Effect of Protein during Hydrothermal Carbonization of Brewer's Spent Grain

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Abstract

This study has two targets: Studying the extraction of the initial protein content from brewer's spent grain and the impact of protein's extraction on the chemical-physical properties of produced hydrochars. The protein was extracted from brewer's spent grains using the pH-shifting method. The extracted protein was quantified and characterized by their amino acid profile. The hydrothermal treatment was applied at 190 °C and 220 °C for 0.5 h, 1h, 2 h, and 4h. The hydrochars and process water were collected and assayed. The hydrochar after protein extraction reveals the lowest yield to hydrochars (67.10 – 45.14 %), higher C/N ratio (19.66 - 21.33) and lower ash content (1.52 – 1.72 wt. %) compared to the hydrochar without extraction.

Keywords:

Hydrothermal carbonization, Brewer's spent grain, Insoluble protein-rich fraction, Amino acid profile

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1. Introduction

The depletion of fossil fuel due to industrial development, uncontrolled and improper waste management, together with the continuous growth of the world population, are important contributors to climate change (Europäische Kommission, 2018). Then, new technologies to get alternative cleaner energy are urgently needed. One of the largest renewable and sustainable energy resources worldwide with considerable potential that can mitigate the current energy crisis is the utilization and conversion of biomass (Europäische Kommission, 2018).

Hydrothermal carbonization (HTC) is a thermochemical wet process for the production of a variety of products, among others higher energy density fuels, at moderated temperatures. At high pressures, usually autogenous, HTC converts wet biomass into a carbon-rich material (hydrochar) (Ekpo et al., 2016; Libra et al., 2014). Still, the complex reaction network produced during HTC is yet not known in detail since it highly depends on the type of biomass used as feedstock (Kruse and Zevaco, 2018)

Hydrochar is considered as an excellent renewable carbon material with a wide range of applications (Gallifuoco et al., 2017; Olszewski et al., 2019a; Poerschmann et al., 2015). Hydrochars can be used for energetic purposes like gasification, combustion, and electricity generation and storage (Kruse et al., 2013; Poerschmann et al., 2015). Hydrochars have also shown superior chemical properties and porous texture morphology that allow them to be used effectively in wastewater treatment applications. This is as feedstock for the production of activated carbon, which can purify water by removing impurities from polluted water (Rodríguez Correa et al., 2018).

In addition to the high energy demand, novel food (and feed) resources are also necessary, especially with high protein content in order to decrease protein malnutrition worldwide (López et al., 2018). It is crucial to develop technologies to recover nutrients such as the protein fraction from waste streams (e.g. brewer’s spent grain). Besides the application of recovered protein in food and

feed sectors, it is also potential to produce nitrogen-containing bulk chemicals in chemistry industry by a biobased protein fraction. Proteins tend to break down with the increment of temperature in nitrites, nitrates, and ammonia. Then, it is necessary to extract before HTC process because of the typical range of operating temperatures (Funke and Ziegler, 2010). One of the most favorable methods for extracting proteins from lignocellulosic materials (e.g. grass) is the pH-shifting (Feyzi et al., 2018). This will not only diminish some nutritional problems but also can decrease the dependence on fossil resources.

Brewer's spent grain (BSG) is the main by-product of the brewing industry, it is essentially the insoluble solid residue that remains after the mashing and wort filtration phase (Montusiewicz et al., 2017). The grains are referred as spent because most fermentable sugars were extracted from them, leaving material without any further valuable in beer production: the outer pericarp seed coat layer of the malted barley grain that consist of husk, pericarp, and fractions of endosperm (Ravindran et al., 2018).

It is essential to know that BSG chemical composition differs with the barley variety, harvest time, malting and mashing conditions, type of adjuncts used, and brewing technology (Forssell et al., 2008). BSG can be generally considered as a lignocellulosic material since it is mainly composed of cellulose, hemicellulose, and lignin, along with considerable amounts of proteins and lipids. (Ikram et al., 2017; Montusiewicz et al., 2017; Ravindran et al., 2018). The main polysaccharides in BSG are hemicellulose, that mainly consists of Arabinoxylan (AX), and cellulose with significant monosaccharides quantities like glucose (Ikram et al., 2017).

Currently, the application of BSG has been limited to bovine feed, mainly because their digestive system allows them to process dried spent grains that mainly consist of needle-sharp particles formed during the mashing process. After fermentable sugars are extracted for beer production, a strong adhesive bond between the grain and its husk is formed, hence it is not suitable for human

consumption (Santos et al., 2003). Moreover, the complex composition and microbial growth susceptibility due to high moisture content makes it difficult to store and transport BSG (Ikram et al., 2017). HTC can overcome these limitations since it needs water as reaction medium, which enables wet biomass to produce high quality fuels and materials (Kruse et al., 2013; Reza et al., 2014). Nevertheless, the high nitrogen content in BSG is not favored in HTC process. A combination of protein extraction and HTC will not only improve the hydrochar properties but also add value to the BSG life-cycle chain.

The aim of this paper is the protein extraction from the original BSG biomass before HTC and evaluating the effect of the protein extraction on the hydrochar properties.

2. Material and method

2.1 Material

The BSG used in this study, was supplied by Hoepfner Brewery factory (Karlsruhe, Germany).

2.2 Experimental procedure

This study was divided into two parts: the extraction and characterization of the protein from BSG and the HTC of three different feedstock. These are BSG complete without prior extraction (BSG_C), BSG, BSG protein extracted residue with acid treatment (BSG_PEA), and BSG protein extracted with acid and washing (BSG_PEAC) (Figure 1a).

2.2.1 Protein extraction and recovery from BSG

Protein isolation method known as the pH-shifting process was adjusted in order to extract the protein fraction from BSG (Figure 1b). This method is based on the differences in solubility that proteins exhibit in water at different pH-values (Cavonius et al., 2015).

Strongly alkaline conditions provide the proteins a negative charge causing repulsion within and between protein molecules, which favors their interaction with water (Cavonius et al., 2015). Firstly, 70g BSG was solubilized in 0.1 M NaOH (Sigma-Aldrich) and adjusted with distilled water to reach

a pH > 11 and a final volume of 700 mL. The suspension was stirred for 2 h at 40 °C and then separated by centrifugation at 13,500 rpm for 20 min at 4 °C (Z 326 K, HERMLE Labortechnik GmbH, Germany). The obtained sediment corresponds to the BSG protein extracted fraction, which was frozen and stored for HTC experiments.

Subsequently, the obtained protein-rich supernatant was adjusted to pH 3.0 with 1.0 M trichloroacetic acid (TCA, Merck). Here, the negative charges in proteins were vanished lowering the pH near to the isoelectric point (pI), thus minimizing the protein interaction with water and becoming insoluble (Cavonius et al., 2015). Hence, the insoluble proteins precipitate and can be recovered after centrifugation at 13,500 rpm for 20 min at 4 °C. The obtained supernatant was discarded and the solid residue containing the precipitated proteins was frozen at -24 °C.

Lastly, the obtained Protein-rich extract (BSG_PRE) was lyophilized in a freeze dryer (ALPHA 1-2LD plus from CHRIST GmbH, Osterode am Harz, Germany) to ensure mild and sufficient water removal. Drying was carried out at 0.5 mbar at mild increasing temperatures -10, -5, 0, and 10 °C. The resulting powders were stored at -15 °C before further analysis.

Acid and biomass washing

This BSG insoluble residue has an alkaline pH which differs from the BSG_C (pH=5.0). BSG_PEA was obtained by adding 50ml of a 1M acetic acid (Sigma Aldrich, ≥99.85%) into the BSG insoluble residue to decrease pH to 4.5. The acid solution was removed by centrifugation (3250 rpm, 7 min), decanting and washing with distilled water to decrease electrical conductivity. After 10 washing-centrifuge times, the electrical conductivity of BSG_PEAC is similar to BSG_C.

2.2.2 Hydrothermal carbonization

HTC of BSG_C, BSG_PEA, and BSG_PEAC was performed in reactors (V2A stainless steel) with an inner volume of 25 ml filled up with 10 g feedstock operated in a batch mode. Each autoclave was waxed with copper paste before being closed in order to avoid seizing and corrosion due to high

reaction temperatures. The reactors were pre-heated 45 min in order to reach the desired temperature, which then was maintained constant. At each temperature (190 and 220 °C) four experiments were conducted at four residence times (0.5, 1.0, 2.0, and 4.0 h) in a gas chromatograph (GC) oven HP 5890 Series II (Hewlett Packard, GC 5890). The oven has an internal sensor for temperature connected to a data logger RSG 30 (Endress+Hauser AG) to get a real-time monitoring. To end the reaction, the autoclave was quenched in a bucket of water until it reached room temperature.

After each reaction, the autoclave was opened in the fume hood to release the gas product. The slurry was then separated by filtration into solid residues (hydrochar) and the process water (PW) using a Büchner funnel with a pre-weighed 40 µm qualitative filter paper (VWR International, Leuven, Germany) connected to a side-arm flask with a vacuum pump. The hydrochars were dried at 105 °C for at least 24 h and stored at room temperature, while the process waters were collected in vials and stored at -24 °C for further analyses.

In order to reduce the particle size of the initial biomass and of the produced hydrochars, they were ground in a CryoMill from Retsch GmbH (Haan, Germany) for 2 min at frequency 30/s and stored in glass vials for further analysis.

2.3 Characterization of proteins, solid and liquid phase

2.3.1 Proteins

2.3.1.1 Amino acids profile

Total amino acids were analyzed according to the European Commission Regulation No 152/2009 (Europäische Kommission, 2018). Before total amino acids analysis, samples are oxidized at 0 °C with a performic acid (Sigma-Aldrich, 96%) and phenol mixture. Then the samples are hydrolyzed with hydrochloric acid (Merck, 37%) for 23 hours, following by pH adjustment to 2.2. The amino acids are separated by ion-exchange chromatography and determined using photometric detection at

570 nm (440 nm for proline) after reaction with ninhydrin (Sigma-Aldrich).

2.3.1.2 pH-dependent behavior

BSG_PRE was suspended in distilled water to obtain a 0.5 wt.% suspension which was then adjusted to different pH-values (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0) by the addition of o-H_3PO_4 (Merck, 85%) and NaOH (0.1N, 0.5 N, 1.0 N, and 5.0 N) (Sigma-Aldrich) respectively. Suspensions were stirred for 30 min and the pH was constantly adjusted to compensate the buffer capacity of proteins. Adjusted suspensions were stored at room temperature and pH was rechecked before further measurements.

2.3.1.3 ζ-potential measurement

The electrophoretic mobility was measured with a particle-electrophoresis instrument (Zetasizer Nano ZS, Malvern Instrument, Malvern, UK). Solutions were transferred into disposable folded capillary cells (DTS1070, Malvern Instruments, Malvern, UK) without further dilution. All measurements were taken at 25 °C.

2.3.1.4 SDS-Polyacrylamide gel electrophoresis

To observe the molecular weight of alkaline extracted protein from BSG, SDS-PAGE carried out on protein-rich extract with Mini-protean II system (Bio-Rad Laboratories, GmbH München, Germany). BSG_PRE was suspended in distilled water then followed by dilution with buffer solution containing 5 % ß-mercaptoethanol (purity ≥98%), 0.5 M Tris-HCl pH 6.8 (purity ≥99.8%), 25 % glycerol (purity ≥99%), 10 % SDS and 5 % bromophenol blue indicator (Merck KGaA Darmstadt, Germany). The protein concentration of the suspension is 5 mg/mL, and 10 µL of sample was loaded into each cell. The electrophoresis was performed at 200 V for 40 min. The gel was stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, GmbH München, Germany) for 2 hours and washed with 15 % methanol and 10% acetic acid solution overnight. A pre-stained marker (Roti®-Mark, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) with a range

from 17 to 245 kDa was used to determine the molecular weight distribution.

2.3.2 Solid phase

The analyses of the BSG_C, BSG_PEA, BSG_PEAC, BSG_PRE and HTC solids were according to standard methods. The ultimate analysis (CHNS) of solid samples was conducted by the elemental analyzer equipped with a thermal conductivity detector (TCD) (EuroEA 3000 Serie, Milan, Italy).

2.3.2.1 Thermogravimetric analysis

Samples were subjected to thermogravimetrical analysis (TGA) in a STA 449 F5 thermogravimetric balance from NETZSCH-Gerätebau GmbH (Ahlden, Germany) in order to obtain knowledge about their thermal stability. Around 20 mg of sample was loaded into differential scanning calorimetry DSC/TG pan Al_2O_3 crucibles. A pretreatment step was implemented to remove possible moisture in the samples by heating them up from room temperature to 105 °C, with a heating rate of 10 K/min over 10 min. Subsequently, the samples were heated up to 900 °C with a constant heating rate of 10 K/min.

2.3.3 Liquid phase

2.3.3.1 High performance liquid chromatography

The chemical compounds in the aqueous phase were filtered with 0.20 µm syringe filters with PTFE membrane VWR International (Radnor, U.S.) to be analyzed by High performance liquid chromatography (HPLC) as described by Arauzo et al., (Arauzo et al., 2018).

2.3.3.2 Total organic carbon

Total organic carbon (TOC) from process water after HTC was measured by TOC Analyzer 5959A (Shimadzu Scientific Instruments, Columbia, MD, USA).

3. Results and discussion

3.1 Protein yield

Wet BSG with a 78 % moisture content was used as extraction feedstock. After extraction BSG,

protein extracted residue (BSG_PE) and the BSG_PRE were obtained. Protein content determination was performed on BSG_C and BSG_PRE with the Kjeldahl method. To calculate the protein content from the Kjeldahl nitrogen content, a factor of 6.25 is applied (D. Breese Jones). The protein content in BSG_C is 22.2% and in BSG_PRE is 60.1%. This means that the protein recovery yield was 23 wt. %. These results are similar to those obtained by Celus et al., (Celus et al., 2007) that reported a protein rich extract with 60 % total protein content after treatment of BSG with NaOH alkaline solution. The remaining 30 % present in BSG_PRE could be attributed to the co-solubilization of nonprotein material like lignin and lipids, which are also soluble in alkali (Rommi et al., 2018).

The relatively low protein extraction yield may be due to two reasons: the biomass pre-treatment and the extraction method used to break down the cell wall. Firstly, some authors like Montusiewicz et al., (Montusiewicz et al., 2017) suggest that BSG has to be milled previous the hydrolysis step in order to soften the grains and reduce the particle size to open up the cell wall structures and reduce the size of particles cellulose. Secondly, the extraction solvent used (NaOH) produced alkali hydrolysis which was only targeting a specific protein fraction present in BSG (globulins and glutelins), which do not represent the complete fractions. According to Thomas B. Osborne (Thomas B. Osborne, 1924), grain proteins can be classified into four major classes in accordance with their sequential extractabilities: albumins (water-soluble), globulins (salt-soluble), hordeins (alcohol-soluble), and glutelins (acid/alkali-soluble). Hordeins and glutelins are the main proteins in BSG, only hordeins account for over 50 % of the total proteins (Vieira et al., 2014), while albumins and globulins compose only around 20 % of the total protein content in BSG as a result of their unavoidable extraction during the brewing process (Ikram et al., 2017; Rommi et al., 2018). Many grain storage proteins such as glutelins are solubilized by weak alkali (Connolly et al., 2013) but hordeins are alcohol-soluble and therefore did not solubilized during the process described here.

Alkaline solutions are the most effective Generally Recognized As Safe (GRAS) solvents for protein extraction from plants and cereals (Connolly et al., 2013), therefore NaOH was chosen as extraction solvent. For the isoelectric precipitation, TCA-induced precipitation was performed. TCA is one of the most frequently used acids for plant protein extraction, which forces the protein to precipitate by isolating the protein-bound water. Additionally, TCA is crucial for conformational changes that cause protein precipitation (Rajalingam et al., 2009). TCA has a higher protein precipitation efficiency (97 %) in comparison with other acids such as phosphoric acid (Polson et al., 2003) but at the same time its use is not environmental friendly. The reason is that salt formation occurs so a washing process might be required. The objective of the protein extraction was to produce a residue with lower protein content.

The elemental composition of BSG_C, BSG_PE, and BSG_PRE are listed in Table 1. It can be seen in Table 1 that the carbon content in protein rich extract is higher than original biomass indicating carbohydrates are isolated in the extraction process, as well. Meanwhile, the nitrogen content in protein extracted BSG residues is lower than the original biomass. Therefore; this residue is suitable for further HTC treatment.

3.2 Protein properties

A slight difference was observed of amino acids profile between original BSG biomass and BSG_PRE as shown in Table 2. The amino acids content in both samples shows the same value as the estimated protein content calculated with the NTP factor 6.25 by the Kjedahl method. The proportions of essential amino acids in BSG_C and BSG_PRE are 35.8 and 35.7% respectively. The most abundant amino acids obtained in this study are glutamic acid (24.1 %), proline (10.5 %), and leucine (7.8 %). These results agree to those obtained by several authors (Connolly et al., 2013; Ikram et al., 2017; Mussatto et al., 2006). The increased proportion of amino acids glutamic acid (Glu) and aspartic acid (Asp) attribute to their maximum solubility under alkaline pH (Ursu et al.,

2014). Meanwhile methionine and cysteine, the sulphur-containing amino acids, were present in the lowest quantities.

Among the nine essential amino acids that human cannot synthesis, BSG_PRE possess a high content of leucine that is an important amino acids used as flavor enhancers in the food industry. Although only specific amino acids can be used in the food industry, others could be used in the chemical industry. Protein can be used to produce nitrogen-containing chemicals due to their amine group ($–NH_2$).

The isoelectric point (pI) of BSG_PRE was observed at pH 3.3 via surface charge analysis obtained by ζ-potential measurements (Figure 2). Macroscopic observations (Figure 2) showed sedimentation and turbidity in all 0.5 wt.% BSG_PRE suspensions, irrespective of the pH value. The turbidity increases at pH 6.0 – 8.0, while the phase separation is more intense in between pH 2.0 – 3.0. At a protein's isoelectric point (pI), the solubility in water is minimal as there are no/low electrostatic repulsive forces and hydrophobic attractive interactions between the protein molecules dominate (Damodaran and Paraf, 1997). The pI of BSG_PRE was determined at pH 3.3. Macroscopic observations of the suspensions show sedimentation and turbidity in all BSG_PRE suspensions, irrespective of pH. One reason could be attributed to a high carbohydrates and fat content in the extract but it is also important to consider that proteins precipitated with TCA cannot be completely dissolved again in solution. According to Rajalingam et al., (Rajalingam et al., 2009) only 20 % of the protein can be recovered in their native form. The solubility of the protein can increase with the addition of a base in pH 7.5 that in this case, agree with the pH-dependent behavior analyzed above.

Protein solubility is the foremost-required property concerning its use as a functional ingredient in foods, since it improves the protein rearrangement capacity and the flexibility to migrate into solvents (Bucko et al., 2016; Feyzi et al., 2018). The presence of polar side-chains at the surface of

proteins, favors water-protein interactions, and thus the solubility of proteins in aqueous solutions (Cozzone, 2005). This physicochemical property, critically affects texture, color and sensory properties of products, including emulsifying, foaming and network-forming properties (gelation). Therefore, proteins with good surface properties and solubility are highly valued food ingredients (Rezig et al., 2013).

It can be noted from Figure 3 that the protein rich-extract from BSG covered a broad range of molecular weights. A higher intensity can be observed at molecular weight 33 and 50 kDa. The techno-functional properties of protein are closely related but not solely with molecular weight (Wang et al., 2006). Protein fraction of high molecular weight, larger than 14.5 kDa, can significantly contribute to better emulsifying and foaming properties (Celus et al., 2007). The protein fraction of molecular weight 50 kDa shows fast increase in foaming capacity in wheat gluten hydrolysate resulting from increase of surface activity (Wang et al., 2006). Meanwhile, the fraction of lower molecular weight can significantly enhance protein solubility (Dai et al., 2019). Fractionation of BSG protein according to its molecular weight and properties will increase its functional value in food industry.

3.3 Hydrochar properties

Based on the fiber composition of the initial feedstock (Arauzo et al., 2018), the operating temperatures were chosen to convert specific biopolymers (cellulose, hemicellulose, protein) in a controllable way. The 4 reaction times were selected during HTC for tracking the decomposition of the biopolymers into the resulted hydrochar. HTC experiments were repeated at least three times to confirm the observed trend. The yield to hydrochar decreased as the severity of the reaction increased (Figure 4). In comparison with the BSG_C, BSG_PEA and BSG_PEAC have notable decrease was observed especially at 220 °C. The lowest hydrochar yield was 45.1 % was achieved

with BSG_PEAC (Table 3), which showed a remarkable constant decrease as the residence time increased. It can be seen that at 220 °C and reaction time of 0.5 and 1h the hydrochar yield of BSG_PEA is higher than BSG_PEAC; however, at 220 °C and reaction time of 0.5 and 1h the BSG_PEAC is higher than BSG_PEA. On the contrary, the increase of the severity trends to increase the conversion of the feedstock to carbon-rich material, with maximum of 68.2 wt. %. This is due to the fact that the product distribution is extremely dependent upon HTC severity as mass yield decreases for all biomass types with increasing reaction temperature (Ekpo et al., 2016). The decrease in hydrochar yield occurs as a result of dehydration and decarboxylation reactions that perform better at higher temperatures; however, this mass loss is counterbalanced by increment of the higher heating value HHV (MJ/kg) (Ekpo et al., 2016; Lucian et al., 2018; Reza et al., 2014).

The HHV of initial three feedstock are similar (~22.5 MJ/kg) and the highest HHV values are found at the temperature of 220 °C, which agrees with those found in the literature (Arauzo et al., 2018; Balogun et al., 2017; Vieira et al., 2014).

The physical properties of the raw BSG feedstock and resulting hydrochars are displayed in Table 3. The proximate analysis shows that the volatile matter (VM) decreases as the severity increased, and the fixed carbon (FC) content increased indicating an increment of the carbonization degree (Lucian et al., 2018). VM of BSG_PEA is slightly higher than in BSG_C and BSG_PEAC. The ash content was always higher in those hydrochars produced from BSG_C feedstock than from BSG_PEA and BSG_PEAC because of the acid addition (Ghanim et al., 2017). The acid solves ash components. Furthermore, the BSG_PEAC has the lowest ash values. A possible explanation is that washing of the biomass with DI have an effect on the removal of the alkaline and alkaline earth salts (Pecha et al., 2015).

The ultimate analysis of the hydrochars (Table 3) shows that the elemental carbon content followed the same trend with the increment of temperature than with the increment of the reaction time, which consequently led to a significant rise in the HHV. This increase is related to poly-condensation reactions (Funke and Ziegler, 2010), decreasing the oxygen and hydrogen content. The nitrogen content in the hydrochar is one of the important key factors to design further applications of hydrochar in different fields e.g. as soil ameliorant or in the energetic sector (Kruse et al., 2016). Table 3 illustrates that the nitrogen content in the hydrochar after HTC. In BSG_PEA and BSG_PEAC it is lower than the one produced after HTC of BSG_C. This could be simply explained by the lower protein content in the initial feedstock before HTC due to protein extraction. One possible reason for the incorporation of nitrogen into hydrochar is via Maillard reaction (Kruse et al., 2005). On the other hand, the table 3 also shows that the nitrogen content in the hydrochar after HTC at 220°C is higher than the one obtained at 190°C, this observation applies to the three feedstock (BSG_C, BSG_PEA, and BSG_PEAC). A possible explanation of this change in the nitrogen content is that higher temperature lead to higher hydrolysis rate of proteins to amino acids, further hydrolysis of amino acids increase the concentrations of NH_3 in the liquid phase, which may eventually lead to higher incorporation of nitrogen to the hydrochar via Maillard reaction (Kruse et al., 2016; Wang et al., 2018). The nitrogen content in the hydrochar is relatively constant. This means that the N-containing groups are more persistent than the O-containing (Olszewski et al., 2019b). However, there is a slight decrease in the nitrogen content during the reaction time, this decrease could be explained by deamination reactions, which cause the transfer of amino group from the solid phase as ammonia to the liquid phase. It is also worth mentioning that in order to give a comprehensive understanding of nitrogen species reaction more investigation on the nitrogen-containing compounds in the liquid phase should be done. The van Krevelen diagram (Figure 5) shows the relationship between O/C and H/C atomic ratios of the raw BSG feedstock, plotted in the

upper right corner and the produced hydrochars. These ratios decreased, as the HTC severity increased by higher in residence time and temperature. Similar results were found by other authors (Lucian et al., 2018). In Figure 5 is shown that hydrochars produced from BSG_C has lower H/C ratios than from BSG_PEA and BSG_PEAC produced at the same operating conditions. On the other hand, BSG_C has higher O/C ratios. There were obtained lower ratios with BSG_PEA than BSG_PEAC as initial feedstock because reduction of hydroxyl groups by water elimination (Uddin et al., 2013).

3.4 Thermogravimetric Analysis

The TGA was performed to evaluate the conversion of biopolymers during HTC process and compare the thermal stability of the initial BSG feedstock and the hydrochars. Three raw feedstock (BSG_C, BSG_PEA, BSG_PEAC) and their hydrochars produced at 190 and 220 °C were selected. Figure 6 a, b, c, and d show the derivative of the mass loss (DTG) curves, which revealing two main peaks at 296 and 355 °C for RAW_BSG_C and RAW_BSG_PEAC. These peaks are associated to the decomposition of hemicellulose and cellulose, respectively. The decomposition of most biomass feedstock occurs within this temperature interval and where many volatile organics are released (Balogun et al., 2017). RAW_BSG_PEA showed a different pattern, with a shifting toward more intensive main peak at around 300 °C and second much less intensive at 340 °C. It is caused by acid treatment and decomposition of original structure of the biomass as a consequence of the rupture of glucosidic bonds (Kruse and Zevaco, 2018). Moreover, with an acidic treatment, more cellulose is degraded than without. The DTG curves for the hydrochars produced at 190 °C (Figure 6 b, d) showed a reduction of the peak, related to hemicellulose peak from raw feedstock. This confirms that hemicellulose is converted at relatively low HTC temperatures (Funke and Ziegler, 2010). TGA of hydrochars, converted at 220 °C, resulted in decreased peak related to cellulose (Figure 6 c, e)

because cellulose starts to decompose at 200 °C (Kruse et al., 2013). The protein extraction and acid treatment had the highest influence on the hydrochar, produced at 220 °C. The peak related to cellulose was the lowest for HTC_220_BSG_C which mean that the cellulose in this material was converted to the highest extent. On the other hand, hydrochar produced after acid treatment (BSG_PEA, BSG_PEAC) showed higher peaks at 350 °C than hydrochars from BSG_C. It may be caused by not fully converted cellulose during HTC or decomposition of newly created structures during HTC, which have similar decomposition temperatures.

3.5 Process water

Table 4 shows the compositional analysis of the obtained PW and results of the TOC and HPLC analysis. During the HTC process, water plays multiple roles as solvent, catalyst, reactant, and product (Gallifuoco et al., 2017). PW contains high concentrations of inorganics and organics compounds that may represent potentially valuable chemicals (Funke and Ziegler, 2010). According to Poerschmann et al. (Poerschmann et al., 2015), around two thirds of the TOC present in the feedstock is fixed into the hydrochar, whereas almost 20 % is transferred to the aqueous product stream. The concentration of TOC in the obtained PW are shown in Table 4 and there is a clear difference between BSG_C, BSG_PEA, and BSG_PEAC. The TOC content decreased as the HTC severity increased, suggesting that organic materials continue to react under more severe conditions (Hoekman et al., 2011). PW obtained from BSG_C has higher content of TOC than from BSG_PEA and BSG_PEAC. This may be due to the protein extraction treatment and acid addition. The main organic chemical compounds dissolved in the process water are acetic acid, formic acid, lactic acid, propionic acid, 5-hydroxymethylfurfural (HMF), and furfural. In the case of BSG_PEAC is found the lowest amount of these compounds. Acetic acid is the compound that showed a higher concentration of 0.196 mol/l with BSG_PEA at 190 °C. In this study, the feedstock condition that

resulted in higher concentrations of organic acids in the process water was BSG_PEA, which was subjected to acid treatment. It is well-known that an acidic condition in the HTC process is required to achieve a simulation of natural carbonization (Funke and Ziegler, 2010). According to several authors (Funke and Ziegler, 2010; Li et al., 2015), the hydrolysis of cellulose performs better under acidic conditions that enhance the reaction rate of the HTC process and have an impact on the products characteristic and distribution. The PW contains most of the nutrients which were originally present in the unprocessed feedstock (Ekpo et al., 2016). The HPLC analysis revealed that low molecular weight (LMW) organic acids were the main derived products present in the PW. LMW organic acids have been expected to originate mainly from carbohydrates that degrade into monomers. Although, proteins and lignin have also been known as sources (Poerschmann et al., 2015). The main organic acids obtained in this study were: acetic acid, formic acid, and lactic acid, while propionic acid, and levulinic acid. These results agree with those obtained by several authors such as (Baccile et al., 2011; Funke and Ziegler, 2010; Hoekman et al., 2011; Kowalski et al., 2013; Li et al., 2015).

The hydrolysis of cellulose produces glucose, which through isomerization to fructose and subsequent dehydration produces HMF. Glucose can undergo retro-aldol reactions to form lactic acid (Li et al., 2015) further rehydration of HMF produces formic acid and levulinic acid (Baccile et al., 2011; Li et al., 2015). According to Kambo et al., (Kambo et al., 2018), the formation of organic acids should increase with an increase in the HTC reaction temperature. It was observed a decrease in the formic acid concentrations as the severity of the process increased. This decrease can be attributed to the decomposition (oxidation) of formic acid into gas products (mainly CO_2) (Kambo et al., 2018). Concerning HMF, the increase in temperature and reaction time reduced its concentration due to a stronger transformation into levulinic acid and hydrochar, whereby the production of la is preferred at low pH. HMF is an interesting chemical used in the industry for the production of

furanoic polyesters and can be used for different applications such as food and flavoring agents, as an intermediate for the production of industrial and pharmaceutical compounds, coating materials, anti-freezers, etc. (Kowalski et al., 2013).

During all experiments, the main error is caused by the heterogeneity of the biomass. Moreover, the analytics (e.g. characterization of the amino acids) may due to being affected by laboratory temperature resulting in a standard deviation (< 0.01). The HPLC results were performed twice with the analytical error from preparation of carrier solution.

4. Conclusions

The protein extraction before HTC is a promising approach to valorize BSG. A protein-rich product was obtained through alkaline extraction and acid precipitation. The essential amino acids were not influenced by extraction and the physical-functional properties revealed its potential use in the food industry and as feedstock. Hydrochar yield, from HTC processing at 220ºC, was decreased after protein extraction; however, hydrochar C/N ratio increased. Under these conditions the lowest ash and volatile matter content were obtained, resulting it better for energy production. The Van Krevelen diagram indicates similar effect of dehydration and decarboxylation pathway independent of the protein extraction process.

Appendix A. Supplementary data

E-supplementary data of this work can be found in online version of the paper

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Figure captions

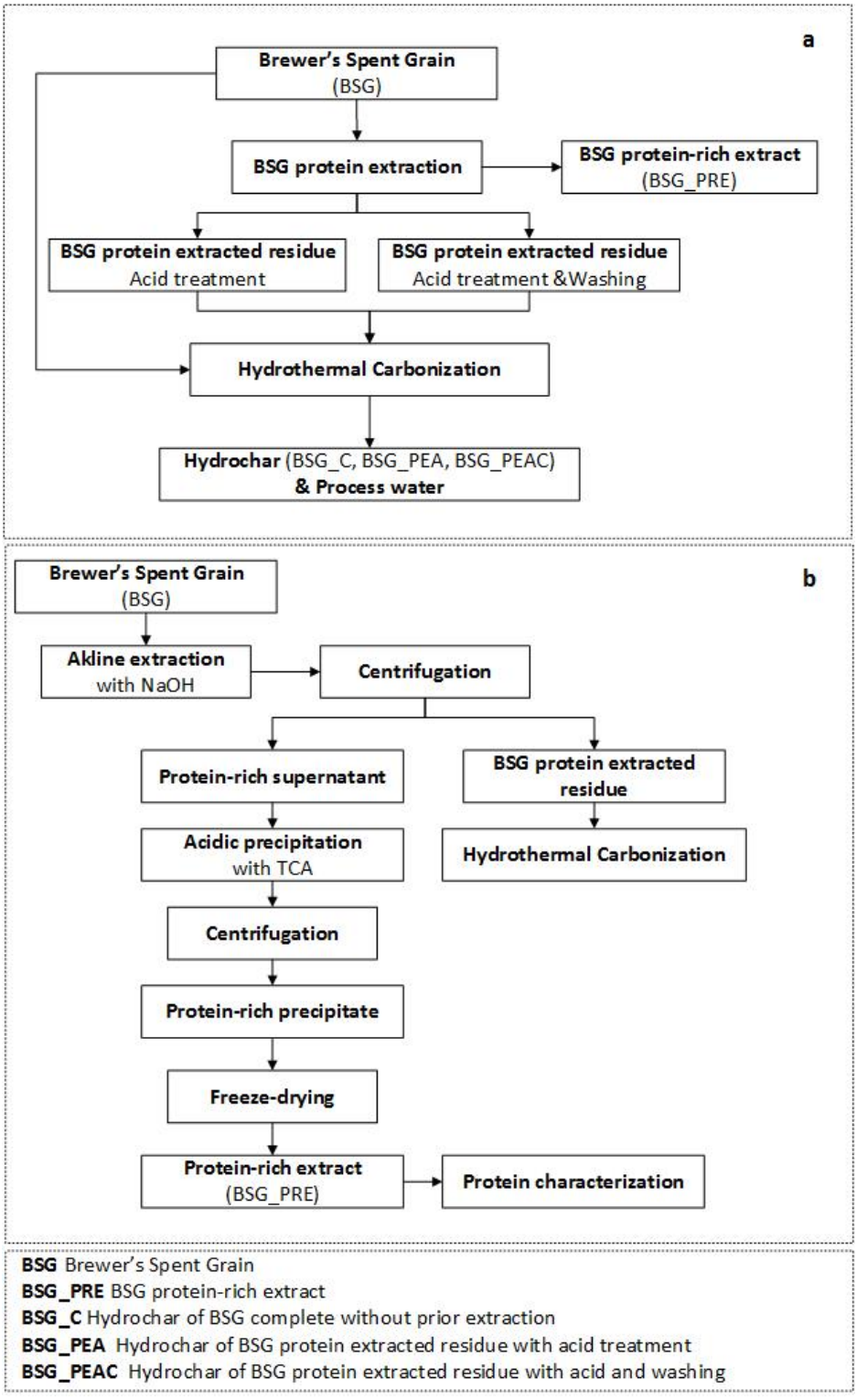

Fig 1. Experimental design (a) and scheme of fractionation protein-rich extract from BSG (b)

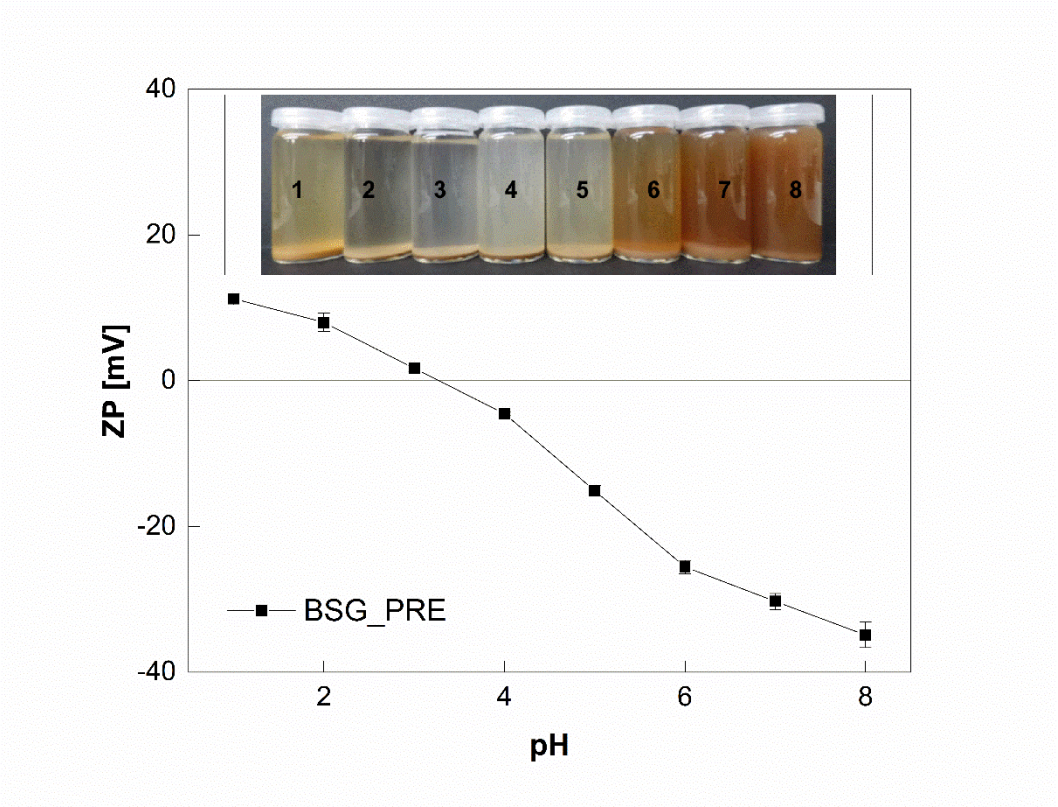

Fig 2. pH-dependent ζ-potential (mV) 0.5 wt% BSG_PRE suspensions at different pH-values

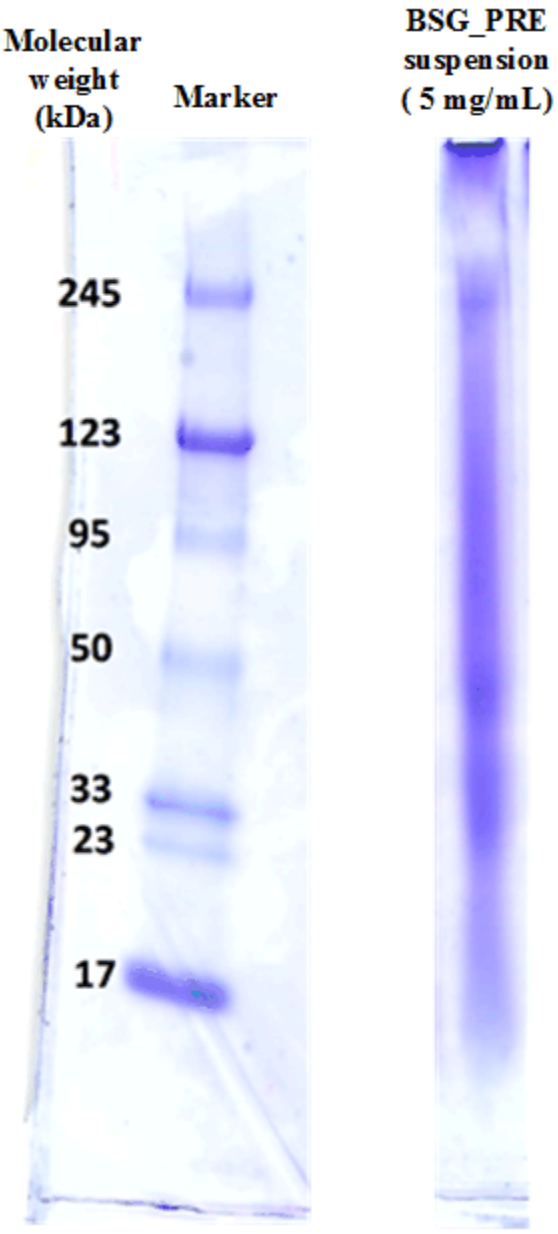

Fig 3. Molecular weight distribution of BSG_PRE.

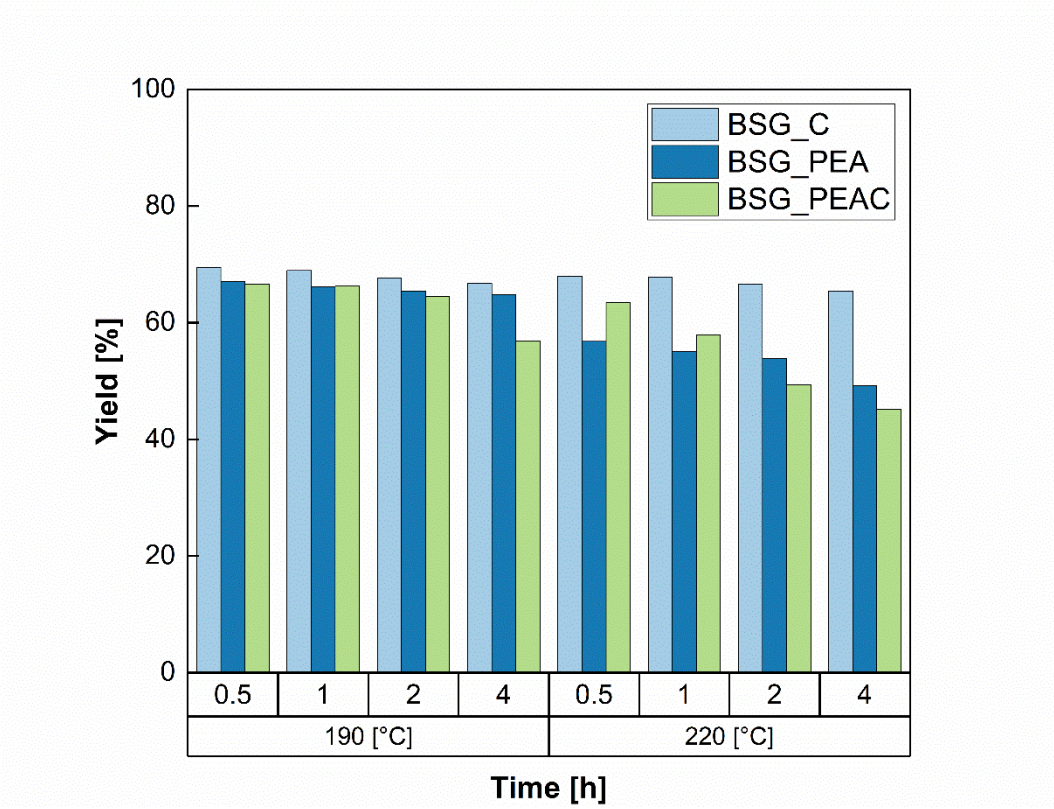

Fig 4. Effect of processing parameters on hydrochar yield

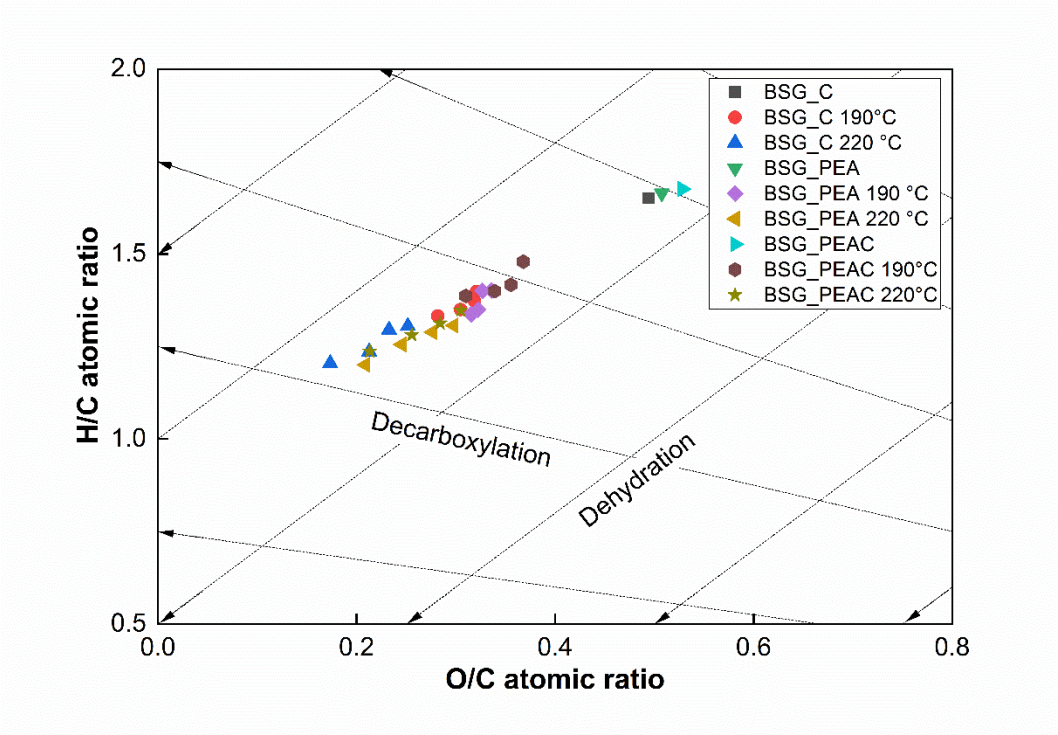

Fig 5. Van Krevelen diagram of initial feedstock and produced hydrochars

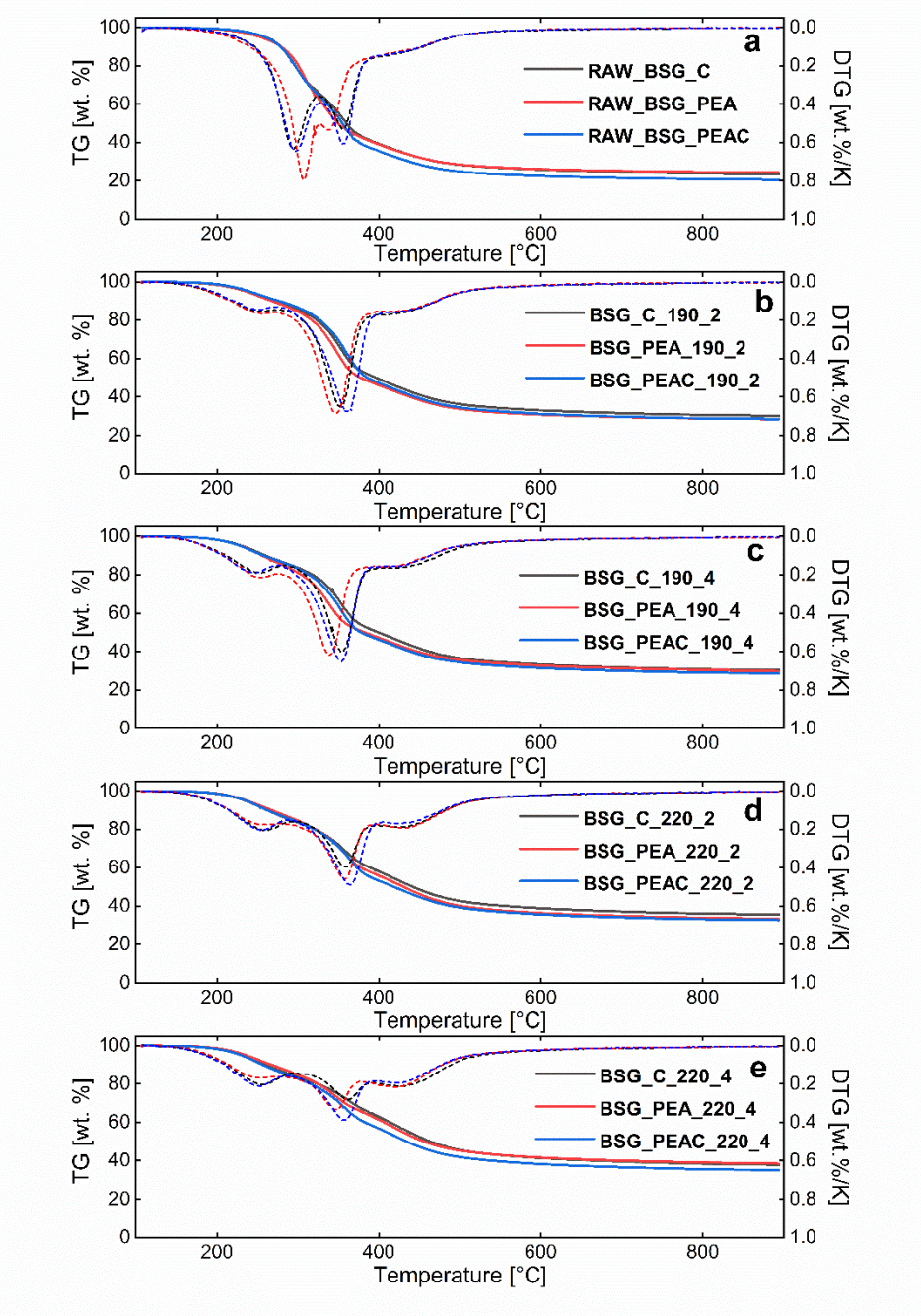

Fig 6. a) Raw BSG_C, BSG_PEA, BSG_PEAC feedstock thermograms, b) Hydrochars produced at 190 °C for 2h, b) Hydrochars produced at 190 °C for 2h, c) Hydrochars produced at 190 °C for 4h, d) Hydrochars produced at 220 °C for 2h, e) Hydrochars produced at 220 °C for 4h.

Table 1. Elemental composition and ash content of BSG_C, BSG_PE, and BSG_PRE (standard deviation with three replications < 0.1).

	Ash	N	C	H	S	O
	wt.% dry basis	wt.% ash free and dry basis				
BSG_C	3.91	4.51	53.02	7.29	0.25	34.93
BSG_PE	2.75	3.63	52.99	7.35	0.17	35.85
BSG_PRE	1.96	3.34	52.33	7.31	0.17	36.86

Table 2. Amino acid profile for BSG and the obtained protein-rich extract (standard deviation with three replications < 0.01).

	BSG_C [%]	% of total amino acids	BSG_PRE [%]	% of total amino acids
Essential amino acids	7.94	35.79	21.48	35.7
Histidine (His)	0.48	2.16	1.48	2.46
Isoleucine (Ile)	0.95	4.28	2.68	4.46
Leucine (Leu)	1.82	8.20	4.68	7.78
Lysine (Lys)	0.80	3.61	2.04	3.39
Methionine (Met)	0.43	1.94	1.16	1.93
Phenylalanine (Phe)	1.26	5.68	3.94	6.55
Threonine (Thr)	0.87	3.92	2.07	3.44
Valine (Val)	1.33	5.99	3.43	5.70
Nonessential amino acids	13.92	62.74	38.94	64.74
Alanine (Ala)	1.3	5.6	2.9	4.8
Arginine (Arg)	1.2	5.4	3.0	5.0
Aspartic acid (Asp)	1.7	7.8	4.5	7.4
Cysteine (Cys)	0.5	2.1	0.7	1.2
Glutamic acid (Glu)	4.5	20.4	14.5	24.1
Glycine	1.0	4.6	2.4	4.0
Proline (Pro)	2.1	9.2	6.3	10.5
Serine (Ser)	1.0	4.6	2.5	4.1
Tyrosine (Tyr)	0.7	3.0	2.2	3.6
Total protein	22.2		60.1	

Table 3. Raw BSG and resulting hydrochars chemical properties as a function of feedstock condition and processing parameters (standard deviation with two replications < 0.01).

Sample	Hydrochar Yield	Proximate Analysis (wt.% dry basis)			Ultimate Analysis (wt.% dry basis)					Atomic			HHV	Fuel
(Condition, °C, h)	(%)	VM	Ash	FC	N	C	H	S	O	O/C	H/C	C/N	(MJ/kg)	Ratio
RAW_BSG_C		77.73	3.91	18.35	4.33	50.94	7.01	0.24	33.56	0.49	1.65	11.75	22.53	0.24
RAW_BSG_PEA		79.79	3.29	16.92	3.51	51.25	7.11	0.17	34.68	0.51	1.66	14.59	22.64	0.21
RAW_BSG_PEAC		80.70	2.12	17.18	3.27	51.22	7.15	0.16	36.07	0.53	1.68	15.67	22.54	0.21
HTC_BSG_C_190_0.5	69.48	72.02	3.66	24.31	4.23	59.33	6.91	0.50	25.36	0.32	1.40	14.04	26.23	0.34
HTC_BSG_C_190_1	68.94	70.55	3.27	26.18	4.07	59.87	6.85	0.48	25.46	0.32	1.37	14.71	26.32	0.37
HTC_BSG_C_190_2	67.71	70.58	3.37	26.05	4.00	60.67	6.82	0.48	24.66	0.30	1.35	15.17	26.66	0.37
HTC_BSG_C_190_4	66.71	68.58	3.58	27.84	3.96	61.88	6.87	0.46	23.25	0.28	1.33	15.64	27.28	0.41
HTC_BSG_C_220_0.5	68.03	67.58	3.57	28.85	4.42	63.42	6.90	0.41	21.29	0.25	1.30	14.36	28.04	0.43
HTC_BSG_C_220_1	67.77	66.50	3.73	29.77	4.68	64.27	6.94	0.42	19.95	0.23	1.30	13.73	28.52	0.45
HTC_BSG_C_220_2	66.60	63.94	3.94	32.12	4.94	65.42	6.74	0.40	18.56	0.21	1.24	13.25	28.83	0.50
HTC_BSG_C_220_4	65.40	61.11	4.04	34.85	5.48	67.63	6.79	0.41	15.65	0.17	1.20	12.34	29.95	0.57
HTC_BSG_PEA_190_0.5	67.10	75.58	2.24	22.18	4.25	59.59	6.96	0.25	26.71	0.34	1.40	14.02	26.21	0.29
HTC_BSG_PEA_190_1	66.22	73.88	2.65	23.47	3.97	59.93	6.99	0.32	26.12	0.33	1.40	15.08	26.43	0.32
HTC_BSG_PEA_190_2	65.36	73.27	2.54	24.19	3.95	60.91	6.78	0.20	25.62	0.32	1.34	15.41	26.57	0.33
HTC_BSG_PEA_190_4	64.85	71.01	2.54	26.45	3.11	61.07	6.87	0.15	26.26	0.32	1.35	19.63	26.66	0.37
HTC_BSG_PEA_220_0.5	56.78	69.68	2.64	27.69	3.21	62.40	6.79	0.16	24.80	0.30	1.31	19.42	27.19	0.40
HTC_BSG_PEA_220_1	55.12	68.86	2.43	28.70	3.38	63.70	6.84	0.16	23.49	0.28	1.29	18.85	27.83	0.42
HTC_BSG_PEA_220_2	53.81	65.69	2.53	31.78	3.55	65.47	6.85	0.16	21.44	0.25	1.26	18.44	28.67	0.48
HTC_BSG_PEA_220_4	49.16	60.34	2.93	36.73	3.88	67.47	6.75	0.17	18.80	0.21	1.20	17.41	29.52	0.61
HTC_BSG_PEAC_190_0.5	66.59	73.58	1.42	25.00	3.26	58.81	7.25	0.40	28.87	0.37	1.48	18.05	26.08	0.34
HTC_BSG_PEAC_190_1	66.26	72.47	2.02	25.51	3.04	59.42	7.01	0.34	28.16	0.36	1.42	19.57	26.08	0.35
HTC_BSG_PEAC_190_2	64.49	70.86	1.83	27.31	2.89	60.47	7.05	0.41	27.36	0.34	1.40	20.91	26.59	0.39
HTC_BSG_PEAC_190_4	56.91	70.65	1.82	27.53	3.03	61.98	7.16	0.39	25.62	0.31	1.39	20.49	27.42	0.39
HTC_BSG_PEAC_220_0.5	63.40	70.24	1.52	28.24	2.94	62.76	7.04	0.22	25.52	0.31	1.35	21.33	27.55	0.40
HTC_BSG_PEAC_220_1	57.92	68.16	1.53	30.32	3.00	64.00	7.00	0.22	24.26	0.28	1.31	21.30	28.06	0.44
HTC_BSG_PEAC_220_2	49.35	66.57	1.62	31.81	3.24	65.57	7.00	0.21	22.35	0.26	1.28	20.25	28.80	0.48
HTC_BSG_PEAC_220_4	45.14	62.73	1.72	35.56	3.47	68.17	7.03	0.22	19.40	0.21	1.24	19.66	30.04	0.57

Table 4. Total organic carbon (TOC) (mg/l) and dissolved organic chemical compound (mol/l) in process water (PW) at different conditions (standard deviation with two replications < 0.005).

Sample	TOC	Acetic acid	Formic acid	Lactic acid	Propionic acid	Levulinic acid	HMF	Furfural
PW_BSG_C_190_0.5	25.50	0.12	0.11	0.07	0.04	0.01	0.01	0.02
PW_BSG_C_190_1	25.00	0.12	0.10	0.07	0.03	0.01	0.01	0.01
PW_BSG_C_190_2	25.78	0.10	0.08	0.04	0.03	0.01	0.00	0.00
PW_BSG_C_190_4	24.50	0.11	0.07	0.05	0.02	0.01	0.00	0.00
PW_BSG_C_220_0.5	24.20	0.11	0.06	0.04	0.02	0.01	0.00	0.00
PW_BSG_C_220_1	23.49	0.11	0.05	0.05	0.02	0.01	0.00	0.00
PW_BSG_C_220_2	23.98	0.12	0.05	0.07	0.02	0.01	0.00	0.00
PW_BSG_C_220_4	22.86	0.12	0.07	0.07	0.02	0.02	0.01	0.00
PW_BSG_PEA_190_0.5	20.15	0.19	0.11	0.05	0.04	0.00	0.01	0.02
PW_BSG_PEA_190_1	21.77	0.18	0.10	0.05	0.03	0.01	0.01	0.01
PW_BSG_PEA_190_2	20.67	0.19	0.10	0.04	0.03	0.01	0.00	0.00
PW_BSG_PEA_190_4	20.47	0.20	0.09	0.04	0.03	0.01	0.00	0.00
PW_BSG_PEA_220_0.5	19.49	0.14	0.02	0.03	0.02	0.00	0.00	0.00
PW_BSG_PEA_220_1	20.15	0.17	0.07	0.05	0.03	0.01	0.00	0.00
PW_BSG_PEA_220_2	20.17	0.17	0.06	0.00	0.02	0.01	0.00	0.00
PW_BSG_PEA_220_4	19.91	0.17	0.06	0.05	0.02	0.01	0.00	0.00
PW_BSG_PEAC_190_0.5	16.11	0.02	0.03	0.02	0.02	0.00	0.01	0.03
PW_BSG_PEAC_190_1	15.73	0.03	0.03	0.01	0.02	0.00	0.01	0.02
PW_BSG_PEAC_190_2	12.87	0.04	0.04	0.02	0.02	0.00	0.00	0.01
PW_BSG_PEAC_190_4	13.38	0.04	0.03	0.02	0.01	0.00	0.00	0.00
PW_BSG_PEAC_220_0.5	13.61	0.03	0.03	0.02	0.02	0.00	0.00	0.00
PW_BSG_PEAC_220_1	12.26	0.04	0.02	0.02	0.02	0.00	0.00	0.00
PW_BSG_PEAC_220_2	11.41	0.03	0.02	0.02	0.01	0.00	0.00	0.00
PW_BSG_PEAC_220_4	11.24	0.04	0.01	0.02	0.01	0.01	0.00	0.00

Supplementary information for

Effect of Protein during Hydrothermal Carbonization of Brewer's Spent Grain

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Table S1 Methods used for characterizing BSG_C, BSG_PEA, BSG_PEAC, BSG_PRE and HTC solids.

Analysis	Parameters	Abbreviations	Methods
Proximate analysis	Moisture	MC	DIN EN 14774-3 (BSG_C, BSG_PEA, BSG_PEAC, BSG_PRE, HTC solids)
	Volatile matter	VM	DIN EN 51720:1978-06 (BSG_C, BSG_PEA, BSG_PEAC, BSG_PRE, HTC solids)
	Ash	Ash	DIN EN 14775:2010-04 (BSG_C, BSG_PEA, BSG_PEAC, BSG_PRE), DIN 51719 (HTC solids)
	Fixed carbon	FC	FC (%) = 100% - MC (%) – Ash (%) – VM (%)
Ultimate analysis	Carbon	C	Elemental analyzer
	Hydrogen	H	Elemental analyzer
	Nitrogen	N	Elemental analyzer
	Sulfur	S	Elemental analyzer
	Oxygen	O	O (%) = 100% - C (%) - H (%)- N (%)- S (%)- Ash (%)
Fiber analysis	Hemicellulose	-	Detergent fiber
	Cellulose	-	Detergent fiber
	Lignin	-	Detergent fiber
Energy content	Higher heating value	HHV	(Channiwala and Parikh 2002)