Technology Innovations and Business Models for Valorisation of Industrial Waste Biomass in Sparsely Located Enterprises (SYMBIOMA)

Report on Extractions of proteins and hemicellulose from brewers' spent grain (BSG) and use of fractions in cosmetic products

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Contents

1 Introduction

Fresh BSG as such has low value. Currently it is mainly used as cattle feed or dumped into landfills. What is left of the barley in BSG are mainly husk, pericarp and seed coat. Therefore, BSG is rich in cellulose (12-25%), hemicellulose (20-25%), lignin (12-28%) and proteins (15-31%) [1]. Proteins component in BSG has potential to be used as a source of human and animal dietary protein, if it can be extracted without too much deterioration in its techno-functional properties. Also, hemicellulose, which in BSG is mainly arabinoxylan, can be used as food ingredient. Isolation of valuable components such as proteins and hemicellulose by extracting is one of the ways to add value to BSG.

Alkaline extraction is a common method for extraction of proteins from vegetable biomass [2]. Alkaline conditions facilitate solubility of proteins to aqueous solution by breaking hydrogen bonds and unfolding protein matrix. Solubility of protein increases with an increase in pH of the solvent also due to the ionization of acidic and neutral amino acids at high pH. Alkali like NaOH and KOH are commonly used for maintenance of basic pH to achieve higher extraction yield. Celus et al. [3] and Vieira et al. [4] used NaOH in different extraction conditions to obtain protein concentrates from BSG. In a so-called pH shift method, proteins initially extracted in alkaline conditions were precipitated by adjusting pH of the extract to 3 or 4 with citric acid. In a method described by Kishi et al. [5], BSG was prior extraction mechanically separated into fibrous fraction, and coarse and fine particulate protein-containing fractions. From the coarse particulate fraction proteins were extracted using 0.05 N - 0.15 N NaOH-solution at a temperature of 60 - 100 °C for a period of 10 - 40 min. KOH and Na_2CO_3 were considered for use in alkaline extraction of wet pale and wet black BSG. Even though KOH was effective, NaOH was determined to be the most effective in extracting proteins from BSG slurry [6]. He et al. [7] have presented a wet fractionation method that utilizes NaOH, $NaHSO_3$ or enzyme treatment to fractionate BSG to high-protein and high-fiber product.

In the following are results from relatively simple experiments aimed to fractionate BSG chemically to a value-added proteins and hemicellulose fractions and to a low value fraction. Added value in one of the fractions should originate from the concentration of proteins into that fraction (hopefully together with other nutritionally valuable components such as fibers), and in other fraction from the concentration of hemicellulose into that fraction. The third low value fraction should contain mainly α-cellulose and lignin.

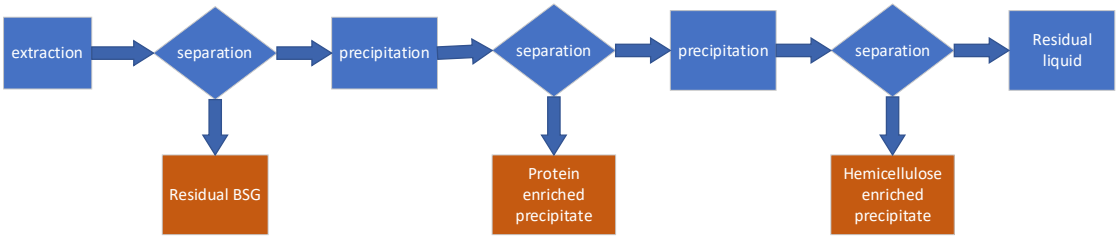
2 Fractionation of BSG

2.1 Laboratory scale extractions of proteins and hemicellulose from BSG

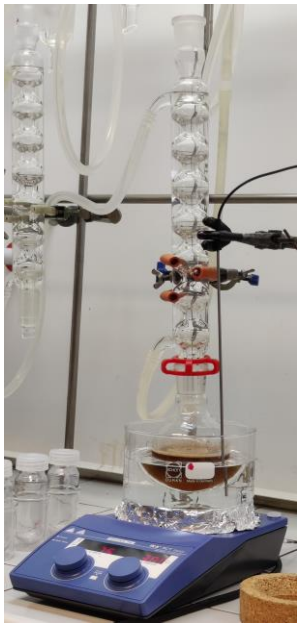
2.1.1 Experimental methods

2.1.1.1 Laboratory scale extractions of proteins and hemicellulose from BSG

Series of small-scale experiments to test different extraction conditions was carried out. In Scheme 1 is presented process steps and products from the extractions. Experiments were carried out in 100 ml round-bottom-flask equipped with condenser, magnetic stirring bar and oil bath for heating as presented in Figure 1. Extraction conditions are presented in Table 1. 3 g (db) of BSG was weighed into the flask. Water was added into the flask so that the solid:liquid -ratio was 1:10. Mixture was heated and stirred for the time stated in Table 1. After heating, temperature of the mixture was brought down to room temperature. From the cooled mixture residual BSG was separated using centrifuge (3,000 g, 10 min) presented in Figure 1. pH of the supernatant was adjusted to 3 using ~2 M HCl. pH-shift led to formation of protein enriched precipitate. Mixture was stored in refrigerator overnight. Following day precipitate was separated using centrifuge (3,000 g, 10 min). Ethanol was added to the supernatant so that EtOH:H_2O ratio was 7:3 to precipitate hemicellulose. Hemicellulose containing mixture was kept overnight in refrigerator. Following day hemicellulose enriched precipitate was separated using centrifuge (3,000 g, 10 min). Residual BSG, protein enriched precipitate and hemicellulose enriched precipitate were dried overnight in 60 °C oven. Dried samples were analyzed for proteins content.



Scheme 1. Process steps and products in small-scale extractions.



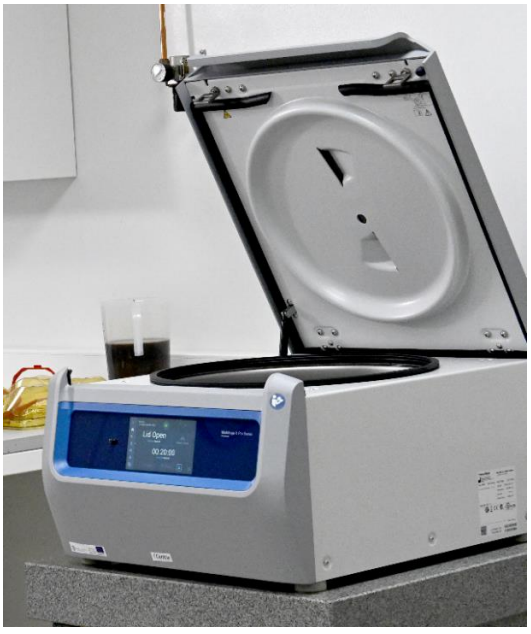


Figure 1. Equipment used in laboratory scale extractions of proteins and hemicellulose from BSG. On the left experimental set up used in extractions, and on the right the centrifuge used in separations of solids and liquids.

2.1.1.2 Proteins content analysis

Protein content of solid samples was analyzed using the Kjeldahl method. Oven dried samples were first digested with 20 ml 74% sulphuric acid and two catalyst tablets (Kjeltabs CX) in TURBOTHERM Kjeldahl digestion block (C. Gerhardt GmbH & CO. KG, Königswinter, Germany) (Figure 2 on the left). Nitrogen content in digested sample was analyzed using VAPODEST distillation system (C. Gerhardt GmbH & CO. KG, Königswinter, Germany) (Figure 2 on the right). Nitrogen content was converted to protein contents by multiplying nitrogen content with a factor of 6.25.

Figure 2. TURBOTHERM Kjeldahl digestion block (C. Gerhardt GmbH & CO. KG, Königswinter, Germany) on the left. VAPODEST distillation system (C. Gerhardt GmbH & CO. KG, Königswinter, Germany) on the right.

2.1.2 Results from the laboratory scale experiments.

In Table 1 is presented results from the small-scale experiments. Extraction temperature, base content, grinding of BSG and mild acid pretreatment were the tested variables. Room temperature was chosen for extraction temperature to see if it is possible to extract proteins and hemicellulose from BSG without use of excessive amount of energy even tough extraction time is then long [4]. From experiments 1, 2 and 4 one can see that the higher the base content (4.0, 0.5 and 1.0 M correspondingly) is the larger both the proteins enriched (1.68, 0.78 and 1.05 g), and hemicellulose enriched (0.64, 0.12 and 0.36 g) precipitates are. Proteins content in proteins enriched precipitate seem to also correlate with the base content of extraction solvent but negatively. Proteins content (7.3%) in the proteins enriched precipitate in experiment 1 is even lower than the proteins content in the BSG used in the experiments (14.9%) (proteins content of the BSG used in experiment 7 was 18.9%). In experiment 2 proteins content is significantly higher (29.6%).

Table 1. Results from the laboratory scale extractions (3 g of dried BSG, solid:liquid -ratio 1:10 unless otherwise stated).

		Fraction		
EXP	**Method**	**Proteins enriched (g), (%)[1], (%)[2]**	**Hemicellulose enriched (g), (%)[3]**	**Residual BSG (g), (%)[4], (%)[1]**
1	2.4 g NaOH (4.0 M) rt, 20 h	1.68 (7.3) (27.2)	0.64 (73)	0.87 (29) (4.4)
2	0.3 g NaOH (0.5 M) rt, 22.5 h	0.78 (29.6) (52.2)	0.12 (13)	1.63 (54) (8.3)
3	0.3 g NaOH (0.5 M) 50 °C, 2 h	0.46 (45.9) (47.7)	0.20 (21)	1.74 (58) (6.7)
4	0.6 g NaOH (1.0 M) rt, 23 h	1.05 (18.6) (45.4)	0.36 (41)	1.15 (38) (7.9)
5	8.6 g wet BSG (dry weight ~3 g), 0.6 g NaOH (1 M), 27 ml H_2O, rt, 23.5 h	1.03 (21.6) (50.0)	0.21 (23)	1.62 (54) (9.3)
6	8.6 g wet BSG (grinded in mortar, dry weight ~3 g), 0.6 g NaOH (1 M), 27 ml H_2O, rt, 23.5 h	1.11 (17.1) (43.1)	0.26 (29)	1.71 (57) (10.2)
7	15 g of dry BSG, 0.75 ml 97 % H_2SO_4, 1h 15 min in 85 °C, cooling to 60 °C, pH to 10.5 with 30 % NaOH, 1 h 15 min. (experiment was done in 600 mL beaker, heating plate was used for heating)	3.65 (33.7) (43.7)	2.20 (45)	7.56 (50) (11.5)

[1]proteins content [2] share of proteins in proteins fraction from the total amount of proteins in BSG
[3] from the total amount of hemicellulose in BSG [4] from the amount of dry weight of BSG at the beginning

Experiment 3 in Table 1 shows that at elevated temperature (50 °C) and in significantly shorter extraction time (2 h) proteins content in the protein enriched precipitate is significantly higher than the proteins content in similar extraction at room temperature (Exp 2) (45.9 and 29.6% correspondingly). Long extraction time even at low temperature seem to favor solubility of other major components in BSG. Wet BSG was used in experiment 5 and wet and grinded in experiment 6 (Table 1). Extraction of wet BSG afforded slightly higher proteins content (21.6%) in proteins enriched precipitate but smaller hemicellulose enriched fraction (0.21 g) than similar extraction with dried BSG (Exp. 4) (18.6% and 0.36 g correspondingly). Grinding of wet BSG decreased the proteins content (17.1%) in proteins enriched fraction but increased the size of the hemicellulose enriched fraction (0.26 g).

Some of the proteins left in BSG after the brewing process are inside cells, and thus not easily accessible to solvents [8]. Disruption of the cell walls with dilute acid (0.5 % H_2SO_4) pretreatment at

elevated temperature (85 °C) to release the proteins inside cells was tested in experiment 7 (Table 1). Acidic solution must be dilute, and treatment cannot be too long if isolation of hemicellulose is also objective. Acids at elevated temperature can catalyze hydrolysis of hemicellulose to smaller oligomeric and monomeric sugars and thus the yield of hemicellulose can be low. During the alkaline extraction step base is consumed and pH of the extraction mixture decreases quite rapidly first and then increasingly slower. In experiment 7 during the alkaline extraction step pH was controlled to stay at around 10.5 by adding 30% NaOH. Acidic pretreatment had a significant effect on the size of the hemicellulose enriched fraction. Size of the fraction in experiment 7 was more than twice the size in experiment 3 from the total amount of hemicellulose in BSG (45% and 21% correspondingly). Proteins content in the proteins enriched fraction in experiment 7 was 33.7% which is clearly lower than in experiment 3 (45.9%). On the other hand, protein content in residual BSG in experiment 7 was slightly higher (11.5%) than in experiment 3 (6.7%). Lower pH during the alkaline extraction step in experiment 7 may have resulted in poorer extraction of proteins and thus lower proteins content in dried proteins enriched precipitate.

2.2 Pilot scale

For pilot scale extraction of proteins and hemicellulose from BSG similar extraction conditions to experiment 7 (Table 1) were chosen. Even though both the proteins and hemicellulose enriched fractions were largest in experiment 1, the amount of NaOH used in the experiment is so high that on a large scale it seems quite impractical. Not only is the amount of NaOH high (4/5 of the weight of the dry BSG) but also the amount of acid needed to adjust the pH to 3 to precipitate proteins fraction is significant. High alkaline content can also severely affect the proteins digestibility and lower the overall quality and acceptability of the extracted proteins [9]. In experiment 4 (Table 1) alkaline content was lower (1.0 M) but the proteins content (18.6%) in protein enriched fraction was only slightly higher than in original BSG (14.9%). In experiment 3 (Table 1) short extraction time at elevated temperature improved both the proteins content (45.9%) in proteins enriched fraction and the size of the hemicellulose enriched fraction when lower amount of NaOH (0.5 M) was used.

2.2.1 Extraction in 200 L reactor

Pilot scale extraction was done in 200 L batch reactor (Figure 3). Reactor was loaded with 35 kgs of thawed wet BSG. Dry weight of BSG was ca. 7 kgs. 42 L of deionized water was added to the reactor. Solid:liquid -ratio was 1:10. Mixture was stirred vigorously. Before adding acid into the mixture, mixture was heated to ca. 75 °C. 0.95 L of 37 % HCl was carefully added into the mixture (HCl content of the solvent was ca. 0.5 %). Temperature was raised to 80 °C for 70 min. Then mixture was neutralized by carefully adding ca. 30% NaOH (acid catalyzed reactions such as hydrolysis of hemicellulose ceases). Mixture was cooled to 60 °C. pH was adjusted with 30 % NaOH to 11.5 for the extraction of proteins. pH was maintained between 11-11.5 for 1.5 hours by adding 30 % NaOH into the mixture. Total consumption of 30 % NaOH was ca. 4 L. Mixture was cooled to ca. 40 °C, after which it was pumped into a barrel for solid-liquid -separation. Following day decanter centrifuge was experimented in separation process.

Figure 3. Pilot scale extraction in 200 L batch reactor. Reactor on the left, dilute acid treatment in the middle and alkaline extraction step on the right.

2.2.2 Separation of residual BSG and precipitated proteins enriched fraction

Separation of solid and liquid matter from the slurry obtained in pilot scale extraction was experimented using Lemitec MD 80 decanter centrifuge (Lemitec GmbH, Berlin, Germany) presented in Figure 4. Separation was not successful. Even though there was a solid discharge, the Liquid discharge appeared almost as cloudy as the extraction slurry. Several operating parameters were changed (acceleration, feed rate, differential speed) without any improvement into separation process. Sample of the liquid discharge from first separation experiment was run through decanter centrifuge without a change in the appearance of the liquid discharge (there was also no solid discharge). Disassembling of the decanter centrifuge for cleaning revealed that there was a buildup of residual BSG inside the screw conveyor of the decanter centrifuge. In Figure 5 is presented the disassembled decanter centrifuge after centrifugation of protein enriched solid fraction. Similar buildup of residual BSG could be observed after the second centrifugation. Solid discharge from the first centrifugation was dried overnight in 105 °C oven.

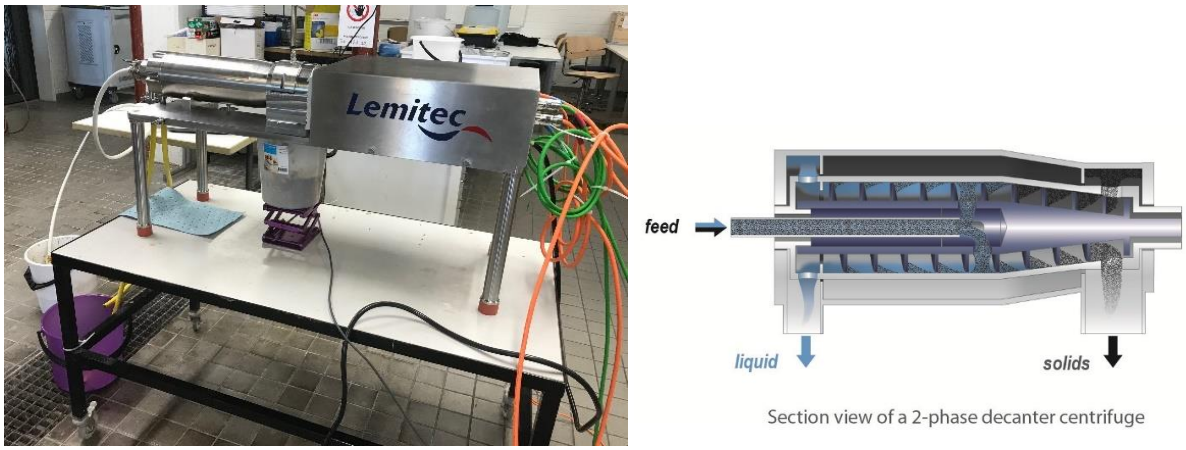


Figure 4. Lemitec MD 80 decanter centrifuge used in separations of solid and liquid matter after large scale extractions on the left. On the right: section view of a 2-phase decanter centrifuge.

Figure 5. Buildup of proteins enriched fraction inside the screw conveyor of the decanter centrifuge during the separation process.

Despite of the difficulties in separation of the residual BSG from the extraction slurry it was decided to continue with the experiment. 1.2 L sample from the extraction slurry after the two centrifugation was treated differently from the main body (69 L) of the slurry. Residual BSG was separated from the 1.2 L sample using centrifuge (18,000 g, 10 min) presented in Figure 1. pH of the clear supernatant was adjusted to about 3 with 2 M HCl. Precipitate that formed due to pH change was separated from the liquid using the same centrifuge (18,000 g, 10 min). EtOH was added to the proteins separated supernatant to precipitate hemicellulose. Into 750 ml of supernatant, 1900 ml of 92 % EtOH (H_2O:EtOH ratio 3:7) was added slowly. White precipitate was separated using centrifuge (18,000 g, 10 min). Solids from the three separation processes (protein enriched extract and hemicellulose enriched extract correspondingly) were dried overnight in 60 °C oven.

pH of the main body (69 L) of the extract was adjusted with 37 % HCl to ca. 3. In Figure 6 are small samples of the extract before and after the pH adjustment. There is a clear change in the color of the extract from dark brown to pale brown. Also, appearance of the solids is different: in alkaline solution the remaining particles appear more swollen than in acidic solution. (Note: in the beaker on the left the amount of solids, if the centrifugation had been successful with the decanter centrifuge, should have been much smaller (in ideal case liquid is clear and there is no solids at all)). Separation of the solids after pH adjustment on a large scale was experimented with decanter centrifuge. Similar problems occurred as did in the separation of residual BSG for the second time. At the beginning of the separation process, supernatant was clearly less cloudy and as the separation process continued supernatant became quite fast almost as cloudy as the slurry entering the centrifuge. There were no solids discharge this time also. Separation efficiency of the process decreased rapidly. In Figure 5 is presented the screw conveyor of the decanter centrifuge after the separation experiment. Clearly there is a significant buildup of the solids inside the decanter centrifuge which lead to poor separation of the solids from the liquid. A 9 L sample from the pH adjusted slurry was separated. Protein enriched precipitate was separated from the liquid using laboratory centrifuge (Figure 1, 4,000 g, 10 min). Separated precipitate was dried overnight in 60 °C oven.

Figure 6. Dark brown alkaline slurry from the pilot scale extraction after the first centrifugation on the left and pale brown precipitated proteins enriched slurry after pH adjustment of the slurry on the left to 3 on the right.

In Table 2 is presented results from the pilot scale extraction. In addition to proteins content, also lignin and α- and hemicellulose contents of the separated fractions were analyzed. There was a significant difference in the compositions of the proteins enriched precipitates. Proteins content in proteins enriched precipitate isolated from the 69 L sample was two thirds of the proteins content of the proteins enriched precipitated isolated from the 1.2 L sample. Also, there was a difference in α-cellulose contents: in 1.2 L sample there was no α-cellulose and in 69 L sample α-cellulose content was 10.2%. Amount of lignin in 1.2 L sample was 43.3 %, 11.5 %-points higher than in 69 L sample. Both samples also contained hemicellulose. More efficient centrifugation clearly had effect on the composition of the proteins enriched precipitates. Hemicellulose enriched precipitate obtained from the 1.2 L sample contained small amounts of proteins and lignin (Table 2). Most likely hemicellulose content in the precipitate was over 90 % since α-cellulose was not found. Residual BSG still had both proteins and hemicellulose left: 13.9 and 25.4 % correspondingly.

Table 2. Results from the pilot scale extraction.

Sample	**Fraction**	**Weight (g)[1], (Yield (%))[2]**	**% proteins**	**% lignin[3]**	**% α-cellulose**	**% hemicellulose**
	Residual BSG	2672.8 (38.2)	13.9	22.6	22.3	25.4
1.2 L	Proteins enriched	874.0 (12.5)	42.6	43.3	0	26.1
	Hemicellulose enriched	690.0 (9.9)	1.8	3.0	0[4]	ND
69 L	Proteins enriched	2151.7 (30.7)	28.9	31.8	10.2	26.6

[1] results are calculated to represent the whole extraction
[2] from the total amount of dry BSG in extraction
[3] acid soluble and acid insoluble lignin combined
[4] For holocellulose analysis (α- and hemicellulose combined) sample dissolved completely. Analysis is a gravimetric analysis. There was no precipitate to weigh.

Note: Due to the compressible nature of the solid matter, there was a buildup of both proteins enriched fraction and residual BSG inside the screw conveyor of the decanter centrifuge. At the beginning of the separation process supernatant was clearly less cloudy than the feed. As the separation process continued supernatant became fast as cloudy as the slurry entering the centrifuge. Separation efficiency of the process decreased rapidly due to buildup of material inside the centrifuge. Buildup of the solid matter inside the screw conveyor could not be avoided. Instead, the buildup material should be exhausted from the centrifuge periodically. After a certain period acceleration inside the centrifuge should be decreased to 50 g for couple of minutes or until there is no solid discharge anymore. Original acceleration should be restored until the liquid discharge becomes cloudy again. To test the instructions for better separation given by the manufacturer of the decanter centrifuge, extraction of BSG in 5 L glass reactor was carried out.

2.2.3 Extraction in 5 L glass reactor

500 g of dried BSG was loaded together with 4440 ml of deionized water (solid:liquid ratio 1:9) into 5 L glass reactor equipped with condenser and mixer (Figure 7). After the mixture was heated to 80 °C, 61 ml of 37 % HCl was added carefully into the reactor (HCl content of the solvent was ca. 0.45 %). Dilute acid treatment in 80 °C was continued for an hour. 100 ml of 30 % NaOH was added from dropping funnel into the reactor to neutralize the mixture (acid catalyzed reactions such as hydrolysis of hemicellulose ceases). Temperature of the mixture was brought down to 60 °C. At that temperature, pH of the mixture was maintained at around 11 by adding 30 % NaOH into the mixture for 1,5 hrs. Total consumption of 30 % NaOH was 190 ml. Mixture was cooled to room temperature.

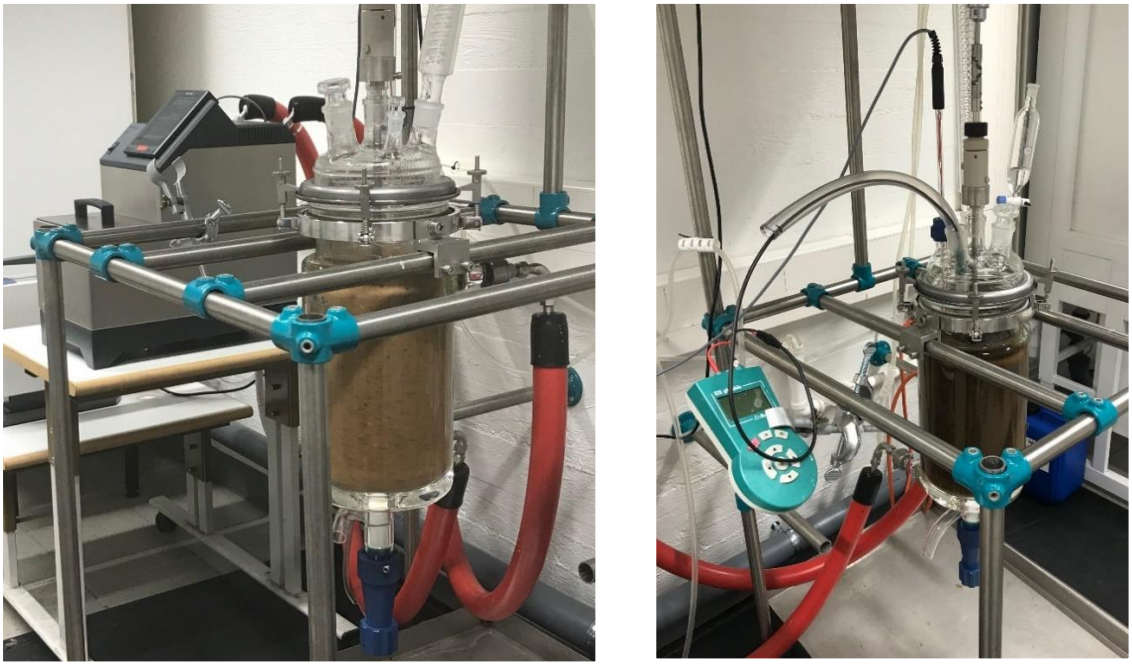


Figure 7. Extraction in 5 L glass reactor. On the left: dilute acid pretreatment of BSG. On the right: during alkaline extraction of the acid pretreated BSG, reactor was equipped with pH-meter and dropping funnel.

Residual BSG in the alkaline mixture from the extraction in glass reactor was separated from the liquid using decanter centrifuge. In Figure 8 on the left in the white bucket, is the mixture from the

extraction, and in purple bucket is the liquid discharge after centrifugation. Liquid discharge contained visibly significantly lesser amount of solid particles than the liquid discharge from the pilot scale extraction. Centrifugation with laboratory centrifuge (Figure 1) proved that the amount of solids in the liquid discharge was minimal. Residual BSG as solids discharge is presented in the middle in Figure 8. Residual BSG was extremely wet and probably contained significant amount of all the dissolved material. Shear forces due to vigorous mixing in pilot scale reactor most likely decreased the particle size of the BSG so that there was a buildup of BSG particles inside the centrifuge. Mixing in glass reactor was not as violent and therefore separation of the residual BSG from the extract was easier to achieve using decanter centrifuge. Residual BSG was dried overnight in 60 °C oven.

pH of the liquid discharge from the centrifugation was adjusted to 3 using 37 % HCl. A precipitate formed and mixture was let to stand for 1.5 hrs before separation. Decanter centrifuge was used for the separation. Soon it became clear that adjustments had to be made since there was no solid discharge and the liquid discharge was as cloudy as the feed. After a certain period (10 to 15 min) acceleration inside the centrifuge was decreased from 4,000 g to 50 g for couple of minutes or until there was no solid discharge. Also, feed of the slurry into the centrifuge was suspended. After the screw conveyor was exhausted from the solid mater, acceleration was brought back to 4,000 g and feed of the slurry was reinstated. Periodical exhaustion of the screw conveyor slowed separation process quite significantly. In Figure 8 on the right is the proteins enriched solid discharge from the separation process. Discharge contained large amount of the liquid as can be seen in the figure. Liquid discharge was recycled back to centrifuge until it was almost clear. Protein enriched solid discharge wad dried overnight in 60 °C oven.

Figure 8. On the left: in white bucket BSG slurry after the extraction and in purple bucket liquid containing soluble material after the centrifugation. In the middle: residual BSG after the separation. On the right: protein-enriched fraction after pH-shift of the liquid in purple bucket and separation of the precipitated proteins together with other solid matter in decanter centrifuge.

Total amount of the liquid discharge after the separation process was 2.8 L. Almost half of the liquids was lost during separation processes. Into a 1 L sample of the liquid discharge, 2.5 L of 92 % EtOH (water:EtOH ratio 3:7) was added from dropping funnel to precipitate hemicellulose. Mixture was stored in refrigerator overnight. Following day precipitated hemicellulose was separated using laboratory centrifuge (18,000 g, 4 min). Hemicellulose enriched precipitate was dried overnight in 60 °C oven.

In Table 3 is presented results from the extraction carried out in 5 L glass reactor. The yield of residual BSG (65.9%) was significantly higher than it was in pilot scale extraction (38.2 %, Table 2). Residual BSG from the extraction in 5 L reactor after centrifugation was very wet and contained probably large amount of dissolved material (washing off dissolved material from the residual BSG may be needed), which increases the dry weight of residual BSG. On the other hand, separation of residual BSG from the pilot scale extraction was not that successful. Supernatant from the centrifugation was very cloudy and contained large amount of residual BSG, which decreased the weight of the dried residual BSG. Proteins content in residual BSG (15.5%) obtained from the extraction in 5 L reactor was slightly higher than proteins content in residual BSG obtained from pilot scale extraction (13.9%). Proteins content in proteins enriched precipitate obtained from extraction in 5 L glass reactor was 36.3%. It is slightly lower than proteins content in proteins enriched precipitate from the 1.2 L sample (42.6%) from pilot scale extraction and slightly higher than proteins content in proteins enriched precipitate from the 69 L sample (28.9%) from pilot scale extraction. All the residual BSG was not separated during centrifugation of the extraction slurry from 5 L reactor. There was some cloudiness in supernatant which after pH adjustment to 3 was separated together with precipitated proteins and other precipitated components. Hemicellulose enriched fraction was slightly smaller (7.6%) and contained a higher amount of proteins (4.3%) than the hemicellulose enriched fraction from the 1.2 L sample from pilot scale extraction (9.9 and 1.8% correspondingly).

Table 3. Results from the extraction carried out in 5 L glass reactor.

Sample	**Weight (g)**	**Yield %[1]**	**% proteins**
residual BSG	329.45	65.9	15.5
proteins enriched precipitate	63.35	12.7	36.3
hemicellulose enriched precipitate	37.80[2]	7.6	4.3

[1]from the amount of dry BSG in extraction

[2]1 L sample from the total of 2.8 L was processed (13.50 g x 2.8 = 37.80 g)

2.3 Pressurized liquid extraction, PLE [10]

Accelerated solvent extractor (ASE, Figure 9) is an automated extraction technique that uses elevated temperatures and pressures higher than the saturation pressure of the solvent at selected temperature to achieve extraction. If water is used as the solvent under high pressure at a temperature between 100 and 374 °C, extraction is called subcritical-water extraction [11]. If temperature is below 100 °C, water-extraction is done in near subcritical conditions. There are several factors that contribute efficiency and reduced solvent use in near subcritical conditions: 1) solvent strength is higher, 2) diffusion rates are faster, 3) solvent viscosity is decreased and 4) solute-matrix interactions are more easily disrupted allowing the analytes to be removed from the matrix more easily [12]. Also, in the case of extracting proteins, lower extraction temperatures have less detrimental effect on the techno-functional properties of proteins [9].

BSG samples used in pressurized liquid extractions were six-month-old preserved BSG samples. Fresh BSG was treated in three ways to preserve the material: 1) A sample of fresh BSG was stored as such in plastic container at -20 °C (prior to extraction, frozen BSG was thawed in 21 °C (RT), 2) sample of fresh BSG was dried in oven in 60 °C for 6.5 h and 3) fresh BSG was preserved by adding formic acid (0.8 ml 98 % formic acid and 3.2 ml of water was mixed with 200 g of fresh BSG). Samples were stored in plastic jar at room temperature (except sample from treatment 1) until being used as such in extractions. Microbial activity (ISO 4833-2:2013) of all the preserved BSG samples was monitored regularly during period of six months to confirm their viability.

Figure 9. ASE-350 Accelerated Solvent Extractor (Thermo Electron Sweden AB, Hägersten, Sweden) on the left. Dried BSG, sample cell used in ASE-extractions, alkaline extract and pH-shift precipitated and dried proteins enriched extract on the right.

Accelerated solvent extractor (ASE) was used in extraction of proteins from brewers' spent grain (BSG) using water as solvent in near subcritical conditions (<100 °C, ~100 bar). 3 g (db) of BSG was placed into the sample cell (Figure). (With dried and ground BSG 6 g of diatomaceous earth was measured into sample cell). Variables in the study were extraction time (20, 60 and 120 min) and NaOH concentration (0.01, 0.05 and 0.1 N) in the extraction solvent. Extractions were performed so that sample in aforementioned conditions was extracted in four different temperatures (21 (RT), 40, 60 and 80 °C). In between each extraction temperature extraction solvent was changed to fresh solvent.

Standard operating conditions were as follows: preheating period (5 min), heating period (5, 15 or 30 min), cycles (4), flush volume (40%), nitrogen purge (110 s), and extraction pressure (103.4 bars). After extractions were completed, extracts were collected in extraction bottles. Dissolved proteins were precipitated from the extracts by adjusting pH to 3 with 2 M HCl, centrifuged (3000 g, 20 min) and dried overnight (16 hrs) in 60 °C oven. Protein contents in dried precipitates and residual BSGs after the extractions were determined using Kjeldahl method.

Effect of ultrasonic and acid pretreatment of BSG on proteins extraction was also experimented. Elmasonic P30H (Elma Schmidbauer GmbH, Singen, Germany) ultrasonic washer (Figure 10) was used for ultrasonic pretreatment of BSG. Operating conditions were as follows: pulse (ON), frequency (37 kHz) and power (100%). Water inside the washer was changed after every 5 min to avoid excess heating. Temperature was 30 °C or below during the treatment. Total time of the treatment was 20 min. After the treatment, water was decanted, and BSG was transferred into 33 ml stainless steel extraction cell. Mild acid pretreatments of BSG (0.5 % H_2SO_4, 100 °C, 20 min in ASE-extractor) prior alkaline extraction was also experimented. Pretreated BSG samples in both cases were then extracted using 0.1 N NaOH and standard conditions presented above: heating period 15 min.

Figure 10. Elmasonic P30H (Elma Schmidbauer GmbH, Singen, Germany) ultrasonic washer.

Combined amount of precipitated proteins from four extractions varied from 21.3 to 65.3% (db) from the total amount of proteins in fresh BSG (186 mg/g (db)) as presented in Figure 11. Alkaline extraction of H_2SO_4 pretreated BSG provided the highest, and 0.01 N NaOH as the extraction solvent the lowest proteins yields. Protein contents in the precipitated and dried extracts was more than three times the protein contents in BSG (18.6%) in 16 of the 41 analyzed extracts. Highest protein contents (69.7%) in dried extract was obtained when dried BSG was extracted with 0.05 N NaOH for 60 min at 40 °C (Figure 12). Water-extraction near subcritical conditions was shown to be a promising method to concentrate proteins from BSG with high protein contents and with low chemicals load.

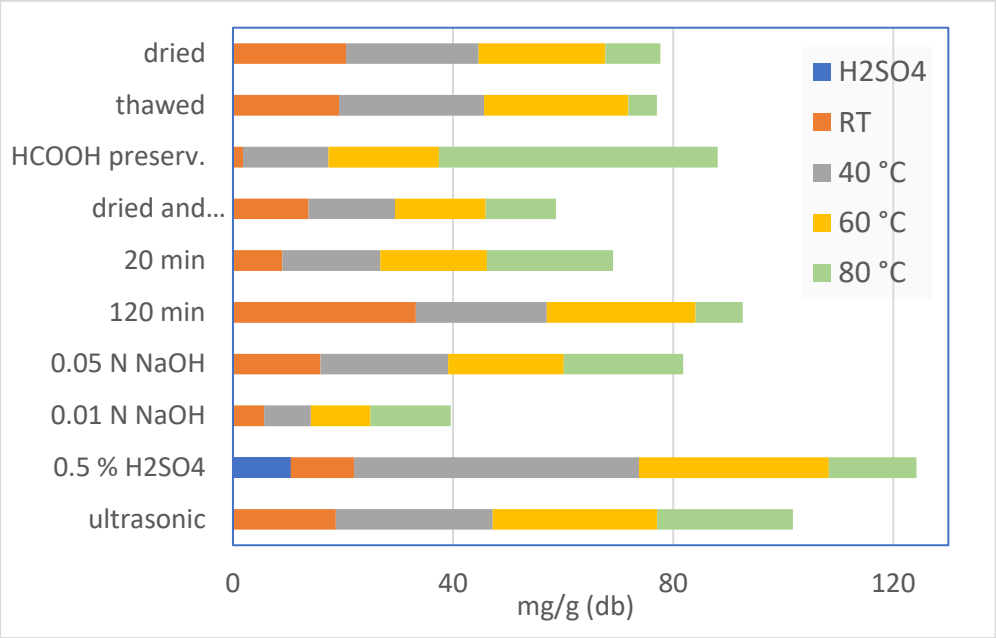


Figure 11. Amount of proteins extracted from BSG (mg/g (db)). Proteins content in dried BSG was 186 mg/g. Dried BSG was extracted with 0.1 N NaOH for 60 min in each extraction temperature unless otherwise stated.

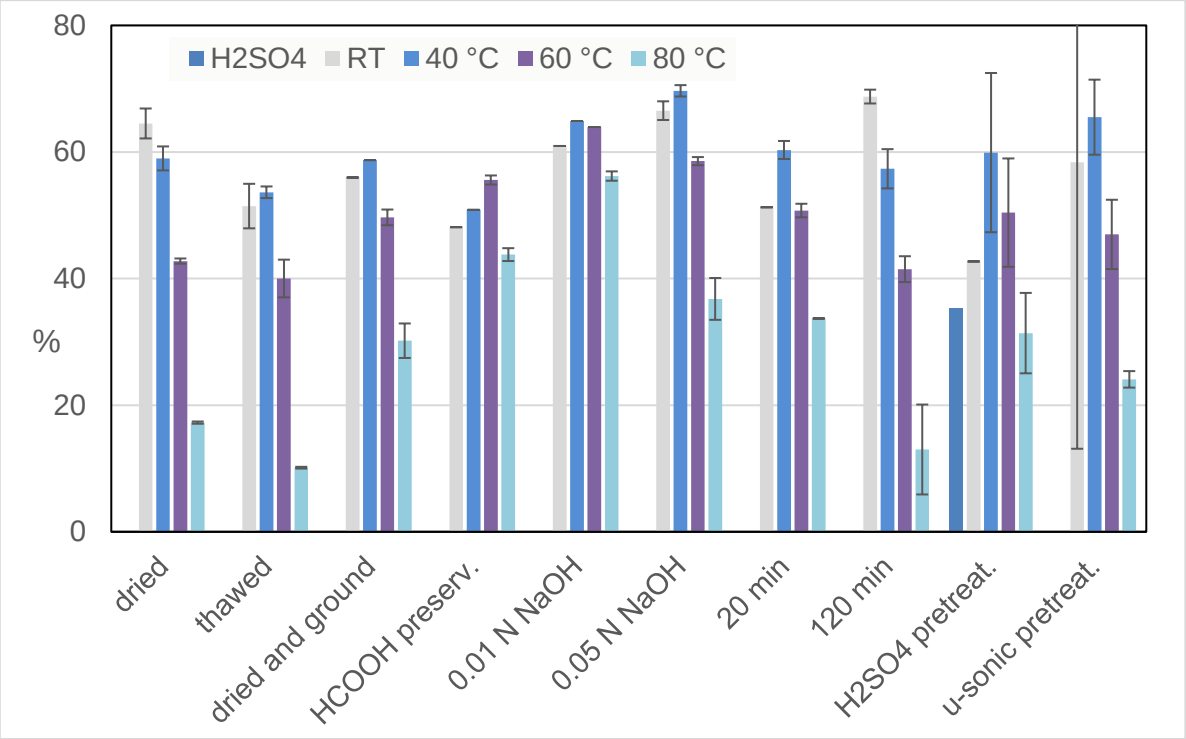


Figure 12. Protein contents in dried extracts. Dried BSG was extracted with 0.1 N NaOH for 60 min in each extraction temperature unless otherwise stated.

2.4 Plausible processes for extracting proteins and hemicellulose from BSG

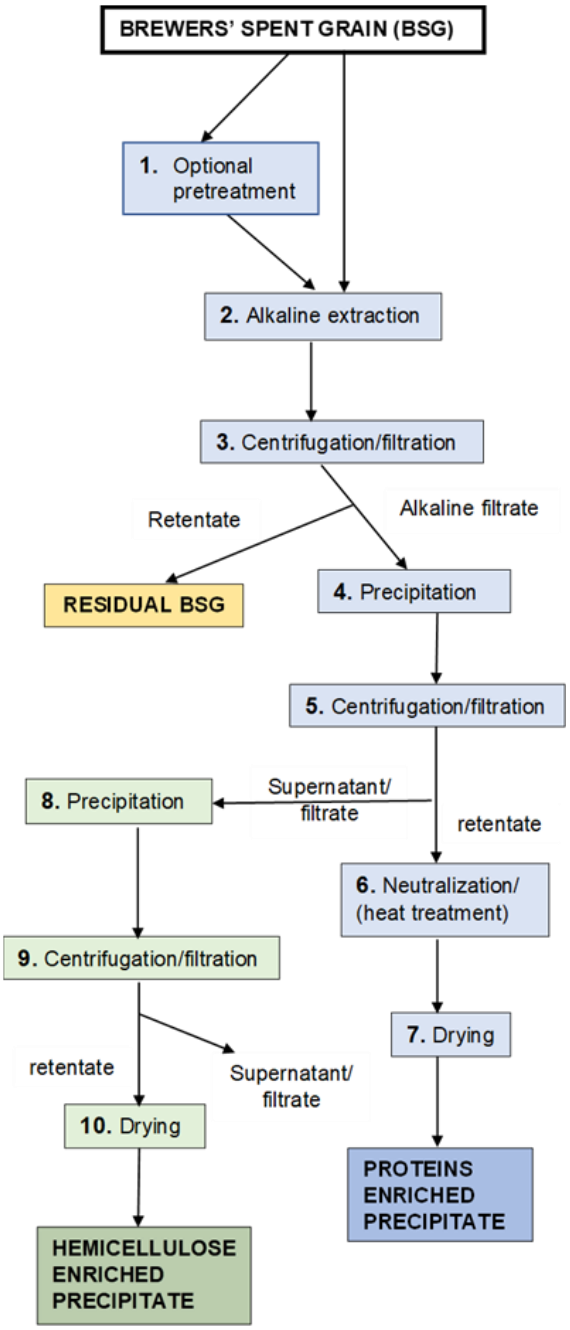
Based on the small-scale and scaled-up experiments presented in this report two plausible large-scale processes for extracting proteins and hemicellulose from BSG are presented. Processes are by no means definitive but more likely starting points for further development. Major difference in the two processes is the pressure during extraction step: in the first one extraction is done in atmospheric pressure, and in the second one at elevated pressure. Due to differences in the reactor types, there are also differences in the separations of residual BSG and proteins enriched and hemicellulose enriched precipitates from the extraction solvent. All the process steps that are presented were not tested. For instance, clarifier was chosen in one of the processes to separate proteins and hemicellulose enriched precipitates from the extraction solvent was based on the observations made during the small-scale and scaled-up experiments. Overall, there are numerous techniques to choose from to separate solid mater from liquid, and numerous techniques to choose from to dry solid matter.

2.4.1 Extraction at atmospheric pressure

In Scheme 2 is presented a flow sheet for a plausible process for extracting proteins and hemicellulose from BSG at atmospheric pressure. In light blue is presented process steps required in extracting proteins and in light green are presented process steps required in isolating hemicellulose from the extraction liquid after proteins enriched precipitate has been separated. Fresh BSG can be used in this process. Drying and/or decreasing the particle size of BSG does not seem to improve extraction of proteins from BSG. Handling of the material is easier if particle size is not decreased.

Following steps are included in the process:

1. Optional pretreatment: dilute acid treatment at elevated temperature in a reactor to improve accessibility of solvent to proteins.



Scheme 2. Flow chart for plausible process for extracting proteins and hemicellulose from BSG.

2. Alkaline extraction: dissolution of proteins and hemicellulose in alkaline conditions (pH = 11-12) at elevated temperature in a reactor.

3. Centrifugation/filtration: separation of residual BSG from the liquid containing dissolved components.

4. Precipitation: adjustment of pH of the alkaline extract to 3 to precipitate dissolved proteins.

5. Centrifugation/filtration/sedimentation: separation of protein enriched precipitate from the mixture.

6. Neutralization/(heat treatment): neutralization of acidic proteins enriched precipitate and dilution to required concentration and viscosity.

7. Drying: spray dryer, ring dryer etc. for removing water from the protein enriched precipitate.

8. Precipitation: addition of EtOH to supernatant/filtrate to precipitate hemicellulose.

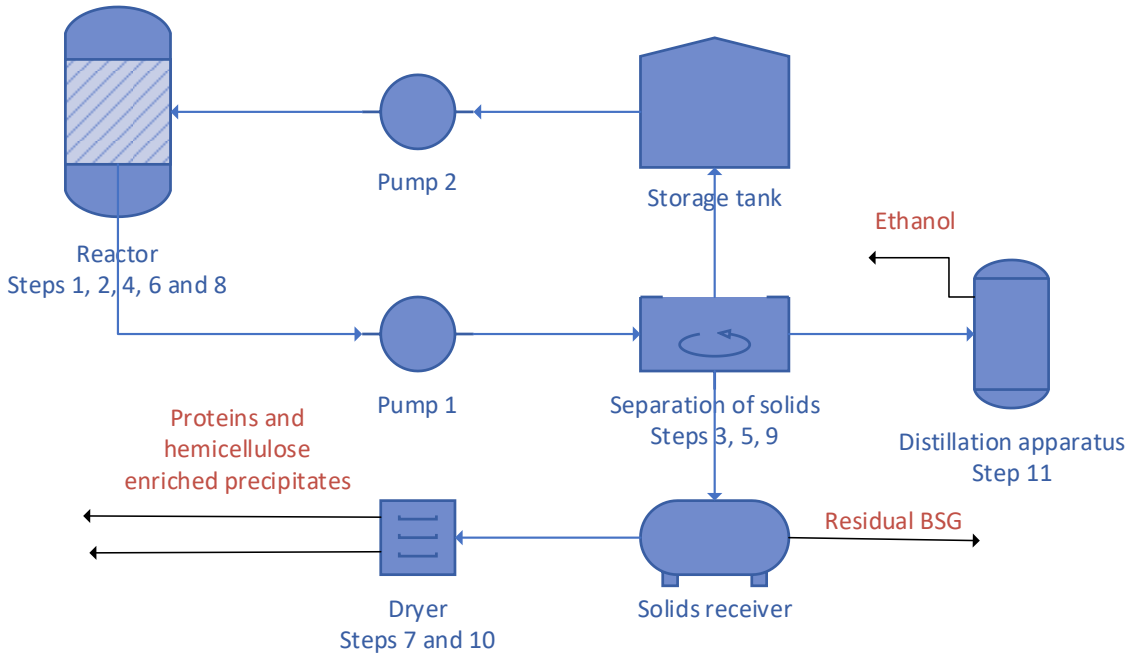
9. Centrifugation/filtration/sedimentation: separation of precipitated hemicellulose from the mixture.

10. Drying: spray dryer, ring dryer etc. for drying hemicellulose enriched precipitate.

    if process includes separation of hemicellulose by adding ethanol in step 8, following step may be needed:

11. Recycling of ethanol by distillation

In Scheme 3 is presented estimate of the process equipment needed in separating proteins and hemicellulose enriched precipitates from BSG. In Table 4 is presented size/capacity of the equipment needed in processing 260 kg of dry BSG (1040 kg wet BSG (moisture content 75%)). Reactor is a 3000 L batch reactor with stirring, heating, cooling and pH-control (solid:liquid -ratio 1:10 during extraction) (Steps 1 and 2). After the extraction, residual BSG is separated using for instance decanter centrifuge (Step 3). Residual BSG as solids discharge from the separation step is collected to solids receiver and liquid containing all the dissolved components is collected into storage tank. From the storage tank liquid is transferred back to reactor. pH of the liquid is adjusted to 3 to precipitate proteins (Step 4). Proteins enriched precipitate is then separated using for instance decanter centrifuge (Step 5). Proteins enriched precipitate is collected as solids discharge from the decanter centrifuge to solids receiver and then dried in a dryer (Step 7). (If neutralization of proteins enriched precipitate is done, precipitate is transferred to reactor and water is added to obtain proper consistency for instance for spray drying. Before drying mixture is neutralized (Step 6)). Liquid fraction (containing dissolved hemicellulose) from the separation process is transferred to storage tank. One third of the liquid is transferred back to reactor. To precipitate hemicellulose 2.3 times the volume of liquid ethanol is added (water:EtOH ratio 3:7) (Step 8). Hemicellulose enriched precipitate is separated using for instance decanter centrifuge (Step 9). Hemicellulose enriched precipitate is collected to solids receiver and then dried in a drier (Step 10). Separated precipitation liquid is transferred to distillation to separate ethanol (Step 11). Steps 8 to 11 is repeated until all the hemicellulose enriched precipitate is obtained.



Scheme 3. Proposal for a process to extract proteins and hemicellulose enriched precipitates from BSG under atmospheric pressure.

Table 4. Estimate of the size/capacity of the equipment if 260 kg of dry (1040 kg of wet) BSG is processed.

Process step	Equipment	Size/capacity
1, 2, 4, 6, 8	Reactor	3000 L
3, 5, 9	Decanter centrifuge	>3 m^3/hr
	Storage tank	3000 L
	Solids receiver	400 kg
7, 10	Belt dryer	50 kg/h
11	Distillation apparatus	6000 L/day

In Table 4 are presented what are the benefits and drawbacks if dilute acid pretreatment is included in the process. Results from the small-scale experiments showed that dilute acid pretreatment increases the amount of dissolved proteins and hemicellulose in the extract after the alkaline extraction (Table 1). Drawbacks may though overweight the benefits. Operating costs are higher if dilute acid pretreatment is included. High temperature is needed during the pretreatment step which increases energy costs. Amount of the chemicals needed in the extraction process also increases. Not only is the acid that increases costs from chemicals but also the use of acid increases the amount of base needed in the extraction step. Amounts are not significant but will have some effect on the operating costs. Proteins content in the protein enriched precipitate is lower if acid pretreatment is included in the process. Lower proteins content of the product can have effect on

the sales since usually selling price of the proteins enriched products positively correlates with the proteins content. Conditions during dilute acid pretreatment are quite harsh: low pH and high temperature. Such conditions can change the techno-functional properties of the proteins, which will limit in which applications proteins enriched products can then be utilized. On the other hand, digestibility of the proteins due to conditions used in acid pretreatment can be enhanced.

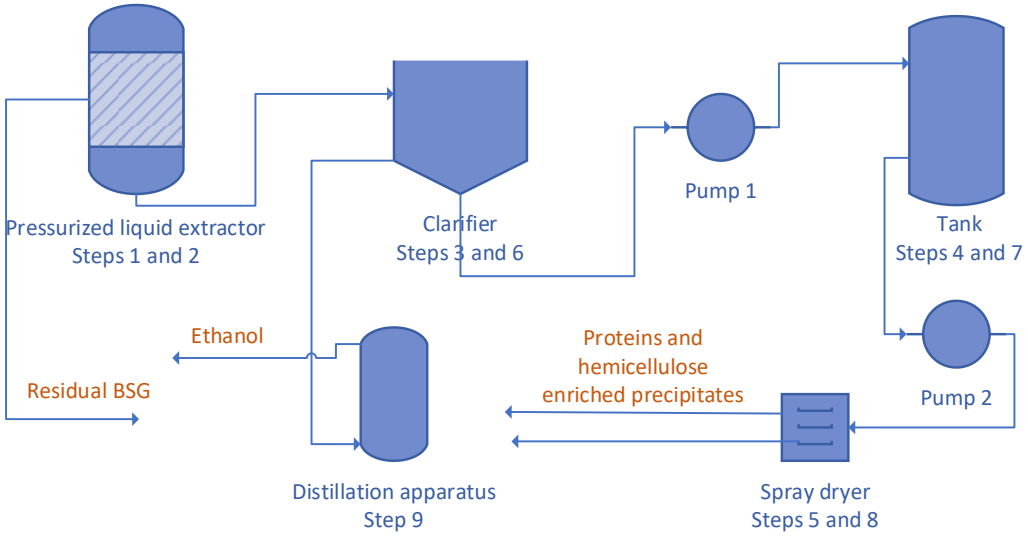
Table 4. Advantages and disadvantages of including pretreatment step in extraction process.

Pretreatment step included (dilute acid treatment for instance in 80 °C for 60 to 75 min)	
Advantages	**Disadvantages**
• more efficient proteins extraction • more efficient hemicellulose extraction	• higher temperatures are needed => higher operating costs • consumption of chemicals higher => higher operating costs • lower proteins content in dried extracts => possibly lower selling price of the product • possibly detrimental effect on techno-functional properties of the proteins => in which applications proteins can be utilized?

2.4.2 Pressurized liquid extraction (PLE)

In Scheme 4 is presented a proposal for a process to extract proteins and hemicellulose enriched precipitates from BSG under elevated pressure. Small-scale pressurized liquid extractions revealed that the proteins content in proteins enriched precipitates was in several extracts (60 - 70 %, Figure 12) higher than proteins content in proteins enriched precipitates obtained from the extractions done under atmospheric pressure (< 50 %, Tables 1, 2 and 3). Extraction cell loaded with fresh BSG is placed into the pressurized liquid extractor. Both dilute acid pretreatment (Step 1) and dilute alkaline extraction (Step 2) can be carried out in same extractor at elevated pressure. Before the extraction cell is filled with alkaline solution, dilute acid solution is removed from the extraction cell. After the extraction, alkaline extraction solution is removed from the extraction cell into a clarifier. Residual BSG stays inside the cell (oppose to process presented in Scheme 3, additional process step for separating residual BSG from the extraction solvent is not needed). In a clarifier pH of the extraction solvent is adjusted to 3 to precipitate proteins. Precipitated proteins are let to settle in clarifier (Step 3). Slurry from the bottom of the clarifier containing the settled proteins enriched precipitate is then pumped into a tank. In the tank, slurry is neutralized and diluted to appropriate concentration (Step 4) for drying in a spray dryer (Step 5). Into the liquid fraction remaining in clarifier after the slurry containing proteins enriched precipitates has been removed, ethanol is added to precipitate hemicellulose. Ethanol is added so that the ethanol:water -ratio is 7:3. Precipitated hemicellulose is let to settle on the bottom of the clarifier (Step 6). After settling, slurry containing precipitated

hemicellulose on the bottom of the clarifier is pumped into a tank. In the tank hemicellulose containing slurry is diluted to proper concentration (Step 7) for drying in a spray dryer (Step 8). Ethanol from the ethanol-water mixture remaining in clarifier after hemicellulose is removed is distilled for recycling in a distillation unit (Step 9).



Scheme 4. Proposal for a process to extract proteins and hemicellulose from BSG using pressurized liquid extraction.

Table 5. Estimate of the size/capacity of the equipment if 260 kg of dry (1040 kg of wet) BSG is processed.

Process step	Equipment	Size/capacity
1, 2	Pressurized liquid extractor	1 T/D (4 x 260 kg/batch)
3, 6	Clarifier	2500 L
4, 7	Tank	1000 L
5, 8	Spray dryer	Water evaporation capacity up to 59 kg/h
9	Distillation apparatus	6000 L/day

In Table 6 are presented some of the major differences between extractions done under atmospheric and at elevated pressure. Solid-liquid -extractions under atmospheric pressure has a long history and the technology is well known. Pressurized liquid extraction on the other hand is still an emerging technology. It has been widely utilized in small-scale extractions, but large-scale applications are difficult to find. Separation of residual BSG after filtration in pressurized liquid extraction is automated. There is a filter on the bottom of the extraction cell and after the extraction liquid is pushed out by pressurized nitrogen. In alkaline conditions BSG swells and forms almost a gel like mixture that does not settle on the bottom of the container. It seems that (vacuum)filtration of residual BSG after extraction under atmospheric pressure is difficult to achieve. Decanter centrifuge

was shown to separate residual BSG from alkaline mixture quite well. Automated separation of residual BSG is though much faster and less energy consuming process than using decanter centrifuge for the same process. For most efficient separation proper operational parameters need to be found in decanter centrifuge and even then, all the solid particles are not necessarily separated from the mixture. Dilute acid pretreatment is not the only pretreatment that can improve extraction of proteins and hemicellulose from BSG. For instance, ultrasonic and microwave assisted extractions have been shown to have effect on the extraction of those components from BSG in alkaline conditions [13]. Both are upcoming technologies that have not yet been utilized on large-scale extractions.

Table 6. Major differences between extractions done under atmospheric pressure and pressurized liquid extraction.

Extraction under atmospheric pressure	Pressurized liquid extraction
• mature technology • additional equipment needed for separation of residual BSG	• emerging technology • separation of residual BSG automatic • ultrasonic and other non-conventional pretreatments easier to incorporate into the process

2.5 Conclusions from extraction studies

Brewers' spent grain is underutilized source of proteins and hemicellulose. Low proteins content (compared to other sources of plant proteins), and high content of lignin, makes the extraction of high proteins content products from BSG challenging. Solubility of both proteins and lignin benefits from alkaline conditions. Precipitation of dissolved proteins by adjusting pH, also precipitates dissolved lignin. Pretreatment of BSG enhances extraction of both components. Currently there are no good methods to separate proteins from lignin in the extracts. Proteins rich high value products seem thus difficult to obtain from BSG using traditional plant proteins extraction methods. Emerging extraction technologies may bring a change. Proteins content in extracts obtained from pressurized liquid extractions was significantly higher.

Low temperature dilute alkaline extraction is not efficient method to extract hemicellulose from BSG. If high temperature dilute acid pretreatment is included in the process to increase extraction yield of hemicellulose, techno-functional properties of the extracted proteins may change, which may limit applications in which extracted proteins can be utilized. There seems to be a balance between high proteins yield and high proteins content in the extract, and on the other hand balance between high proteins extraction yield and techno-functional properties in the extracted proteins. The more efficiently proteins are extracted from the BSG, the harsher the extraction conditions, and thus lower proteins content in the extract, and more changes in the techno-functional properties in proteins. To obtain a commercially viable extraction process one should find a solution how to obtain high extraction yield for proteins, high proteins content in the extract and no changes in the techno-functional properties of proteins.

3 Fractions of spent grains (BSG) as ingredients for cosmetic products

Spent grain is a lignocellulosic material, the major constituents of which are fibre (hemicellulose and cellulose), protein and lignin (Fig. 1). Hemicellulose is mostly consisting of arabinoxylan and is present at a level of up to 29 % on a dry weight basis. Another significant constituent is lignin, representing 14 % of total dry weight. The protein content is present at levels of 15 % per dry weight basis. Also starch representing 16 % content of dry weight is interesting raw material in cosmetics.

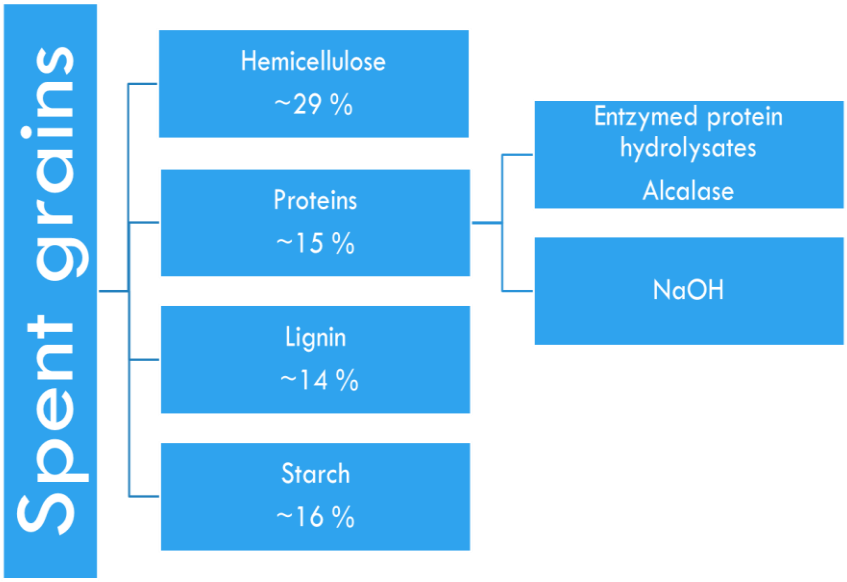


Figure 1. Contents of spent grain.

3.1 Spent grain – direct side stream

As a whole *Hordeum Vulgare* spent grains can be used directly after drying in different particle sizes and use as mechanical scrub material. Extract is an extract of the cereal grass of the barley, Hordeum vulgare l., Graminae (CosIng)

- different particle sizes
- mechanical exfoliation response = peeling dead skin cells
- humectant = hygroscopic material for moisturing skin
- Viscosifying benefits = texture of the product structure

Figure 2. Different fractions of spent grain. Dried spent grain (left), NaOH extracted protein (middle) and enzyme treated protein hydrolysates (right).

3.2 Cellulose and hemicellulose

Cellulose and hemicellulose are natural cellulose fiber sourced from sustainable side streams. Both raw materials show superior absorption properties for cosmetic oils and human sebum and provides the skin with a matte appearance. It can be incorporated into skin care emulsions, but also powders. With a rising focus on sustainable ingredients, it is a perfect alternative for microplastics in leave-on applications.

Hemicellulose is water solubility, biodegradability and amorphous, flexible structure, an interesting raw material for high-value cosmetics. Its polysaccharide structure can be hydrolyzed to sugars, which can be used as humectant in cosmetics. Polymeric, natural hemicellulose can be used as an environmentally friendly and inexpensive emulsifier to stabilize cosmetic products. Hemicellulose is used as fillers, lubricants, disintegrants, binders, glidants, solubilizers, and stabilizers in creams, suspensions, or solutions. Additionally, biodegradable and bioadhesive polymers may play an important role in the development of cosmetic delivery systems, especially for controlled active ingredient release.

O/W emulsion

▪ Recommended usage concentration: 1-5%

▪ Cellulose can be added to the water phase, (hot) oil phase or, alternatively, after homogenization at approx. 40 °C

W/O emulsion

▪ Recommended usage concentration: 1-2% (higher concentrations might lead to formulation destabilization, due to the high water binding efficacy of the fiber)

▪ Cellulose is preferably added as last ingredient to the hot oil phase or, alternatively, after water addition to the pre-emulsion, before homogenization

Water-free

▪ Recommended usage concentration: 5-20%

▪ Cellulose is blended with other powders/particles before addition of liquid ingredients

Example: Face lotion (w/o)

Phase	Ingredients	w/w %
A	Castor Oil	0.40
	Cera Alba	0.60
	Cetearyl Alcohol	1.00
	Caprylic/Capric Triglyceride	7.5
	Isoamyl Laurate	6.00
	Simmondsia Chinensis (Jojoba) Seed Oil	5.00
	Cellulose	1.00
B	Water	72.5
	Glycerin	5
C	Preservative	1

1. Heat phase A to approx. 85 °C
2. Add phase B (room temperature) slowly while stirring
3. Homogenize
4. Cool with gentle stirring below 30°C
5. Add phase C and homogenize again

3.3 Proteins

Proteins have been considered useful ingredients for creating a suitable environment for healthy skin and hair because of their ability to bind water with the horny layer of skin and its annexes.

Active function:

- moisturizing agents
- Increase elasticity
- Improve skin tightness
- cells growth stimulation

- softness and smoothness to skin
- anti-irritating effect
- buffering properties
- viscosity control

Used amount is in consentrations 0,1-2 %.

Example: Face mask (gel)

Phase	Ingredients	w/w %
A	Water	87.3
	Aloe Barbadensis	1.0
	Allantoin	0.3
	Glycerin	1.5
	Betaine	3.7
	Protein	5.00
	Xantan	0.2
B	Preservative	1

1. Mix ingredients in order mixing until clear. Heat phase A to approx. 70 °C, so that xantan makes a gel.

2. Cool below 40°C and add preservative

3.3.1 NaOH extracted protein

Barley+ Sodium hydroxide = exctracted protein

- Antimicrobial
- Antioxidant
- Humectant

Example: Face mask (gel)

Phase	Ingredients	w/w %
A	Aloe Barbadensis juice	81.3
	Gelatine/agar agar/xanthan gum	1.0
	Allantoin	1.5
	Sorbitol/erytritol	5.0
	Glycerin	5.0
	Betaine	3.0
	Xantan	0.2
B	Barley bran protein	2
	Preservative	1

1. Mix ingredients A. Heat phase A to approx. 70 °C, so that xantan makes a gel.

2. Cool below 40°C and add barley bran protein and preservative

3.3.2 Protein hydrolats

To make proteins suitable for use in water-based cosmetics, it is necessary to convert them into a soluble form, which is easier to manipulate and is more practical for formulating purposes. This form is obtained by the hydrolysis procedure, ie, by cleavage of the protein macromolecule by disruption of some of the peptide bonds. Cutting native proteins into smaller pieces with water and a catalyst will produce water soluble peptides.

- Enzyme alcalase
- Exfoliating effects
- Protein hydrolysis changes the molecular weight and charge
- More solubility to water, viscosity, sensory properties, and emulsifying and foaming behaviour

Example: Face serum, o/w

Phase	Ingredients	w/w %
A	Plant extract (plant-water 1:20) Allantoin Hydrolysated protein Xanthan	84 1.0 1.5 0.5
B	Glyceryl stearate SE Simmondsia Chinensis (Jojoba) Seed Oil Squalan	3.0 3.0 3.0
C	Preservative	1.0

1. Heat phase A to approx. 70 °C
2. Heat phase B to approx. 70 °C

2. Add phase B to phase A slowly while stirring

3. Homogenize

4. Cool with gentle stirring below 30°C

5. Add phase C and homogenize again

Example: Hair conditioning treatment

Phase	Ingredients	w/w %
A	Water	82.8
	Glycerin	2.0
	Creatine	0.5
	Hydrolysated protein	0.5
	Xanthan	0.2
B	Cetearyl Alcohol	7.0
	Castor oil	6.0
C	Preservative	1.0
	Lactic Acid	q.s.

1. Mix and heat phase A to 60°C
2. Mix and heat phase B to 60°C
3. Add phase B to phase A slowly with stirring.
4. Add preservative, adjust pH with lactid acid to 4.5-4.8

3.4 Starch

Technical function starches in cosmetics offer a very wide range of application. they constitute an alternative to talc. Starches are also effective at effecting foam profiles improving the characteristics of the “bubble size”, resulting in a creamer denser foam, whilst leaving a silky skin feel afterwards. Additionally a natural alternative to opacifiers where they provide a milky look formulation.

Active function:

- greasiness, and tacky formulation reduction
- lightening of heavy occlusive formulas
- smooth, powdery, and silky feeling
- spreading improvement “ball bearing effect”
- longevity in moisturization
- viscosity control

Example: Face cleaning bar

Phase	Ingredients	w/w %
A	Water	52.9
	Starch	4.0
B	BTMS, Behentrimonium Methosulfate and Cetearyl	11.0
	Cetyl Alcohol	3.8
	Simmondsia Chinensis (Jojoba) Seed Oil	10.0
	Hemp oil	5.0

	Shea butter	1.6
	Coconut oil	3.2
	Cera alba	5.4
	Myristyl myristate	1.3
	Zinc ricinolate	0.3
	Tocopherol acetate	0.5
C	Preservative	1.0
	Lactic Acid	q.s.

3. Mix carefully starch to water. Heat phase A to approx. 70 °C
4. Heat phase B to approx. 70 °C

2. Add phase A to phase B while stirring

3. Homogenize

4. Add phase C and homogenize again

3.5 Conclusions - possibilities in Cosmetic products

Spent grain can be use in several different products.

- Skin creams
- Hair shampoos
- Hair Conditioners
- Shower gels
- Peeling products
- Makeup and hair-coloring products (brown colour)
- Anti-odor products like deodorants and antiperspirants
- Color cosmetics
- Dry shampoos
- Powders

References

[1] K. M. Lynch, E. J. Steffen, E. K. Arendt, Brewers' spent grain: A review with an emphasis on food and health. J Inst. Brew. 122(4) (2016) 553-568. https://doi:10.1002/jib.363

[2] F. Hou, W. Ding, W. Qu, A. O. Oladejo, F. Xiong, W. Zhang, R. He, H. Ma, Alkali solution extraction of rice residue protein isolates: Influence of alkali concentration on protein functional, structural properties and lysinoalanine formation. Food Chemistry. 218 (2017) 207-215. https://doi.org/10.1016/j.foodchem.2016.09.064.

[3] I. Celus, K. Brijs, J. A. Delcour, Enzymatic Hydrolysis of Brewers' Spent Grain Proteins and Technofunctional Properties of the Resulting Hydrolysates. J. Agric. Food Chem. 55 (2007) 8703-8710. https://doi:10.1021/jf071793c

[4] E. Vieira, M. A. M. Rocha, E. Coelho, O. Pinho, J. A. Saraiva, I. M. P. L. V. O. Ferreira, M. A. Coimbra, Valuation of brewer's spent grain using a fully recyclable integrated process for extraction of proteins and arabinoxylans. Ind. Crops Prod. 52 (2014) 136-143. http://dx.doi.org/10.1016/j.indcrop.2013.10.012

[5] S. Kishi, T. Kimura, T. Minami, H. Kobayashi, Process for producing protein rich product, fibrous product and/or vegetable oil from brewer's spent grain. (EU Patet No. EP0369818A2). European Patent Office. https://patents.google.com/patent/EP0369818A2/en, 1990

[6] A. Connolly, C. O. Piggot, R. J. FitzGerald, Characterisation of protein-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain. Int. J. Food Sci. 48, (2013) 1670-1681. https://doi:10.1111/ijfs.12137

[7] Y. He, D. D. Kuhn, J. A. Ogejo, S. F. O'Keefe, C. F. Fraguas, B. D. Wiersema, Q. Jin, D. Yu, Haibo, H, Wet fractionation process to produce high-protein and high-fiber products from brewer's spent grain. Food Bioprod. Process. 117 (2019) 266-274. https://doi.org/10.1016/j.fbp.2019.07.011

[8] P. Niemi, C. B. Faulds, J. Sibakov, U. Holopainen, K. Poutanen, J. Bucher,. Effect of a milling pre-treatment on the enzymatic hydrolysis of carbohydrates in brewer's spent grain. Bioresour. Technol. 116 (2012) 155-160. https://doi.org/10.1016/j.biortech.2012.04.043

[9] M. Kumar, M. Tomar, J. Potkule, R. Verma, S, Punia, A. Mahapatra, T. Belwal, A. Dahuja, S. Joshi, M. K. Berwal, V. Satankar, A. G. Bhoite, R. Amarowicz, C. Kaur, J. F. Kennedy, Advances in the plant protein extraction: Mechanism and recommendations. Food Hyrdrocoll. 115 (2021) 106595. https://doi.org/10.1016/j.foodhyd.2021.106595

[10] M. H. Junttila, Extraction of brewers' spent grain in near subcritical conditions: A method to obtain high protein contents extracts. J. Agric. Food Res. 10 (2022) 100378. https://doi.org/10.1016/j.jafr.2022.100378

[11] M-J. Ko, H-H. Nam, M-S. C, Subcritical water extraction of bioactive compounds from *Orostachys japonicus* A. Berger (Crassulaceae). Sci Rep 10 (2020) 10890. https://doi.org/10.1038/s41598-020-67508-2

[12] S. C. Mandal, V. Mandal, A. K. Das, Essentials of botanical extraction: Principles and applications, first ed., Academic press. 2015.

[13] C. Barrios, M. Fernández-Delgado, J. C. López-Linares, M. T. García-Cubero, M. Coca, S. Lucas, A techno-economic perspective on a microwave extraction process for efficient protein recovery from agri-food wastes. Ind. Crops. Prod. 186 (2022) 115166.
https://doi.org/10.1016/j.indcrop.2022.115166