Potential for the valorization of brewer's spent grains: a case study for the sequential extraction of saccharides and lignin

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Abstract

This study highlights the possibility of using brewers' grains (BSGs) for the successive extraction of the main lignocellulosic biopolymers, namely cellulose, hemicelluloses and lignin. An exhaustive chemical characterization revealed a variability of composition in distinct batches of BSGs, depending on their origin and the brewing process used. In particular, the protein content can vary from 13 to 23 wt.%, which is accompanied by a change in the hemicelluloses content from 9 to 23% (in the samples of our study). By applying a two-step aqueous treatment, involving an acid (1.25% v/v aq. H_2SO_4) and a base (3% w/v aq. NaOH) at a temperature of 120°C and fixed reaction time of a few tens of minutes (15 to 90 min), more than 80% of hemicelluloses could be recovered. Cellulose could be isolated at more than 68%, while a high purity lignin could be recovered from a lignin-rich fraction (70wt.%). Our work also suggests that the variability of the chemical composition of these brewers' grains is a hindrance to achieving process standardization and large-scale exploitation. The pooling of various materials is therefore not a recommended option, and the preliminary chemical analysis of the composition is therefore a prerequisite for an efficient extraction process.

Keywords: brewer's spent grains, lignin, cellulose, extraction.

1. Introduction

Food and beverage industries, and the co-products they generate, are often seen as the solutions of choice in the definition of new value chains in bioeconomy strategies, whether for the production of new molecules, materials and/or energy solutions. This is particularly true for the brewing sector, which for more than a decade has been involved in research efforts to valorize its by-products, particularly brewer's spent grains (BSGs). Indeed, BSGs represent a huge volume, cumulating at approximately 85% of total by-products generated by the brewing industry. In 2011, an estimated 185 Mt of beer were produced worldwide, among which 21% were generated in the European Union (Heuzé et al., 2017). Depending of both the brewing method and beer type, we can estimate that for each hectoliter of beer brewed, 20 kg of BSGs are produced. Worldwide, it represents 35 to 40 Mt of BSGs on an annual basis, among which 8 Mt are produced in EU (Fărcaş et al., 2015). Up until this day, BSGs are mainly used as animal feed, especially for ruminants, due to their high content in proteins and fibers. (Mussatto, 2014) (Crawshaw, 2003).

In a logic of development of new materials or biobased technological solutions, BSGs appear however nowadays as a relevant input, available all year long and with a moderate cost. Nevertheless, various technical barriers hinder their expansion towards an industrial exploitation in connection with the bioeconomy. Indeed, as a result of their high moisture and fermentable sugars content, BSGs are a highly perishable feedstock (Ikurior, 1995) (Santos et al., 2003). Furthermore, they represent a huge volume, making their transport logistics expensive. When devoted to animal feed, BSGs are commonly stored by silage, which is a convenient option to preserve BSGs before their usage (Geron et al., 2008) (Heuzé et al., 2017). However, for any other industrial applications, it seems more relevant to dry BSGs to avoid rotting, which could lead to a fluctuation in the chemical quality and composition of BSGs. Currently, most breweries that wish to reduce the moisture of BSGs, while also increasing the efficiency of their mashing procedure, use mash filter presses to reduce the humidity of the grains to around 60%, before drying them further to humidity levels below 10 wt.% (Santos et al., 2003).

Beside traditional uses as feed, BSGs find also other potential applications, mostly still under development (at a lab- or pilot-scale). For instance, Davila and coworkers underlined in 2016 the technical feasibility to produce, using a cascade and integrated approach, valuable biobased

compounds. Practically, dried BSGs from a unique batch (and origin) are submitted to a highly concentrated acid pretreatment to isolate a glucose-rich and a xylose-rich aqueous fractions, both submitted independently to further fermentation into respectively bioethanol, polyhydroxybutyrates (PHBs) and xylitol (Dávila et al., 2016). Outeiriño et al. proposed a three-step protocol to provide consecutively a cocktail of xylanases (obtained through solid-state fermentation using with *Aspergillus brasiliensis*), lignin-rich materials (after the set-up of a ionic-liquid process) and carbohydrates-containing solutions (Outeiriño et al., 2019). Even if it is supposed to be economically competitive, the difficulty of recycling ionic liquids makes the approach not very strategic from an industrial point of view. George et al. described in 2017 a "biorefinery model" based on the production of xylitol and polylactic acid (PLA) (George et al., 2017). An improvement of this methodology was published in 2020 by Akermann and coworkers for the efficient production of lactic acid through simultaneous saccharification and fermentation (Akermann et al., 2020). In 2021, Ong et al. proposed to investigate deeply the fate of BSGs using as a substrate for solid-state fermentation (Ong et al., 2021). Guarda and co-workers published in parallel in 2021 the two-step conversion of BSGs (involving a highly concentrated acid pretreatment and a fermentation route) into mostly volatile fatty acids (Guarda et al., 2021). As can be seen, most of the research findings published to date concern bacterial fermentation-oriented approaches of the carbohydrates present in SBGs and isolated after catalyzed hydrolytic pretreatment (at high or low temperatures) of BSGs. All these published conclusions state on the reliance on the market-value of the outputs, making them feasible or not. Also, the energetical cost has a strong influence on the economic viability, and integrating heat production should be a key factor when considering these models. Almost all studies conducted to date have focused on a single batch of BSGs. The issue of batch composition variability is often overlooked in the choice of conversion processes, and in the treatment of the generated data. We anticipate at this point that variability in the composition of these BSGs may have an impact on the efficiency (both economic and technical) of the conversion options for these brewing by-products. Thus, we propose in this study to verify the chemical composition of different batches of BSGs from different breweries, whether industrial or smaller capacities, and to verify if the application of a pretreatment phase under acidic conditions, as mentioned in the above-mentioned published works, can be influenced by a possible variability in chemical composition.

To do that, we evaluated herein the potential of BSGs generated in a specific geographic area, the Greater Region (Europe), as starting lignocellulosic materials for the individual recovery of biobased components, namely cellulose, hemicellulose and lignin. The Greater Region is indeed a strategic territory, with an extensive brewing activity. We have identified 163 commercial breweries distributed between Belgium (36 breweries in Liege and Luxembourg Provinces), Germany (56 units in Saarland and Rheinland-Pfalz), France (63 breweries in Meuse, Meurthe-et-Moselle, Vosges, and Moselle), and Luxembourg (8 industrial units). **Figure 1** illustrates the distribution of these breweries throughout the Greater Region.

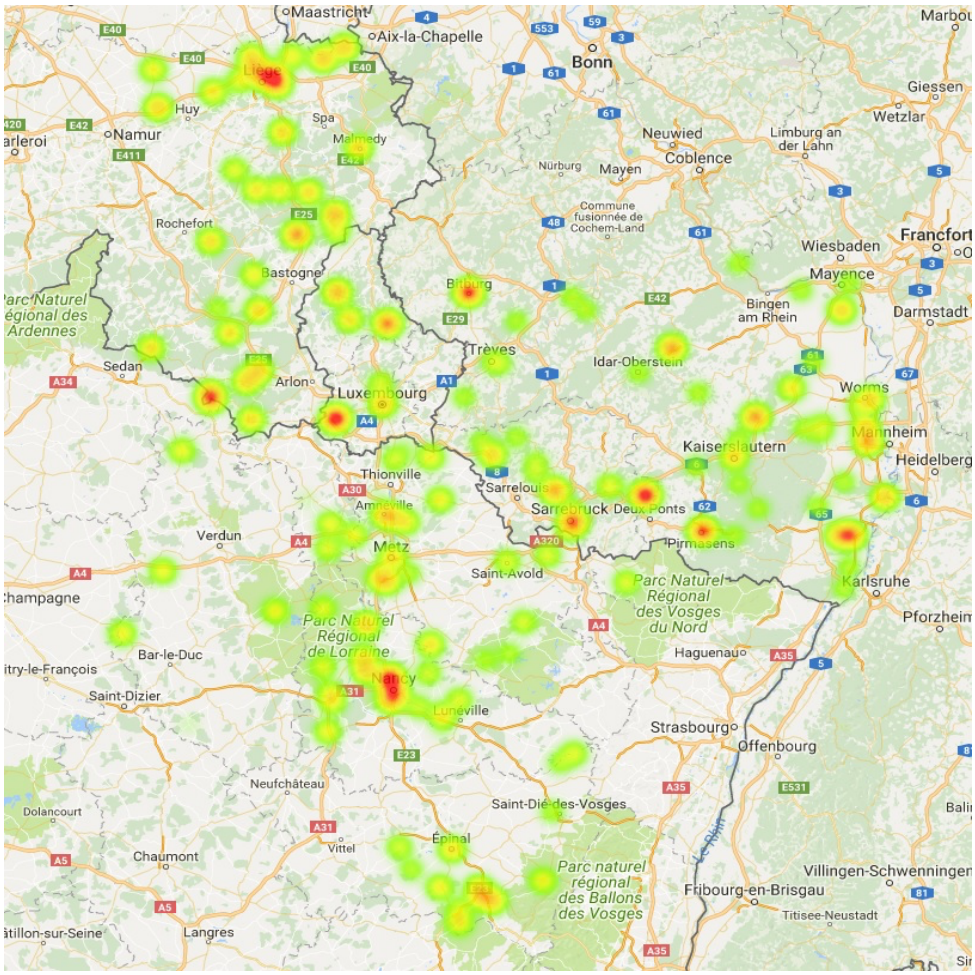

Figure 1. Geographical distribution of breweries in the Greater Region. Green to red gradient depicted the production volume from “low” to “high”.

Our work has identified a cumulative production in 2018 of 17,236,959 hL of beer on an annual basis (data collected in 2018). To a first approximation, this corresponds to a production of about 344,000 tons of BSGs per year. The establishment of centralized biorefineries working on the valorization of

these BSGs, using a unified and continuous process, could produce a significant added value to the residues produced by these breweries, while generating interesting biochemical compounds. However, this can only be considered if the variability of BSGs is quite low, which will be verified in this study.

2. Materials and methods

2a. Sample collection and preparation

BSGs samples were collected between 2018 and 2020 in a commercial large-scale brewery, namely the Brasserie d'Orval S.A. (Villers-devant-Orval, Belgium), and used as benchmark for this study (samples denoted "OR"). After collection, BSGs samples were pressed using a De Smet Rosedowns MINI100 screw press at 150 rpm. The "press liquor" was frozen and the pressed BSGs were then further dried in an oven at 50°C for 2 days, in order to achieve a total humidity level below 5wt.%. Prior to characterization, all samples were dried at 40°C for 3 days before being grinded at 0.5 mm using a Fritsch Pulverisette 19 Cutting Mill. For comparative characterization, two other batches of spent grains were obtained through the microbrewery at the University of Kaiserslautern, brewed on April 2018: KL1.1 represents spent grain obtained after brewing beer using 100% barley malt, and KL1.5 represents spent grain obtained after brewing beer using 50% barley malt and 50% wheat malt. Another sample of BSGs containing 14% of rye malt was also characterized, which was obtained after homebrewing on March 2021 (RB).

2b. Compositional analyses

All analytical analyzes were carried in triplicate, values are expressed as the mean ± standard deviation. Results were expressed as % of dry matter.

Dry matter was determined based on the weight loss after 24h at 105°C in an oven. Ash content was determined after calcination of the sample for 6 h at 575°C, using a Nabertherm Germany B18 muffle furnace, according to the NREL method (Sluiter et al., 2004).

Extractives.

The total water-soluble and ethanol-soluble compounds (including polyphenols, free carbohydrates, and terpenoids) were quantified using a Soxhlet apparatus according to the NREL method without modification (Sluiter et al., 2008). The extraction was performed in two successive steps of 6 h each.

First, 70 mL of distilled water was used. The water solution containing the extracts was then freeze-dried and weighed. Then, extraction was performed using 70 mL of ethanol (97%), and the obtained ethanol solution was then evaporated under pressure, and subsequently weighed.

Polysaccharides (cellulose and hemicellulose) and lignin quantification.

The **lignin content** of the solid residues was determined using the Klason procedure according to previous published protocols (Berchem et al., 2020). Practically, around 300 mg of the dried Soxhlet residue (depleted of extractives) was weighed and hydrolyzed using 3 mL of a 72% H_2SO_4 solution during 1h at 30°C. Then, distilled water was used to dilute the solution to a 4% H_2SO_4 concentration, and hydrolysis was resumed in CERTO CLAV (Model CV-22-VAC-Pro) autoclave at 121°C for 60 min. The mixture was filtered through a P4 tared crucible, dried at 105°C during 24h and weighed to gravimetrically determine the total lignin content. The hydrolysate obtained after filtering was preserved and used for total sugars determination.

Cellulose and hemicellulose contents were obtained on the basis of the quantification of the constituent monosaccharides, namely glucose for cellulose, and the sum of arabinose, xylose, rhamnose, mannose and galactose for hemicellulose according to an adapted published protocol (Berchem et al., 2020). First, 100 µL of an internal standard solution (2-deoxy-D-glucose, 3g/L) was added to 900 µL of the Klason hydrolysate. Then, 2 drops of NH_4OH 15M were added to neutralize the mixture, and 2 mL of $NaBH_4$ was added in order to reduce the monosaccharides. After incubation at 40°C during 90 min, 0.6 mL of acetic acid, 0.4 mL of 1-methylimidazole and 4 mL of acetic anhydride were added for the acetylation of the reduced monosaccharides into alditol acetates. Finally, 10 mL of distilled water and 3 mL of dichloromethane were added, and 1 mL of the dichloromethane phase was retrieved and injected using an Agilent (7890B series) gas chromatograph equipped with a flame ionization detector. A high-performance capillary column HP1-methylsiloxane (30 m x 0.32 mm, 0.25 µm) was used to separate the components and the results were analyzed using the Lab ChemStation software.

Estimation of the protein content.

Protein content was determined according to the Kjeldahl method, using a nitrogen conversion factor of 6.25 as validated by Mathias and co-workers (Mathias et al., 2015). Around 80 mg of the milled and dried sample was mineralized with concentrated H_2SO_4 (95%) and a CTQ catalyst (1.5 g K_2SO_4 + 0.045 g $CuSO_4.5H_2O$ + 0.045 g TiO_2) using a TECATOR 105 heating device at 360°C for 2h. The sample was then titrated with a 0.01 M H_2SO_4 solution using a Kjeltec 2300 (Foss) apparatus.

2c. Integrated extraction process

BSGs were submitted to a sequential treatment, involving aqueous acid (H_2SO_4) followed by an alkaline (NaOH) process, as schematized on **Figure 2**.

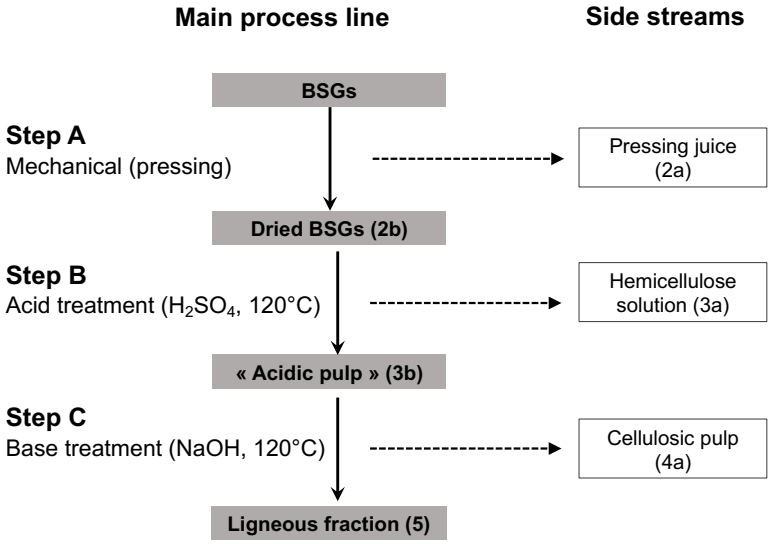

Figure 2. Schematization of the treatments applied to BSGs (allowing sequential separation of hemicellulose, cellulose and lignin). The different process phases are denoted “Step A” to “Step C”.

Practically, BSGs were pressed to reduce humidity content (step A). Then, the dried SBGs were submitted to an acid treatment (step B) involving a 1.25% (v/v) aq. H_2SO_4 in a Parr Series 4580 HT reactor (8mL per gram of BSGs) at 120°C for 17 min. After reaction, the medium was cooled down, and the solid fraction (called “acidic pulp”) was separated from the liquid phase by centrifugation using a Rousselet Robatel basket centrifuge. The recovered acidic pulp was then dried at 40°C for 3 days. The liquid fraction was recuperated, identified as the “hemicellulosic solution”, and stored at 5°C.

For the subsequent alkaline treatment (step C), a 3% (w/v) NaOH solution was added to the dried “acidic pulp” (10 mg per gram of acidic pulp) in the high-pressure Parr reactor. The reaction was maintained at 120°C for 90 min according to a previous published protocol (Maniet et al., 2017). After treatment and cooling, the reaction mixture was filtered through a 0.125 mm sieve. The solid fraction was then washed thoroughly with distilled water, and centrifuged until the washing liquid showed no coloration. The solid fraction was then dried at 40°C during 3 days and identified as the “cellulosic fraction”. The liquid fraction was used for the isolation of lignin, after precipitation by correction of the pH to a value of 2. The newly formed precipitate was separated by centrifugation and dried at 40°C.

3. Results and discussion

BSGs recovered from a large-scale brewery (samples “OR”) were subjected to an extensive chemical characterization. The results are presented in **Table 1** and highlight that the component-by-component chemical variability was quite moderate, notably all over the year, meaning that the composition of the BSGs was fairly identical. This consistency in composition is related, among other things, to the standardization of the brewing process in large volume commercial units.

Table 1. Chemical composition of BGSs, estimated in g/100g of dry matter.

	Content (%)
Proteins	20.3 ± 0.4
Hemicellulose	22.7 ± 0.5
Cellulose	11.1 ± 0.7
Lignin	13.8 ± 1.0
Extractives	35.5 ± 1.9
Ashes	4.7 ± 0.4

The proteins content reached a value of about 20.3%. Structural lignocellulosic biopolymers, namely cellulose, hemicellulose, and lignin, accounted for nearly 48%. Extractables compounds represented a significant part of the total weight of BSGs (35.5%), and consisted in secondary metabolites (mostly polyphenols, and terpenoids as previously evidenced by Stefanello and coworkers) and free carbohydrates (Stefanello et al., 2018). The inorganic materials complete this chemical composition and culminated at around 5%.

The technical feasibility of our selective and cascade extraction protocol (depicted in **Figure 2**) was then checked for the step-by-step recovery of structural polysaccharides (cellulose and hemicellulose) and lignin. Practically, although the process was achieved in an aqueous phase, our protocol contains a drying phase of BSGs (step A) required to better preserve this product over time, and consistent with a storage strategy in industrial conditions. The reduction of humidity was ensured by pressing BSGs using a screen press, which allowed a reduction of the moisture content from 80 wt.% to 60 wt.% (consistent with what is practiced on an industrial scale for the conservation of BSGs). The liquid recovered after this step (called “pressing juice, 2a”) possessed a stable concentration of total sugars of 28.6 g/L. This value is quite close to the one presented by Akermann et al in parallel works, where the press liquor was used for the production of lactic acid (Akermann et al., 2020). Further reduction of

the moisture content of our samples was achieved by oven-drying, reducing the moisture content below 10 wt.%.

After successful drying of the BSGs, the dilute acid treatment using H_2SO_4 (step B) allowed to isolate a liquid fraction (called "hemicellulose solution, 3a") and a solid residue ("acidic pulp, 3b"). The "crude" BSGs contained 22.7±0.5% of hemicellulose. After passing through a screw to reduce moisture, the hemicellulose content dropped to 19.5±0.3% (meaning a reduction of 14.2% of the hemicellulose content) (**Table 2**). The acid treatment, on the other hand, significantly reduced the hemicellulose content to 4.4±0.2%, which implies that more than 80.5% of the hemicellulose were removed, and passed into the liquid phase. It appeared also that this treatment also had a minor influence on the proteins whose content dropped from 18.9±0.5 after pressing to 16.1±3.0% after contact with the acid agent. The chemical composition of the liquid phase ("hemicellulose solution, 3a") was found containing about 6.6% arabinose, and 11.7% arabinose, together with huge amount of 5-hydroxymethylfurfural, levulinic acid and formic acid (obtained as a mixture, with a 5-HMF content reaching up 9.8%).

Table 2. Chemical composition of the obtained fractions after pressing and dilute acid treatment.

	Hemicellulose content (%)	Hemicellulose removal* (%)	Protein content (%)	Ash content (%)
Untreated BSGs (1)	22.7 ± 0.5	--	20.3 ± 0.4	4.7 ± 0.4
Pressed BSGs (2b)	19.5 ± 0.3	14.2	18.9 ± 0.5	3.5 ± 0.1
Acidic pulp (3b)	4.4 ± 0.2	80.5	16.1 ± 3.0	5.7 ± 0.3

* Hemicellulose removal was determined based on hemicellulose content of the initial untreated BSG

Figure 3 illustrates the C5 and C6 carbohydrates (free sugars and/or constitutive of cellulose and hemicellulose) composing the solid fraction recovered after the acid treatment. Our results underlined that the pressing phase (step A) modified the overall content in both xylose and arabinose. As the (absolute) percentage of arabinose reached 7.20±0.19% in the starting BSGs, it decreased to 6.09±0.94% in the pressed BSGs and to 3.49±0.09% in the solid residue obtained after the H_2SO_4 treatment. The same trend was recorded for xylose, which decrease from 13.66±0.24% in the crude BSGs to 13.03±1.81% in the pressed BSGs. The content of xylose was found insignificant in the solid fraction obtained after the contact with aq. H_2SO_4. Our results demonstrates also that the solid residue

after the acid treatment was quite rich in D-glucose, with a content reaching 85.8% of the total carbohydrates content, emphasizing that this acid step was able to isolate a cellulose-rich final fraction.

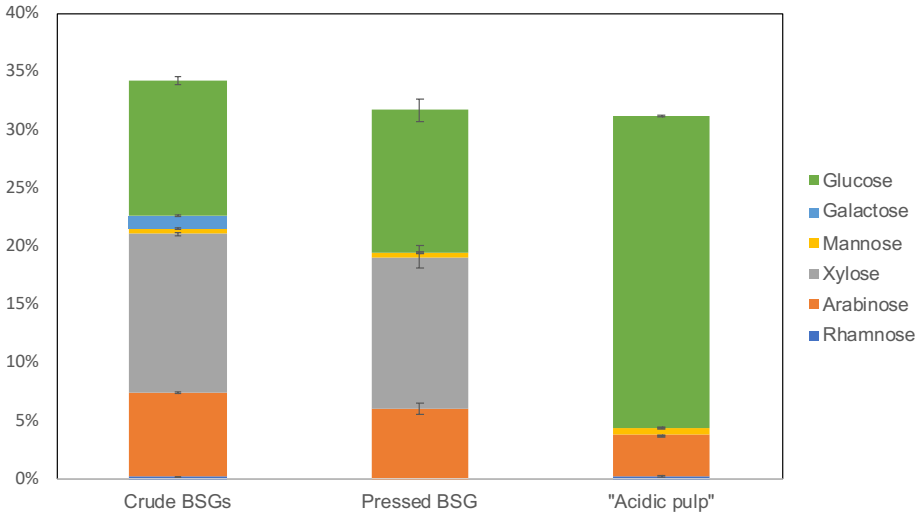

Figure 3. C5 and C6 carbohydrates composition of “crude” BSGs, BSGs obtained after pressing (solid, 2b) and BSGs obtained after the acid treatment (“acidic pulp, 3b”).

The dried “acidic pulp, 3b” was then submitted to an alkali treatment involving NaOH (step C). After the treatment, both a solid fraction (called “cellulosic pulp, 4a”) and a liquid were isolated and deeply analyzed. The cellulosic pulp showed a 68.6±2.3% content in cellulose, together with minor amounts of proteins (3.9±1.3%) and hemicellulose (3.4±1.0%) (**Figure 4**). The lignin content was dropped to 7.2±0.4%, meaning that the alkali treatment was efficient to ensure an optimal delignification of about 71.2%.

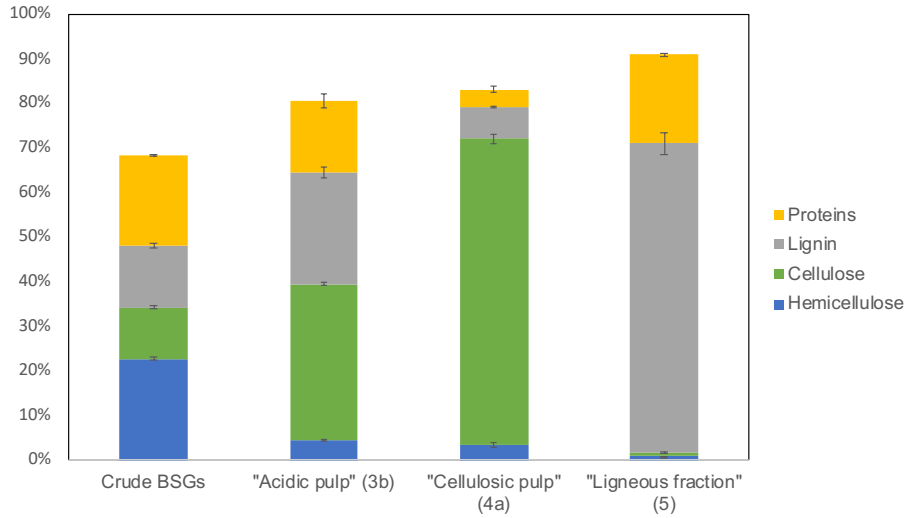

Figure 4. Structural polysaccharides (cellulose and hemicellulose), lignin and proteins contents in the starting BSGs, the acidic pulp (3b) and both the cellulosic pulp (4a) and the lignin-rich fractions obtained after the alkali treatment.

A lignin-rich solid fraction (5) was obtained after adjusting the pH of the liquid fraction at 2 (found as the most suitable pH value to recovered the maximum yield in lignin). The isolated solid was characterized by a lignin content reaching more than 69.4%. This lignin was however contaminated with proteins (20%), which coprecipitated with lignin at pH 2, whilst polysaccharides (cellulose and hemicellulose) accounted for less than 2% of the total weight. About 9% of the composition was identified as inorganic salts, that could not be removed during the washing phases. However, ultrafiltration was found a convenient option to overcome the “protein issue”. Avoiding a co-precipitation, it allowed us to obtained a high purity lignin (>98%) that could be a good candidate for value-added applications (considering the intrinsic cost of the ultrafiltration approach).

If we calculate the mass balance on the whole production chain (including steps A, B and C presented in **Figure 2**), it appears that for one ton of wet BSGs (with a humidity content of 80%), we can obtain 150 kg of dried BSGs after pressing and drying (step A). Applying the successive acid and alkali treatments (steps B and C), we can thus technically isolate about, notably, 20 kg of cellulose and 20 kg of lignin corresponding to recovery rates of respectively 50.1 and 41.8%. Hemicelluloses accounting for 60.5% could be recovered (partially degraded) in an aqueous liquid phase after the H_2SO_4 treatment.

277 The estimated amount of waste is relatively small and represents inorganic salts and aqueous effluents
278 obtained in intensive neutralization and washing phases preliminary to the chemical characterizations
279 mentioned in this work.
280
281 It should be mentioned that these results have not been optimized, but were rather screened to verify
282 technically the possibility of a larger scale operation. Moreover, to our opinion, the exploitation of BSGs
283 as an input for the extraction of valuable biopolymers (polysaccharides, or lignin) is subject to critical
284 points that deserve attention. The major point of criticism is related to the variability of BSGs
285 compositions that may be available on the market. Indeed, our results underlined that a significant
286 and/or important variability could be observed in the chemical composition of BSGs from different
287 origins as evidenced in **Table 3**.
288
289 **Table 3.** Chemical compositions of various BSGs obtained from several breweries located on the
290 Greater Region territory. OR stands for "Orval residues", used as benchmark for this study; KL1.1 and
291 KL1.5 are BSGs obtained from a micro-brewery and RB are side-products from a home-made
292 production. Results are expressed in g/100 g of dry matter.

	OR	KL1.1	KL1.5	RB
Proteins	20.3 ± 0.4	22.7 ± 0.8	21.1 ± 0.6	13.4 ± 1.5
Hemicellulose	22.7 ± 0.5	17.6 ± 1.5	15.3 ± 1.4	9.7 ± 1.8
Cellulose	11.1 ± 0.7	10.4 ± 1.0	13.1 ± 1.0	15.1 ± 0.9
Lignin	13.8 ± 1.0	17.2 ± 1.2	12.0 ± 0.9	9.1 ± 0.4
Extractives	35.5 ± 1.9	27.0 ± 1.9	38.4 ± 2.2	41.0 ± 1.7
Ashes	4.7 ± 0.4	5.6 ± 0.0	2.7 ± 0.1	3.0 ± 0.2

293
294 This variability is related to the brewing method used, but also and above all to the nature of the cereals
295 used for the production of the beer. Thus, if we compare results provided in **Table 3**, one could observe
296 that the lignin content could fluctuate from 9.1 to more than 17.2%, whilst the hemicelluloses could
297 account for 9.7 to 22.7%. If we explore in more details these results, we can notice that, although OR
298 and KL1.1 are both obtained from beer brewed using only barley malt, the brewing process (large-scale
299 vs. microbrewery) could lead to variations mostly in both the hemicellulose (22.7 vs. 17.6%) and lignin
300 (13.8 vs. 17.2%) contents. When considering KL1.5, obtained also in a microbrewery but from 50%
301 wheat malt and 50% barley malt, we could underline that, here also, significant differences are detected
302 (notably in the cellulose and lignin contents). However, no significant differences were found in the
303 proteins content between the OR, KL1.1 and KL1.5 samples (respectively 20.3%, 22.7% and 21.1%).

If we analyzed then BSGs obtained after a home-brewing process using 14% rye malt and 86% barley malt (sample denoted “RB”), we observed a noticeable difference in the global amounts of lignin and hemicellulose. Moreover, the proteins level dropped to 13.4%, which could be explained by the lack of control and standardization of the home-brewing process used for our study.

As emphasized, BSGs have a compositional variability, which can be significant, and which is related to the nature of the cereals used during manufacture and/or the nature (and know-how) of the brewing process. if the two-step extraction process (acid then basic) can be applied to KL1.1 and KL1.5 samples with comparable recoveries, without loss of efficiency and yield (results not presented here), it proves to be ineffective for BSGs with protein levels that deviate from the 20% value (which is the case for the RB sample). To overcome this drawback, some samples of BSGs from different origins could be pooled and processed at a single high-capacity industrial site. However, our previous work has shown that this pooling was not strategic, but rather the opposite, especially for samples with fluctuating protein levels, which is the case in this study (Berchem et al., 2020).

Our second point of critical reflection is based on the projection of the results obtained (which, even if they have not been optimized, highlight certain trends). The perspective of these results in an industrial logic must be analyzed in a reasonable way. Indeed, if we consider on the scale of the Greater Region that nearly 344,000 tons of BSGs are produced on an annual basis, this would represent an annual production of approximately 6,800 to 13,600 kg of cellulose and 6,800 to 17,000 kg of lignin. Without structural and/or functional differentiation, these two molecules would be produced with insufficient contents to reach competitive profitability thresholds. The same reasoning can be obtained for hemicelluloses, whose composition, rich in xylose and arabinose, is not differentiated from other fractions obtained from cereals by distinct processes (Aguedo et al., 2013).

4. Conclusions

Brewers' grains are relevant agri-food by-products, particularly in terms of volume, in certain territorial areas including the Greater Region (Europe). These brewers' grains (BSGs), which are lignocellulosic materials, are often seen as strategic in certain applications related to the bioeconomy. Our work has shown that a two-step extraction in aqueous (acid then alkali) medium, preceded by a pressing phase,

allowed to recover sequentially hemicelluloses (or hemicellulose-constitutive saccharides), then the cellulose and the lignin. The quantities of lignin and cellulose obtained reach about 20 kg each after treatment of nearly one ton of BSGs (with an initial moisture content of 80%).

Although interesting from a mass balance point of view, this process suffers from various points of criticism which deserve to be underlined and which could constitute an obstacle to the economic deployment of BSGs in a biorefining option. The first is that, even though abundantly exploited in the state of the art, the acid pretreatment process generates notable amounts of furanic compounds, known to be fermentation inhibitors. Either the process must be optimized to minimize this formation (if the targeted applications are fermentation derived molecules like lactic acid or ethanol for instance), or the process must be improved to increase the extraction rate of lignin, which would then be considered as the target molecule. The intrinsic characteristics of this lignin (molecular, supramolecular and therefore applicative) must however be studied more deeply. The second fact to highlight is the intrinsic variability in the chemical composition of BSGs, being the result of differences in the cereal varieties selected by the brewers and/or differences in brewing practices. While BSGs from large industrial breweries show stable chemical compositions over time, BSGs obtained from microbreweries or from individuals show some notable variations, especially in lignin and protein contents. If the “pooling strategy” which consists in mixing together several BSGs samples coming from several origins could offer a strategy, its application to the case of BGSs is not possible when too much protein variation is detected. This implies that if brewers' grains are to be used as inputs in a bioeconomy option, the chemical analysis of these BGSs remains a prerequisite in order to exclude sample batches whose chemical composition deviates from a "standard" defined mostly by BSGs coming from large industrial capacity brewing activities.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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