaction mixture was first hydrolysed, either at 100 °C for 1 h, or at 121 °C for 17 min. The hydrolysate was then separated and the resulting solid material was submitted to a second hydrolysis step, under the same conditions, except that the treatment time was always 1 h. The experiments at 100 °C were performed in glass flasks equipped with reflux condensers and heated by electric mantles.

### Delignification

Delignification treatments were applied either to the raw spent grains or to the cellulignin

obtained after acid hydrolysis. Acetosolv delignification was performed with 95% acetic acid and 0.2% HCl. 5 g (DW) of biomass were mixed in a flask with delignification media, to give a 1:10 solid-to-liquid ratio. The flasks containing the reaction mixture were held for 2 h at 121 °C in an autoclave. By the end of the treatment, the material was vacuum-filtered and the filter cakes were washed first with an acetic acid solution and then with distilled water, and dried at 105 °C overnight.

For alkaline delignification, the solids were treated with a 2% w/v NaOH solution at a solid-to-liquid ratio of 1:20, at 120 °C for 90 min, following the protocol developed by Mussatto *et al.* for barley spent grains.<sup>16</sup> Then, the pulp was separated by filtration, washed with water to remove the residual alkali, and dried at 105 °C overnight. The samples of the filtrates were subjected to posthydrolysis with a 4% (w/w) H<sub>2</sub>SO<sub>4</sub> solution at 121 °C for 15 min, to establish the amount of polymeric sugars.

#### Analysis of treated solids

The hemicelluloses and cellulose in the treated solids were determined as easy-to-hydrolyse and difficult-to-hydrolyse polysaccharides, respectively. A modification of the method described by Bazarnova,<sup>17</sup> based on two-step acid hydrolysis, followed by spectrophotometric quantification of the total reducing sugars<sup>18</sup> was used. In the first hydrolysis step, the biomass was treated with dilute hydrochloric acid for hydrolysing the easily hydrolysable polysaccharide fraction, whereas in the second step, cellulose and the difficult-to-hydrolyse fraction of hemicelluloses were hydrolysed with 72% sulfuric acid. Lignin was determined gravimetrically as the solid residue remaining after total hydrolysis of polysaccharides.

#### Lignin precipitation

Acetosolv filtrates were concentrated to 1/4 of the initial volume, using a rotary evaporator (IKA RV 05 Basic, Germany). The concentrated liquors were mixed with 10 volumes of water, heated in a water bath at 80 °C for 2 h and left to stand at room temperature for 24 h. Then, the precipitated lignin was recovered by filtration, washed and dried at 105 °C until constant mass. In the alkaline delignification filtrates, lignin was precipitated by acidification to pH 2.0 with HCl, followed by holding at 80 °C for 2 h, and left to stand for 24 h at room temperature.

## RESULTS AND DISCUSSION

### Chemical characterization of raw material

The analysis of the raw material revealed that 60% of the dry matter content of the spent grains was composed of polysaccharides (Table 1). The cellulose

content (15.1%) was comparable within the range (15-17%) reported in literature for other sorts of barley spent grains.<sup>16-22</sup> However, the content of hemicelluloses (32.5%) was slightly higher than the formerly published values (24-29%). The contents of starch and lignin differed considerably from those reported previously. Starch content in this work was 12.5%, whereas, for other sorts of spent grains, it was markedly lower<sup>19</sup> or not reported.<sup>10,22</sup> The lignin content (13.4%) was considerably lower than in other sorts of barley spent grains (25-28%).<sup>10,19,22</sup>

Table 1  
Chemical composition of barley spent grains  
(standard deviations are shown in parentheses)

Component	Content, %
Hemicelluloses	32.5 (1.6)
Cellulose	15.1 (0.7)
Starch	12.5 (0.3)
Lignin	13.4 (1.9)
Extractives	12.9 (0.7)
Ash	3.4 (0.1)
Unidentified	10.2

The composition of barley spent grains is known to vary considerably for different sorts, as a consequence of the harvesting time, malting and mashing conditions, as well as of the quality and kind of adjuncts added to the brewing process.<sup>23</sup> It has been shown that different lots of spent grains generated in the same brewery can significantly differ.<sup>24</sup> The higher starch content found in this work is a result of an incomplete hydrolysis in the mashing process, and of the heterogeneity of the raw material used in the brewing process, which includes dehusked rice as an adjunct. The lower lignin content is a consequence of a lower percentage of husks, caused by a higher presence of incompletely hydrolysed grains. The high starch content is a positive feature in ethanol production. However, the presence of starch introduces additional difficulties in the hydrolytic processing of spent grains, since it can be destroyed under the acid conditions applied for pretreatment and cellulose hydrolysis.

### Fractionation of spent grains

Three different approaches were used for fractionating the spent grains into their main components. First, an acid hydrolysis approach was performed, for solubilising the

easily hydrolysable polysaccharides, namely starch and hemicelluloses. Then, lignin isolation using either sodium hydroxide or acetosolv treatment was investigated. Finally, an approach combining acid prehydrolysis and delignification was also applied.

#### Acid hydrolysis

Acid hydrolysis was applied to saccharify the hemicelluloses and starch contained in the raw material. Two different, single- and two-step schemes of dilute-acid hydrolysis were used. In the single-step process, the hydrolysis conditions were selected on the basis of the optimal results for hemicellulose solubilisation reported by Mussatto and Roberto.<sup>15</sup> The yield of solids resulting from hydrolysis was 58.8% (Table 2). The content of the easy-to-hydrolyse fraction decreased from 45% in the raw spent grains to 20.3% in the treated material. Almost 3/4 (73.5%) of the easily-hydrolysable polysaccharides were removed (Fig. 1). Lignin solubilisation was negligible, its content increased to 32.5%, as a result of polysaccharides solubilisation. The results on solids yield and solubilisation of polysaccharides and lignin agree well with previous reports on acid hydrolysis of spent grains performed under the same conditions.<sup>10,15</sup>

When the resulting solid material was subjected to a second hydrolysis step, additional removal of hemicelluloses took place, reaching an overall solubilisation around 89% (Fig. 1). As a result, the overall yield of solids decreased to 51.7%, the content of the easy-to-hydrolyse polysaccharides in the cellulignin decreased to 9.8%, and the lignin content increased to 51.1% (Table 2).

Simultaneously, another two-step hydrolysis, using 100 °C as the reaction temperature in the first step, was also performed. The comparison of the yields of both two-step hydrolysis processes shows that solubilisation in the second step was higher for the process, whose first step proceeded at 100 °C. The yields of solids in the second step were of 69.7 and 88.3% for the materials previously treated at 100 and 121 °C, respectively (Table 2). This is a consequence of the lower amount of material solubilised in the first step at 100 °C, compared with the process at 121 °C. Prehydrolysis at 100 °C practically removed the whole starch fraction, whereas

prehydrolysis at 121 °C removed an important part of hemicelluloses in addition to starch. These results are well supported by the differences between the concentrations of reducing sugars in both hydrolysates.

It is noteworthy, however, that overall solubilisation of the easy-to-hydrolyse polysaccharides was slightly higher for the process with prehydrolysis at 100 °C than for the one with prehydrolysis at 121 °C (Fig. 1, Table 2). Considering that starch was totally removed during the first step, hemicellulose solubilisation in the second step was of 90% for the process, with prehydrolysis at 100 °C, and of 84% for the process with prehydrolysis at 121 °C. A possible explanation for this phenomenon is that a lower temperature treatment could have exerted some pretreatment effect on the material, thus potentiating the hydrolysis of hemicelluloses in further processing, whereas prehydrolysis at 121 °C could have somehow affected the substrate, making it more difficult for further hemicellulose hydrolysis. In accordance with the solubilisation of polysaccharides, the concentration of reducing sugars both in the second step and in the overall process was higher for the scheme using prehydrolysis at 100 °C (Table 2).

#### Delignification

Alkaline delignification, previously investigated for barley spent grains,<sup>16</sup> was compared with the acetosolv treatment, a method seen as efficient for wood,<sup>25,26</sup> even if, so far, it has not been evaluated for spent grains.

Although the processes were directed towards lignin removal, the solubilisation of other components also occurred, leading to low pulp yields (Table 3). Alkaline delignification was more effective than the acetosolv treatment. Using sodium hydroxide, approximately 95% of the initial lignin was removed (Fig. 2), the residual lignin content in the pulps being of only 3.5% (Table 3). The low concentration of reducing sugars in the liquid fraction indicates a low degree of hydrolysis of polysaccharide, which were not hydrolysed to simple sugars, but only solubilised, forming oligosaccharides. On the other hand, acetosolv was less selective, leading to a considerable degree of polysaccharide hydrolysis, removing only 34% of lignin,

while the resulting pulp still contained 29.9% lignin.

### Combined fractionation approach

A sequential fractionation approach, in which dilute-acid hydrolysis was carried out

prior to delignification, was considered for improving the effectiveness of the acetosolv treatment. The same method was applied to alkaline delignification, which was used as a reference.

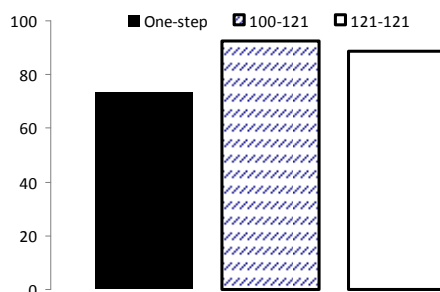


Figure 1: Solubilisation of easy-to-hydrolyse polysaccharides during acid hydrolysis (% of the initial value)

Table 2  
Yield and composition of spent grains processed by different dilute-acid hydrolysis schemes (standard deviations are shown in parentheses)

Hydrolysis scheme	Yield of solids, % (w/w)			EHPS <sup>1</sup> content, % (w/w)	Lignin content, % (w/w)	TRS <sup>2</sup> in liquid fraction, g/L	
	First step	Second step	Overall			First step	Second step
One-step (121°C)	58.8 (2.3)	-	58.8 (2.3)	20.3 (0.0)	32.5 (0.2)	13.5 (0.2)	-
Two-step (121-121°C)	58.8 (2.3)	88.3 (0.1)	51.7 (2.3)	9.8 (0.8)	51.1 (0.4)	13.5 (0.5)	10.4 (0.0)
Two-step (100-121°C)	64.1 (0.2)	69.7 (0.5)	44.7 (0.4)	7.6 (0.0)	43.3 (0.4)	10.0 (0.4)	20.8 (0.8)

<sup>1</sup>Easy-to-hydrolyse polysaccharides, <sup>2</sup>Total reducing sugars

Table 3  
Yield of solids and lignin content in barley spent grains processed by acetosolv and alkaline delignification (standard deviations are shown in parentheses)

Delignification method	Pulp yield, % (w/w)	Lignin content, % (w/w)	TRS <sup>1</sup> in liquor, g/L
Acetosolv	29.6 (0.0)	29.9 (1.0)	9.3 (0.8)
NaOH treatment	17.0 (0.8)	3.5 (0.4)	2.1 (0.2)

<sup>1</sup>Total reducing sugars

Lignin removal increased from 34%, in direct acetosolv delignification, to 70-74%, in combined treatments, especially when two-step hydrolysis schemes were used (Fig. 2). However, the lignin content in the resulting pulps was still considerable, and the content of difficult-to-hydrolyse polysaccharides was well below 50% (Table 4). On the other hand, alkaline delignification was still much more effective than the acetosolv treatment. Acid prehydrolysis was also beneficial for alkaline delignification. Lignin removal increased from 95% in direct alkaline delignification,

to more than 99% in the acid hydrolysis-assisted alkaline treatment (Fig. 2). The best results were obtained with materials previously subjected to single-step acid hydrolysis. The pulp obtained in this process was very rich in cellulose (84.4% of difficult-to-hydrolyse polysaccharides), and its lignin content was considerably lower.

Two different methods were used for lignin precipitation. In the alkaline liquors, lignin was precipitated by adjusting pH to 2.0. In the liquid fraction obtained by acetosolv, the pH was very acidic, and lignin was precipitated by water addition, after

concentrating liquors by evaporation. The recovery of the dissolved lignin was considerably higher for alkaline processes than that for acetosolv treatments, and it was higher when acid hydrolysis was included in the treatment schemes (Fig. 3). In the liquors obtained by direct alkaline delignification, around 41% of the

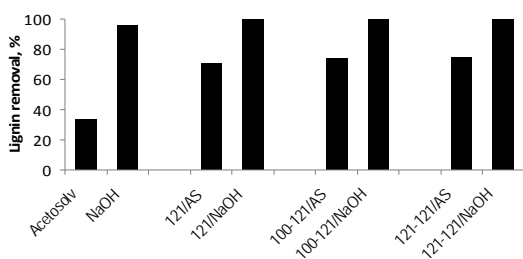


Figure 2: Lignin removal after direct delignification and combined treatments. 121/AS and 121/NaOH, combined processes including one-step hydrolysis and, respectively, acetosolv and NaOH delignification; 100-121/AS, 100-121/NaOH, 121-121/AS and 121-121/NaOH, combined processes including two-step hydrolysis and delignification

solubilised lignin was precipitated. Lignin recovery increased to 85-99% when the NaOH treatment was preceded by acid hydrolysis. The highest lignin recovery was achieved in the treatment including acid prehydrolysis at 121 °C, before the alkaline process.

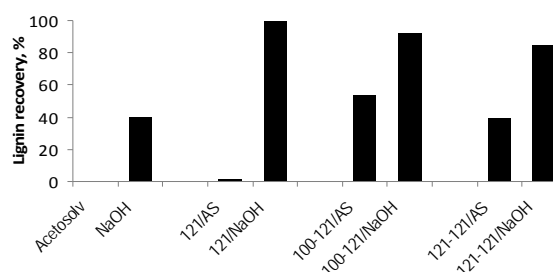


Figure 3: Lignin recovery after precipitation from liquors. Same symbols as in Fig. 2

Table 4  
Yield and composition of pulps obtained by combined acid hydrolysis-delignification schemes (standard deviations are shown in parentheses)

Hydrolysis temperature, °C	Delignification method	Pulp yield, %	Lignin, %	EHPS, <sup>1</sup> %	DHPS, <sup>2</sup> %
121	Acetosolv	31.7 (1.2)	29.8 (1.0)	9.0 (0.4)	28.8 (1.2)
121	NaOH	12.9 (0.4)	0.6 (0.0)	8.3 (0.3)	84.8 (3.5)
100-121	Acetosolv	29.6 (0.9)	28.4 (0.8)	7.4 (0.3)	41.2 (1.3)
100-121	NaOH	10.7 (0.4)	0.4 (0.0)	13.0 (0.5)	70.6 (3.2)
121-121	Acetosolv	25.2 (1.1)	27.4 (1.0)	7.2 (0.2)	41.4 (1.8)
121-121	NaOH	11.0 (0.5)	1.1 (0.0)	13.5 (0.6)	74.2 (3.0)

<sup>1</sup>Easy-to-hydrolyse polysaccharides, <sup>2</sup>Difficult-to-hydrolyse polysaccharides

Lignin recovery in all acetosolv experiments was low. Although visual inspection of the liquors revealed a relatively good lignin precipitation after water addition, the precipitated material was hardly separable by filtration. Some improvement was observed for combined treatments, especially for those including two-step acid hydrolysis. The best recovery of acetosolv lignin, 54% of the removed lignin, occurred for the process including consecutive acid hydrolyses at 100 and 121 °C before acetosolv (Fig. 3). The low lignin recovery achieved in the acetosolv treatments of spent barley grains seems to be a particularity of this raw material, since good results have

been previously reported for other materials.<sup>25</sup> To achieve a higher lignin recovery, for future experiments it is recommendable to use other separation methods, for instance centrifugation.

## CONCLUSIONS

The barley spent grains used in this work have a higher content of carbohydrates and a lower lignin content than other sorts of barley spent grains reported in literature.

Acetosolv delignification was not effective when it was directly applied to barley spent grains, but its effectiveness increased when combined with dilute acid prehydrolysis. The best fractionation strategy

using acetosolv was to perform consecutive hydrolyses at 100 and 121 °C, followed by delignification. This led to a nearly total removal of starch in the first hydrolysis step, to 90% hemicelluloses solubilisation in the second step, and to a relatively high formation of reducing sugars and 74% lignin removal in the acetosolv step.

The effectiveness of sodium hydroxide treatments for the delignification of both raw and acid-pretreated barley spent grains was confirmed. The recovery of the solubilised lignin was better for alkaline delignification than for acetosolv treatment.

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