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## Processing of brewing by-products to give food ingredient streams

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6 measurements.

### 7 Abstract

8 Very large amounts of brewer's spent grains (BSG) are produced in the world which is usually  
9 considered as a waste, or animal feed, rather than food for humans. Here we report for the first time  
10 a new process at pilot scale for the separation of brewer's spent grain and trub to solid and liquid  
11 streams that can be used in foods. A new type of continuous rotary drum press was used to process  
12 hot BSG to produce a liquid filtrate and a filter cake stream. Analysis showed that of the starting  
13 mass of BSG (ca. 120 kg), the liquid filtrate composed 50% of the mass and the filter cake fraction

14 composed 50% of the mass. The dry weight (DW) content of the BSG increased from 23% to over  
15 35%. This led to concentration of insoluble dietary fibre (from 38% to 54%) and phenolics in the  
16 filter cake (from 102 to 150 mg/100 g DW as gallic acid equivalents). No fractionation of soluble  
17 species such as proteins occurred. Centrifugation of the filtrate from the rotary drum press led to a  
18 clarified supernatant stream and a paste. Concentration of insoluble dietary fibre and phenolics  
19 occurred in the paste (from 5 to 14% of DW and 61 to 114 mg/100 g DW as gallic acid equivalents)  
20 whereas soluble fibre and protein did not selectively partition. Given that the unit operations used  
21 here are scaleable and approved for food production, an industrially feasible route now exists to  
22 process brewers spent grains to ingredients.

23

## 24 **Introduction**

25 During beer production, four main separate streams are generated: Wort which is fermented to beer,  
26 brewer's spent grains (BSG), denatured protein precipitate after boiling (trub), and excess yeast.  
27 Beer is the main product and the other streams are often perceived as by-products with little, or  
28 even negative value [1]. In particular, the authors' discussions with many microbreweries in  
29 Denmark shows that those in rural areas can often have BSG removed at no cost, whilst those in  
30 urban areas must pay for disposal. Some large breweries are, however, able to sell BSG for a low  
31 price. Barley malt usually comprises the main ingredient in the mashing process and the total mass  
32 of BSG generated per brew varies from 20-30 weight-% of the original total mashing weight,  
33 depending on the lautering efficiency. This relatively high waste fraction makes BSG a very  
34 abundant (ca. 38.6 million tons per year) and cheap material, suitable for exploitation [1].  
35 Furthermore, for every 100 liters of beer produced, 0.2 kg of trub and 2.5 L of excess yeast [2, 3]  
36 are also produced. Given that approximately over 400 million hL of beer were produced in the EU  
37 in 2016 [4], the corresponding amounts of BSG, yeast and trub can be estimated to be extremely  
38 large.

39 A logistical issue arises for production companies exceeding 1 million hectolitres of beer annually,  
40 as the sheer volume of BSG becomes neither feasible nor viable to transport and sell as an  
41 agricultural feedstock [5, cited in 6]. Furthermore, BSG is subject to rapid microbial contamination  
42 due to the rich availability of nutrients [7, 8], which can quickly become a hygienic problem for a  
43 brewery. Thus with such large volumes, a limited shelf life and a traditional view that it does not

44 constitute a foodstuff, an oversupply of BSG quickly reduces its value, and focus is placed on it as  
45 an animal feed, as fertiliser, or waste to landfill. With this in mind, some methods have been  
46 advanced to store large quantities of BSG over longer periods of time for livestock feed [8]. BSG  
47 has been suggested for a range of other applications such as for using it as a biosorbent for cleaning  
48 wastewater of copper(II) [9] or in solid-state fermentation processes to enrich the protein content  
49 [10].

50 A window of opportunity exists at the end of the mashing process however, where BSG remains hot  
51 (> 70 °C) and exposure to air has been minimal. In this state, there is little microbial contamination  
52 and the BSG can be considered for food use [7]. BSG as a food has received increased attention due  
53 to its potential to reduce the environmental impact while potentially easing the economic strain on  
54 the brewing companies. Some previous studies have examined the potential for using BSG as a  
55 human food additive. However in most cases, they have examined the composition of BSG and  
56 from this drawn conclusions about the potential nutritional value and applications, but they have not  
57 satisfactorily addressed the industrial challenges associated with downstream processing of BSG [7,  
58 11].

59 In this study, we examine for the first time, a new way of continuously processing brewer's spent  
60 grains directly after lautering to give food ingredients. A new type of continuous rotary drum press  
61 is used at pilot scale, followed by continuous disc-stack centrifugation. We examine two different  
62 filter pore sizes (100 micron and 300 micron) simultaneously in the press and analyse the fractions  
63 produced. We also apply this to the trub produced after wort boiling.

64

## 65 **Materials and Methods**

### 66 **Materials:**

67 Barley malt was obtained from Sophus Fuglsang Maltfabrik A/S, Denmark. It was 2-row spring,  
68 Pilsner malt 8070, production date 10-2016, with the following characteristics as stated by the  
69 supplier: Degree of utilization, 100%; European Brewing Convention (EBC) colour, 3.7; dry  
70 weight, 81.4%; protein, 9.7%. Microcrystalline cellulose powder, 20µm, immunoglobulin G from  
71 bovine blood were obtained from Sigma-Aldrich, as were other chemicals which were of analytical  
72 grade.

73

74 **Methods**

75 Production of BSG by mashing.

76 A 600 L mashing system constructed in-house, based on patent WO2013024065 was used for all  
77 mashing. It works in the same way as the lab-scale device reported by us previously [12] and a  
78 schematic is shown in Figure 1. In brief, it consisted of a 600 L mashing tank (1.3 m high, 0.8 m  
79 diameter) with six cylindrical stainless steel filters (each being 0.3 x 0.07 m; with a 200 micron pore  
80 size) at the top and six at the bottom connected by a recirculation loop in which there was a large  
81 peristaltic pump (Bredel 50, AxFlow AB, Sverige) and heat exchanger (water heated). During  
82 mashing, wort was drawn at a rate of ca. 50 L/min through one set of filters by the pump, passed  
83 through the heat exchanger to maintain the desired temperature and then back through the other set  
84 of filters and into the mashing vessel. When a pressure difference of 2 bar was observed in the loop  
85 due to fouling of one set of filters, the circulation direction was reversed, cleaning the filters, but  
86 progressively fouling the set now on the intake side. This pattern of flow reversal was repeated  
87 (every ca. 5 minutes) until mashing was completed (see Figure 1).

88

89 To start the mashing process, the malt was milled with a Sommer, Piccolo 15 S 400 V (Germany)  
90 two roller mill at a setting of 5 (1.25 mm distance between rollers). In a typical process, the  
91 mashing vessel was filled with heated tap water (e.g. ca. 450 L) and ca. 84.5 g of citric acid was  
92 added to reduce the pH to 5.8. When the water was at 56°C, the required amount of milled malt was  
93 added (e.g. 90 kg), during which the temperature dropped to ca. 54°C. Mashing then proceeded with  
94 the following temperature profile: 15 minutes at 54°C where limit dextrinase is active, 60 minutes at  
95 65°C where beta-amylases mostly are active, and then 5 minutes at 72°C, where the alpha-amylases  
96 are most active. Subsequently the wort was pumped out through the bottom set of filters to the  
97 boiling kettle and 150L of hot water (72°C) was used to sparge the spent grains, which was also  
98 pumped to the boiling kettle. The total time for draining the wort and then sparging was ca. 15  
99 minutes. The spent grains were then removed through a man-hole on the side of the mashing vessel,  
100 weighed (ca. 120 kg), and whilst still hot (ca. 65°C), fed to the rotary drum press.

101

102 Processing brewer's spent grains with a rotary drum press.

103 For these experiments, the hot BSG was manually filled into buckets and then fed into the rotary  
104 drum press from the top via an Archimedes type screw integrated into the central chamber (Figure  
105 2). The rotary drum press was obtained from Dacofi ApS (Denmark) and consisted of 2 stainless

106 steel filter drums opposing each other and two rubber coated pressing drums opposing each other.  
107 For this work it was configured with one 100 micron pore size filter drum and simultaneously with  
108 a second 300 micron pore size filter drum. The length of each filter and diameter were 0.48 m and  
109 0.2 m respectively, giving a total area of each filter of 0.3 m<sup>2</sup>. These filter and pressing drums were  
110 arranged between a top and bottom plate such that they touched each other forming a water tight  
111 central chamber. Thus the area of each filter actually in contact with the spent grains being added  
112 was 0.11 m<sup>2</sup>. The motor driven screw traversed the length of the central chamber and observations  
113 showed that the BSG filled the space, and was in contact with the whole filter area available. Liquid  
114 filtrate from the BSG passed through the filter and was collected from inside by a manifold and  
115 peristaltic pump (one for each filter) and the filter surface became fully coated in BSG. The filters  
116 and rubber drums all rotated such that the BSG-fouled filter surface was pressed by the rubber roller  
117 releasing more liquid into the filters and leaving a dewatered filter cake that rolled out of the central  
118 chamber and was removed with scrapers (Figure 2) and collected for analysis. Essentially no BSG  
119 adhered to the rubber rollers. The rotational speed of the feeding screw (30 rpm), the filters (6.8  
120 rpm) and rubber drums (6.8 rpm) was the same in all experiments. The peristaltic pumps (Realax  
121 RP40, AxFlow AB, Sverige) connected to the manifold from each filter were calibrated to run at the  
122 same rate, which in both cases was in excess of the filter flux. Samples were stored at -20°C for  
123 analysis, except for those used for centrifugation, which were processed directly. An overview of  
124 the fractionation process is shown in Figure 3.

125 In parallel to processing the BSG, 260 L of the ca. 500 L wort was boiled at 100°C for 60 minutes  
126 together with 200 g of Perle hops pellets and 200 g of Hallertuer mittelfrüh hops pellets (both from  
127 the 2016 harvest and supplied by Caldic Ingredients Denmark A/S) in a hops basket. Perle  
128 contained 4.5%  $\alpha$ -acids, Hallertuer mittelfrüh 3.5%  $\alpha$ -acids. After boiling, the wort was transferred  
129 to a fermenter, leaving the trub precipitate sludge behind. The trub was then collected and fed into a  
130 cleaned rotary drum press in the same way as described for processing BSG (Figure 3).

#### 131 Centrifugation of filtrates

132 Two types of centrifuge were used to process the filtrates from the rotary drum press (see Figure 3).  
133 The lab centrifuge (Varifuge 20 RS, Heraeus Sepatech Group) processed 4 x 0.5 litre tubes at 4000  
134 rpm, equivalent to 3935 g. The disc stack centrifuge (SAOH 205, Westfalia Separator AG) was  
135 operated continuously to process all of the filtrate from the 100 micron and 300 micron filters. It  
136 was operated at 10000 rpm giving a relative centrifugal force of 17664 g. A two-phase split of the

137 liquid filtrate could be produced and water jet injection was used to release the particle laden phase.  
138 Moreover, when the 300 µm filtrate was processed, the two valves controlling the “heavy” and  
139 “light” outlet flow rates were adjusted such that a very thick paste was continuously produced  
140 comprising the accumulated dense particles from the filtrate. All samples for analysis were  
141 promptly frozen. Observations showed that significant sedimentation was seen if samples of the  
142 filtrate were stored at 4°C for a number of days. This suggests that simple decantation may also be a  
143 possible clarification method.

144

#### 145 **Analysis.**

##### 146 Solvent extraction of proteins

147 For all solid samples *i.e.* malt, BSG, pressed-BSG and the paste from centrifugation, 100 mg (dry  
148 weight equivalent) was mixed in a vortex mixer with 3.0 ml of either solvent A (5.0% (w/v) NaCl),  
149 B (55.0% (v/v) 1-propanol) or C (6 M urea, pH 8.4) at room temperature (RT) for solvents A and C  
150 and 60°C for solvent B and then extracted by subsequent shaking for 10 min. The suspensions were  
151 centrifuged (1500 g; 10 min; RT) and the supernatant was then diluted to 10 ml with the respective  
152 extraction solvent [13]. Solvent A was used to extract the albumin and globulin fraction that is  
153 water and salt soluble, solvent B was used to extract the hordein fraction that is alcohol soluble and  
154 solvent C was used to extract the glutamin fraction, as described by the Osborne method for  
155 fractionation [13,14].

156

##### 157 Protein concentration

158 Total protein was determined using both the Kjeldahl and Dumas methods in order to be able to  
159 compare the suitability of the two methods for BSG analysis as recently reported by Kupetz et al.  
160 [15]. The Kjeldahl assay was used to measure total nitrogen using a method reported in  
161 Oehlenschläger [16]. A conversion factor of 6.25 was applied, which is equivalent to 16% nitrogen  
162 content in the protein. The reference protein used was IgG.

163

164 The Dumas assay was conducted using an automated technique with an Rapid Max N Exceed  
165 (Elementar), in which the organic sample was combusted in a small crucible at 900°C and the  
166 protein content in the sample determined using the accompanying software. Aspartic acid was used  
167 to calibrate the instrument. Jones [17] and the rapid MAX N exceed® technical notes [18] suggest a

168 N/p-factor of 5.83 is most appropriate for barley and other cereals. However, the N/p-factor of 6.25,  
169 known as the Kjeldahl factor, has been widely adopted by other workers and was used in the current  
170 work.

171

172 The Bradford assay was used to measure soluble protein and the Coomassie plus reagent (Thermo  
173 Scientific), was used according to the manufacturer's instructions. The color was measured after 10  
174 minutes incubation at room temperature using a Shimadzu UV-1201 VIS spectrophotometer at  
175 595nm. IgG was used as standard.

176

177 The Amido black assay was used when sample size was limited (i.e. from the Osborne fractionation  
178 method) and prior to trypsin digestion for mass spectrometry. This was performed as reported by  
179 Popov et al. [19], with minor modifications for smaller volumes. The amido black 10B dye (Sigma  
180 Aldrich), was dissolved in a washing solution of 90% methanol and 10% acetic acid to give 0.26  
181 g/L, left to stand at room temperature for 4 h then filtered to give the staining solution. Liquid,  
182 extracted or enzyme hydrolysed samples (50µg) were mixed with 300µl of stain solution for 5 min  
183 in a 2 ml 'Eppendorf' centrifuge tube. The mixture was then centrifuged for 5 minutes at 14000rpm  
184 in a bench top 'Eppendorf' centrifuge. The supernatant was discarded and 500µl of washing  
185 solution (90% methanol and 10% acetic acid) added. The new mixture was centrifuged and the  
186 supernatant was discarded, the step was repeated until the supernatant became colourless (two  
187 washes). The pellet was subsequently dissolved in 250µl of 0.1N NaOH and the adsorbance  
188 measured at 615nm. A standard curve with BSA was used as reference.

189

#### 190 Liquid chromatography–mass spectrometry (LC-MS) for protein identification

191 All samples were precipitated and digested with trypsin before analysis using the method reported  
192 in Glatter et al. [20]. In brief, the precipitate was dissolved in 8M Urea, 50mM Tris-HCl pH 8.0  
193 with 41 mM DTT and incubated for 45 min at room temperature. Sufficient iodoacetamide was then  
194 added to give a concentration of 91 mM and the sample incubated for 1 h before starting digestion  
195 overnight with a protein : trypsin ratio of 50:1. After incubating overnight at 25°C the reaction was  
196 stopped by adding 10% TFA to give a concentration of ca. 0.4% TFA. Subsequently they were  
197 analysed with an EASY-nLC II 1000 nanoflow liquid chromatograph coupled to a Q Exactive™  
198 Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, both of them from Thermo Fisher Scientific.



199 The results from the mass spectrum were compared to data retrieved for barley from the Uniprot  
200 database. The proteins were identified by using MaxQuant proteomics software.

201

#### 202 Total Phenolic Content (TPC)

203 The method was performed as described earlier [21, 22], giving a value for the total content of  
204 phenolic compounds in gallic acid equivalents (GAE) on a dry weight (DW) basis. In brief, a 100µl  
205 sample was mixed with 0.75 ml of a 1:10 diluted Folin-Ciocalteu reagent and incubated at room  
206 temperature for 5 minutes. Sodium carbonate (0.75 ml of a 6% solution) or sodium bicarbonate  
207 (there was no difference in using either, data not shown) was added to the mixture and further  
208 incubated at room temperature for 90 minutes before measuring the adsorbance at 725nm and  
209 comparing to a calibration curve made using a solution of gallic acid. For solid samples, an  
210 extraction was performed using methanol, rather than water or ethanol, as advanced by previous  
211 workers [20]. Methanol (5 ml) was added to 0.2 g of solid sample and sonicated for 30 minutes  
212 then centrifuged at 3500 rpm for 10 minutes, and the supernatant collected. The supernatant was  
213 subsequently evaporated by continuous nitrogen flow. The extraction process was repeated twice,  
214 and the final dry sample sediment was weighed. This sediment was redissolved in 5 ml methanol  
215 and a 100µl aliquot was then subjected to similar processing as the liquid samples.

216

#### 217 Iron (Fe<sup>2+</sup>) chelating antioxidant activity

218 The method advanced by Farvin et al. [23] was employed. In brief, 110 µL of the extract (at a  
219 concentration range of 1–2000 µg/ml) was transferred to a microtiter plate, where 110 µL of  
220 deionised water and 20 µL of 2 mM ferrous chloride were added. After 3 min, 20 µL of 5 mM  
221 ferrozine was added, the mixture shaken and then incubated at room temperature for 10 min. The  
222 absorbance was then measured at 562 nm using a spectrophotometer (Shimadzu UV 1201 UV-VIS).  
223 A blank was made using distilled water instead of sample. EDTA at a concentration of 200 µg/ml  
224 was used as reference. Controls were made for each extract without adding ferrozine.

225

#### 226 DPPH radical scavenging antioxidant activity

227 The method of Farvin et al. [22] was used, in which 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution  
228 (100 µL, 0.1 mM in 96% ethanol) was mixed with 100 µL of extract made in the same way as for  
229 total phenolics determination (at a concentration range of 1–2000 µg/ml). The mixture was shaken  
230 and left for 30 min at room temperature. The absorbance of the resulting solution was measured at

231 517 nm using a spectrophotometer. For the blank, distilled water was used instead of the sample and  
232 a sample control was also made for each fraction by mixing 100 µL of sample with 100 µL of 96%  
233 ethanol. A reference with butylated hydroxytoluene (BHT) at a concentration of 200 µg/ml was also  
234 made in a similar manner. Radical scavenging activity for the individual concentrations tested the  
235 capacity expressed as the concentration of extract required to scavenge 50% of DPPH radicals  
236 (EC50) were calculated as reported in Farvin et al. [22].

237

#### 238 Soluble sugars

239 A GlycoChrom Analyzer with refractive index detector was used, running a mobile phase of 5mM  
240 sulfuric acid at 0.6 mL/min on an Animex HPX-87H column (300 x 7.8 mm), at 60°C. Peak areas  
241 were determined and related to standard curves made with pure sugars.

242

#### 243 Dietary Fibre

244 Samples were freeze-dried before transporting to Finland for analysis, which was performed using  
245 the AOAC Method 2011.25 and the Megazyme total dietary fibre assay kit (Megazyme, Ireland)  
246 according to the manufacturer's instructions. Upon arrival the samples were ground and starch and  
247 protein in them was degraded using  $\alpha$ -amylase, amyloglucosidase and protease. The samples were  
248 then filtered twice through a crucible filter (coarse, ASTM 40-60 µm). The insoluble dietary fibre  
249 (IDF) was collected as residue and all soluble fibre were located in the filtrate. Ethanol was added  
250 to the filtrate causing precipitation of the water-soluble polysaccharides (SDFP) which were  
251 collected on a filter crucible. The second filtrate, containing only the oligosaccharides (SDFS) was  
252 desalted and analysed by HPLC using Sugar-Pak® column (6.5 x 300 mm; Waters Corporation,  
253 Milford, MA, USA). The two residues, IDF and SDFP, were dried and weighed, and their ash and  
254 residual protein contents were analysed using a muffle furnace (550°C, 3 h) and a Kjeldahl Auto  
255 Sampler Analyzer Unit (FOSS, Kjeltex 8400, Hillerød, Denmark), respectively. Thus, each fibre  
256 content could be calculated by subtracting the residual protein and ash content from the total mass.  
257 The total dietary fibre amount in the samples was determined as IDF + SDFP + SDFS.

258

#### 259 Particle Size Distribution

260 The particle distribution of both liquid streams and their centrifugation products were determined  
261 using a Malvern® Mastersizer 2000 particle analyser APA2000 with Hydro 2000G sample  
262 dispersion accessory filled with distilled water. The refractive index of cellulose (1.46) was used

263 and a 20 $\mu$ m microcrystalline cellulose powder from Sigma Aldrich was used as a gross check.  
264 Results are expressed as volume percent.

265

#### 266 Free amino nitrogen (FAN)

267 The FAN analysis was performed as described in Analytica-EBC by the European Brewery  
268 Convention (EBC 9.10.1), with slight modifications as described below. Liquid samples were  
269 centrifuged to remove particles and were diluted 1:100, before being analysed. Glycine standards  
270 were used.

271

#### 272 Viscosity

273 A StressTech rheometer (Rheologica Instruments AB, Lund, Sverige) with a 4 $^{\circ}$  cone plate (C40)  
274 was used; or for the solids containing paste a coaxial cylinder piston (CC25) was used. A shear rate  
275 interval of 1–777.2  $s^{-1}$  was chosen and the StressTech software was used to determine viscosity as  
276 a function of shear rate.

277

#### 278 Other analyses

279 Specific gravity was measured by weighing precisely 100 ml of liquid in a volumetric flask at 20 $^{\circ}$ C.  
280 The dry matter fraction or dry weight (DW) was obtained via drying to constant weight at 100 $^{\circ}$ C.  
281 No human or animal trials were conducted in this study.

282

### 283 **Results**

#### 284 Fractionation of BSG

285 Hot BSG (ca. 65 $^{\circ}$ C) was taken directly from the mashing process following sparging and lautering  
286 and pressed with the rotary drum press. Two streams were produced simultaneously, from each of  
287 the 100 micron and 300 micron filters, namely a dewatered solids (i.e. a filter cake) and a liquid  
288 filtrate stream. The flux of the filtrate from each filter was measured during operation and found to  
289 be constant over the time measured and the result for the 100 micron filter is shown in Figure 4. The  
290 calculated rate of liquid permeate was 134 kg/h (129 L/h) from each filter and the 120 kg of BSG  
291 was processed within the 560 s shown in Figure 4. Given that the surface area of the filter exposed  
292 to the spent grains is 0.1096 m $^2$ , then the flux of each filter can be calculated to be 1181 L/m $^2$ /h.  
293 The composition of the filtrate from the 300 micron filter was compared at the start of processing,  
294 in the middle and at the end of processing. Small increases in total nitrogen, FAN, soluble protein,

295 glucose and maltose and specific gravity were seen (Table 1), most likely due to classification of  
296 the BSG during mashing and lautering in the brewing process. Therefore in the further work,  
297 samples for analysis were taken from the complete pool of the relevant fraction after all BSG had  
298 been processed.

299 Six separate batches of BSG of between 104 and 120 kg were processed, each made from an  
300 identical mashing process with the same pilsner malt batch, and similar results were obtained for  
301 each. A complete summary from one of the batches is presented in Table 2. Here it can be seen that  
302 fractionation of some components in the BSG occurred. Processing by the rotary drum press led to  
303 close to 50 % of the initial BSG mass ending in a liquid filtrate stream and 50% as a filter cake  
304 stream from either the 100 or the 300 micron filter (Table 2, see also Figure 3). Consequently the  
305 filter cake produced had the dry matter content increased from ca. 23% in the BSG to over 35%  
306 from either of the filters. In preliminary experiments, increasing the pressure between the filters and  
307 rubber rollers was found to be able to increase the dry matter content in the filter cake to 42%, but  
308 at the risk of denting the filter types used in the current work. As may be expected, the 100 micron  
309 filter was better at excluding solids, as shown by the composition of the filtrate collected from it  
310 which contained 8.9% of the solids. In contrast, that from the 300 micron filter contained 13.42% of  
311 the solids (Table 2).

312 Fractionation of antioxidants and some fibre types was also possible by the rotary drum press. Up-  
313 concentration of total phenolics was seen in the filter cake from both filters, increasing from ca. 100  
314 mg GAE/100 g DW in the BSG to ca. 150 mg GAE/100 g DW in the filter cake. Consequently the  
315 total phenolics decreased in the liquid fractions. EC50 for DPPH was lower in the filtrate (1 mg/L)  
316 than the filter cake (2500 mg/L), i.e. there was a higher antioxidant activity in the filtrate. For the  
317 antioxidant activity shown by iron chelation there was no change in the fractions compared to the  
318 BSG (20% versus 20.6%). Furthermore an up-concentration of IDF fibre was seen in the filter cake,  
319 rising from ca. 38% of DW to ca. 50%, and decreasing drastically to between 5 and 9% of DW in  
320 the liquid fractions (Table 2). This is consistent with the increase in dry matter content and  
321 phenolics in the filter cake. A slight up-concentration of SDFS fibre was seen in the filtrate from  
322 both filters compared to the starting BSG and the filter cake. For SDFP fibre and total nitrogen  
323 measured by the Kjeldahl or Dumas, no preferential fractionation was seen to the filtrate or filter  
324 cake streams. For soluble protein and soluble sugars similar concentrations were seen in both liquid  
325 fractions (Table 2). The Kjeldahl and Dumas methods gave results which were not statistically

326 different and it was concluded that the much more rapid Dumas method can substitute the Kjeldahl  
327 in future work, which is in agreement with the recent findings of Kupetz et al. [15]. The overall  
328 mass balance for the BSG batch processed in table 2 was found to close within 0.5% and for the  
329 other BSG batches, it closed within 4%.

### 330 Fractionation of filtrate streams using laboratory and continuous disc-stack centrifugation.

331 Pressing of the BSG with the rotary drum press gave a liquid filtrate stream from each of the 100  
332 and 300 micron filters, which accounted for approximately 50% of the starting BSG mass. These  
333 filtrate streams were essentially opaque and contained soluble and insoluble substances. For some  
334 applications, such as in drinks, it would be beneficial to have clarified liquids. Centrifugation is  
335 easily scaleable and it was therefore investigated as a means of further fractionating the filtrates.  
336 First a large laboratory centrifuge was employed, capable of centrifuging ca. 800 mL of filtrate in  
337 each bucket. The trials were done in duplicate for two of the BSG batches treated by the rotary  
338 drum press and the results were then scaled for the total amount of each filtrate. The results for both  
339 batches were similar and those corresponding directly to the results presented in Table 2 are shown  
340 in Table 3. Two trials were also conducted (with all the filtrate from 2 separate batches of rotary  
341 drum press processed BSG) with a small continuous disc stack centrifuge to confirm that the  
342 filtrates can be processed continuously in this way, and the results from one of those are also found  
343 in Table 3. The results confirm that the filtrates contained large amounts of particles, rather than  
344 colloids. The 300 micron filter did contain more solids than that from the 100 micron filter, leading  
345 to ca. one third of the volume ending as a thick paste-like pellet, which contained two thirds of the  
346 mass from that filtrate. In contrast the paste from centrifuging the 100 micron filtrate contained one  
347 quarter of the wet mass and ca. one half of the mass from that filtrate. The paste from each filtrate  
348 had a total nitrogen content of over 32% of the dry mass, when measured by Kjeldahl or Dumas  
349 methods, and ca. 4-5% in the supernatant (Table 3). Antioxidant activity as measured by total  
350 phenolics, was also much higher in the paste than the supernatant or the filtrate. However, DPPH  
351 EC50 was much higher (i.e. lower activity) in the paste than in the filtrate (data not shown). All the  
352 IDF fibre was found in the paste, whilst there was no difference in SDFP or SDFS fibre  
353 concentrations in the different fractions. Only small amounts (< 0.3 g/L) of soluble protein were  
354 found in any of the centrifuged fractions. When processed by the disc stack centrifuge, a very  
355 similar overall picture to that seen with the laboratory centrifuge was seen for the properties  
356 measured (Table 3). The majority of the dry matter and total nitrogen were recovered in the paste

357 fraction. Furthermore, centrifuging of the filtrate from the 300 micron filter resulted in more solids  
358 in the paste fraction than that from the 100 micron filter.

359 The viscosity of the filtrates and fractions produced by the lab centrifuge were also measured (Table  
360 3). The filtrate produced by the 100 micron filter had lower viscosity than that from the 300 micron  
361 filter and led to a much lower viscosity in the heavy fraction after centrifugation, than the 300  
362 micron filtrate. Supernatants derived by centrifugation from both filtrates had viscosity similar to  
363 water.

#### 364 Particle size distributions in the fractions produced

365 The dry matter content in the filtrates from the rotary drum press showed that the 100 micron filter  
366 excluded more dry matter than the 300 micron filter (Table 2). It could thus be expected that there  
367 would also be a difference in the size distribution of particles passing through the 100 micron filter  
368 compared to the 300 micron filter. This was tested by analysing filtrates from all four experiments  
369 with a Malvern Mastersizer. Similar trends were seen for all of the BSG batches processed,  
370 however only those corresponding to the data in Table 2 will be presented here. The results in  
371 Figure 5 for the filtrate show a difference in the profile of the size distributions from the 100 versus  
372 300 micron filter (Fig 5A) and suggest a lower average particle size in the former (ca. 2 micron –  
373 900 micron versus 2 micron - 1100 micron). Using the software in the particle sizer an average  
374 particle size based on a mode value (i.e. the most frequent particle size occurrence) gave an average  
375 of ca. 30 microns from the 100 micron filter and ca. 363 microns from the 300 micron filter. It is  
376 interesting to note that particles much larger than the nominal pore size of the filters were detected.  
377 It can be speculated that the particles in solution are not spherical but rather are rod shaped, or that  
378 the pressing action of the filter extrudes particles. It can also be speculated that agglomeration  
379 between proteins and polyphenols in the filtrate led to formation of particles in the time between  
380 undertaking the filtration and analysis, during which time the samples had been frozen. Cursory  
381 observations under a light microscope showed a variety of particle types, but this was not examined  
382 further.

383 Centrifugation of the filtrates shifted the particle size distributions dramatically. In the supernatants  
384 from the 100 or 300 micron filtrates a Gaussian type distribution with small shoulders (Figure 5B)  
385 and a modal particle size of 13.18 micron was found for both. In contrast the paste fractions from  
386 the 100 micron filtrate shifted to give a close to symmetrical distribution (Figure 5B) with a modal

387 particle size of 79.43 microns and the paste fraction originating from the 300 micron filtrate had a  
388 modal particle size of 478 micron. Similar effects were seen when the continuous disc stack  
389 centrifuge was used (Figures 5C and 5D). Here, centrifugation of a 100 micron filtrate with modal  
390 particle size of 26.3 micron (Figure 5C) lead to a supernatant fraction with a modal size of 8.71  
391 micron and a paste fraction with modal size also of 30.2 (Figure 5D). The filtrate from the 300  
392 micron filter had a double peaked size distribution and two modal sizes of 26.3 and 363 micron  
393 (Figure 5C) which lead to a light and heavy fraction with a similar bi-modal distribution and sizes  
394 of 13.18 and 316.2 for the light fraction and 11.48 and 316.2 microns for the heavy fraction after  
395 centrifugation (Figure 5 D).

#### 396 Fractionation of proteins

397 The filters used in the rotary drum press cannot be expected to fractionate soluble species, however  
398 given that BSG is a by-product of a hot water extraction during mashing, it can be expected that  
399 insoluble and or denatured proteins may remain, which could potentially be fractionated. To analyse  
400 for this, the streams from the rotary drum press were extracted using a modified Osborne method to  
401 yield three fractions corresponding to albumins/globulins, hordeins and glutuelins, respectively. The  
402 protein concentrations were measured with the amido black method. The results showed that there  
403 was no significant change in the concentration of these protein groups in filtrate from the rotary  
404 drum press compared to the BSG (data in supplementary Table S9). Liquid chromatography- mass  
405 spectrometry was also used to determine if there was any difference in the fractions produced by the  
406 rotary drum press or centrifugation. When the data was compared with the Uniprot database for  
407 barley and extracted from the MaxQuant software, the majority of the barley proteins were present  
408 in all liquid fractions and no significant differences could be determined (data not shown).

#### 409 Fibre

410 Fractionation of different fibre types was observed using the rotary drum press (Table 2). In  
411 particular, a large up concentration of fibre in the filter cake was seen, and a subsequent reduction  
412 in the filtrate. The 100 micron filter was able to retain more of the fibre than the 300 micron filter  
413 (Table 2). Centrifugation of the filtrate led to all of the insoluble fibre segregating to the heavy paste  
414 fraction, indicating that it was primarily present as particles (Table 3). There was little difference in  
415 soluble dietary fibre fractions in the filtrates or filter cake (Table 2), and centrifugation had no  
416 fractionating effect upon these (Table 3). The amount of fibre found in BSG and the filter cake  
417 (42.35 and 57.35% on DW basis, respectively) is similar to that reported by other workers such as

418 Stojceska [24] (53.39% for BSG) and the differences are most likely due to the different malts and  
419 mashing conditions used. The AOAC method 2011.25 separates fibre mainly in terms of solubility  
420 and roughly by size (large polysaccharides vs. oligosaccharides). A detailed identification of the  
421 types of fibres present and their dietary function is extremely complicated and deemed to be outside  
422 the scope of the current work. An overall model to describe fibre has been made by previous  
423 workers [25, 26]. Thus no attempts were made in the current work to further identify the types in  
424 the fibre fractions. Nevertheless, based on the use of barley malt in the current work, it can be  
425 expected that both soluble and insoluble fibre types would contain arabinoxylan of low or high  
426 degrees of polymerisation. Beta-glucan can be expected in the soluble fibre fraction, and this  
427 together with arabinoxylan have been shown to contain features that provide health benefits for  
428 humans upon consumption [16]. Water-soluble polysaccharides and other types of hemicelluloses  
429 and pectin, as well as gums and oligosaccharides can also be expected in the liquid fractions. Water-  
430 soluble oligosaccharides can mainly be expected to be fructo-oligosaccharides. Lignin is classified  
431 as a non-carbohydrate fibre, but fractions of lignin may be included as dietary fibre by the analytical  
432 method used.

433

#### 434 Processing of Trub

435 The precipitate resulting after boiling of the wort is commonly called trub and is known to contain  
436 denatured proteins, and is present as a slurry after pumping the wort out to the fermenters.  
437 Surprisingly, processing of this by-product has received very little attention in the literature. In this  
438 study we collected the relatively small amount of trub slurry (ca. 27 kg) and processed it with the  
439 rotary drum press. The results in Table 2 are shown for one of two separate experiments where  
440 similar results were found. It can be seen that most of the liquid passed through the 100 or 300  
441 micron filters, and that a small filter cake fraction could be recovered (0.6-1.5 kg), which had a  
442 greatly increased protein concentration compared with the trub and over 10 fold higher than in the  
443 filtrate.

444

#### 445 **Discussion**

446 Previous reports on the use of brewers spent grains for foods are primarily centred around drying  
447 the BSG directly from the brewing process, grinding it and then incorporating it directly into a food  
448 product, see for example Özvural et al. [27] and Stojceskaa et al. [24]. In a few cases enzyme aided



449 fractionation has been studied, e.g. by Forssell et al. [28], however it is a much longer (> 8h) and  
450 more complicated process than that studied in the current work, which also involves mixing the  
451 BSG with warm water before hydrolysis, after which a carbohydrate soluble fraction, protein  
452 soluble fraction and a hydrolysis residue were obtained and freeze-dried. [28]. El-Shafey et al. [8]  
453 used a membrane filter press with a woven polypropylene filter cloth, in a small-scale complicated  
454 process with many unit operations to dewater BSG for animal feed. The process had a 3 h cycle  
455 time (*i.e.* it was not a continuous process in contrast to that in the current work) and in it, the BSG  
456 was also mixed with water to create a slurry, then filtered, water washed, membrane squeezed and  
457 vacuum dried. The amounts of dried BSG (2.46 kg) produced were orders of magnitude lower than  
458 presented in the current work but moisture levels were much lower (20-30%). Recovery, study or  
459 applications of the liquid streams were not considered [8]. In a more recent study, the same workers  
460 refined the process and were able to eliminate dilution of the starting BSG and to reduce the  
461 moisture levels in the filter cakes to 12-15% by increasing squeezing pressure and using improved  
462 thermal vacuum drying [29]. When a BSG with 25% solids was squeezed at 60 bar for 3-5 h, 59%  
463 of the water (*i.e.* ca. 7 L) could be removed [29].

464 Perhaps the study closest to what we have reported here is that of Finley et al. [30] from over 40  
465 years ago, in which 45 gallons (ca. 170 L) of brewers spent grains were pressed in a type 3A  
466 Davenport press. They did not process the trub, nor analyse for fibre, antioxidants, sugars etc as was  
467 done here. They found that approximately 42% of the BSG mass was squeezed out as a press water  
468 (with 3% solids), and 58% ended as a press cake; in the latter the water content was 70%.  
469 Comparison with Table 2 in the current work shows that the rotary drum press used here was able to  
470 press more water from the spent grains (50% of the mass) and yielded a filter cake with higher  
471 percentage dry weight (35% versus 30%). In the study of Finley et al. [30], the press water was  
472 centrifuged in an International size 2 centrifuge with solids collecting bowl and 5% of the mass  
473 ended as a pellet and 95% as a clarified press water, which contained 1.6% solids [30]. The clarified  
474 press water was added to beer wort before boiling and could account for 11% of the total water used  
475 in the brew. They concluded that beer produced in this way was indistinguishable from  
476 conventional beer. The sediment from centrifugation was freeze-dried to yield a protein concentrate  
477 with 50-55% protein and when mixed with other ingredients could be used in an extruder to  
478 produce different foods. The solid fraction of spent grains was dried for animal feed [30]. In the  
479 current work, the filtrate and filter cake fractions contained ca. 17-20% of dry matter as protein  
480 (Table 2) which increased to 32-34% of dry matter in the paste after centrifugation (Table 3).

481 Up-concentration of total phenolics (to 150 mg GAE/100 gDW) was seen in the filter cake from the  
482 rotary drum press (Table 2) as compared to the BSG (100 mg GAE/100 gDW). Centrifugation of  
483 the filtrate led to up-concentration of total phenolics in the paste fraction (from ca. 63 to 114 mg  
484 GAE/100 gDW). These concentrations are almost twice as high as Socaci et al. [31] reported (ca. 60  
485 mg GAE/100 gDW) in water extracted BSG samples, but very similar to what was found with the  
486 best extraction mixture of acetone and water (114 mg/100 gDW). Socaci [31] also measured  
487 antioxidant activity with DPPH, but reports the values as percentage of radical inhibition rather than  
488 as EC50 values which we report. They found values of 15% for water extracted BSG samples,  
489 which could be increased to 45% when acetone-water mixtures were used. The results from other  
490 workers [28, 31, 32] therefore suggest that the process we have examined here could be augmented  
491 by BSG pretreatments (such as solvent extraction or enzymatic hydrolysis) yielding even higher  
492 values for functional food ingredients and could be considered in a further work. A number of  
493 workers have reported that BSG extracts contain metabolites with potentially useful functionalities  
494 [31,32].

495 The filtrate and filter cake produced by the rotary drum press, and subsequent centrifugation in this  
496 work were derived from BSG collected hygienically from a microbrewery running under safe food  
497 (HACCP, i.e. Hazard analysis and critical control points) principles, using equipment made with  
498 food approved contact materials. They therefore retain their status as food and can be used as food  
499 ingredients. Nevertheless, as with BSG itself [33], the fractions are prone to microbial  
500 contamination and must be used in foods immediately, or stabilised to prevent their deterioration.  
501 However more work needs to be conducted to determine the most industrially relevant (with respect  
502 to costs and functionality of the fractions) means of achieving this. We have shown here that the  
503 fractions can potentially provide a source for dietary fibre, antioxidants (polyphenols), protein and  
504 some sugars. Amateur tasting by the authors reveals the fractions to be neutral in flavour, with  
505 cereal notes, and very low bitterness. The concentration of the soluble sugars in the liquid streams is  
506 influenced greatly by the efficiency of the mashing and lautering process in the brewery the BSG  
507 originates from. After clarification of the filtrates by centrifugation, they could potentially be added  
508 to wort for beer making as proposed by Finley [30], however given that breweries do not make the  
509 same beer everytime, it is speculated that unwanted flavour components may need to be removed if  
510 the process was to be generic and applicable to every batch of beer. Soluble protein levels in the  
511 liquid fractions, however are too low to make them interesting as the basis for protein containing  
512 drinks, and would require up-concentration, for example by ultrafiltration. A variety of preliminary

513 experiments have been conducted with the fractions produced here together with commercial  
514 kitchens, canteens and event organisations which show wide acceptance of their use in a variety of  
515 foods. In particular to replace wholly, or in part, water or flour added during production of foods  
516 such as bread, sausages, yoghurt, cakes, and crisp bread. Preliminary results suggest the fractions  
517 can be used to enhance these foods, by altering water retention, mouthfeel, 'crumb', colour and  
518 crispiness [34,35] and are in good agreement with other authors findings using unfractionated BSG  
519 [27,32,36]. A comprehensive study to examine the functional effects of their use in foods is outside  
520 the scope of the current study and will be presented in a later work.

521 To conclude, the results presented here demonstrate that 50% of the mass of brewers spent grains  
522 can be continuously pressed out as a liquid filtrate. It is also possible to fractionate specific  
523 compounds into the filtrate or filter cake produced. However soluble species cannot be fractionated  
524 given the filter pore sizes of 100 and 300 micron employed here. For concentrating or fractionating  
525 soluble protein we suggest that an ultra-filtration process could be added after the centrifugation  
526 step. Some differences in fractionation performance were seen by employing a 100 micron filter  
527 versus a 300 micron filter and the latter allows greater throughput. The process we have examined  
528 here, is an easy add-on to any existing brewery, many of which already have disc-stack centrifuges  
529 for yeast separation. All that is required is to transfer the BSG in a hygienic way, directly from the  
530 lauter tun or mash-filter press into the rotary drum press. Given that the rotary drum press used here  
531 has already been scaled-up and trialled at 80 metric T/h (for potato pulp processing (G Larsson  
532 Starch Technology AB, Sweden, unpublished results)) it seems reasonable that a full scale BSG  
533 processing line could easily be implemented at any micro-, craft- or full-scale brewery. BSG could  
534 be easily processed by a rotary drum press and centrifuge to food ingredients within the ca. 2 h  
535 window that exists between the mashing and lautering process of one brew and that of the next.

#### 536 **Conflict of Interest**

537 The authors declare that they have no conflict of interest.

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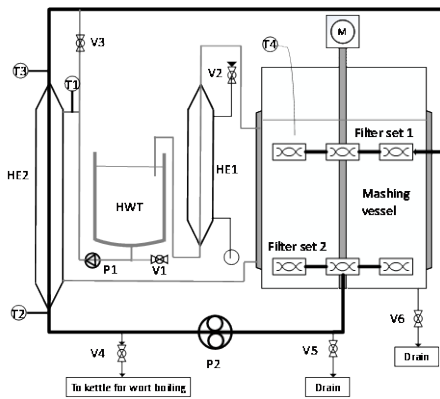
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622 **Figures.**

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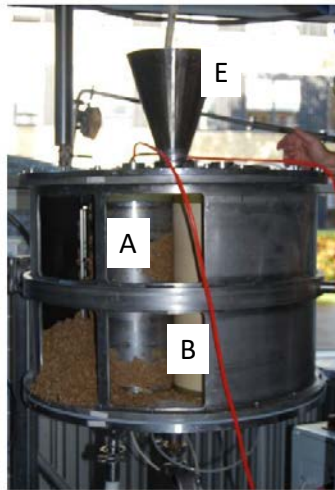
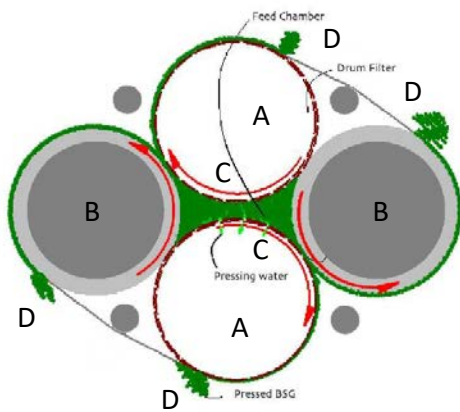
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627 **Fig. 1** Schematic of the mashing system used. The hot water tank (HWT) is filled via valve 1 (V1)  
628 then the water is circulated by pump P1 and heated in heat exchanger HE 1 via steam admitted by  
629 V2. When at the correct temperature the mash kettle is filled with heated water through filter set 1  
630 after opening V3 and malt is added. Mashing commences by closing V3 then drawing wort out of  
631 filter set 2 using peristaltic pump P2 then passing it through HE2 to regulate the temperature and  
632 then back into the mashing vessel through filter set 1. When filter set 2 blocks up with malt  
633 particles, the circulation direction is reversed by P2. When mashing is finished, the wort is pumped  
634 out using P2 via V4 to the boiling kettle. Sparge water heated in HWT is added via V3 and filter set  
635 1. T1-T4 are temperature sensors, V1-V6 are valves, M is a motor to raise or lower the filter sets.

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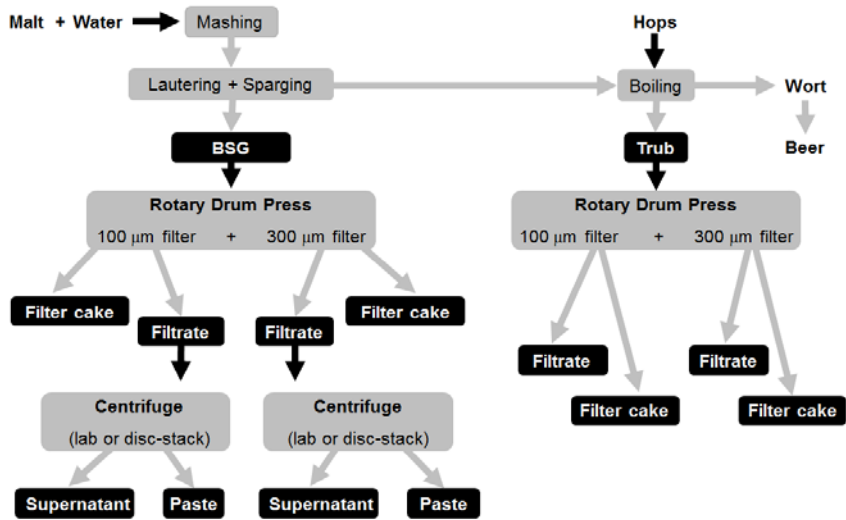


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639 **Fig. 2** Rotary drum press used to process BSG. Left hand side: Top schematic view. Right hand  
 640 side: Picture from side of rotary drum press used in the current work. Legend: A filter drum, B  
 641 rubber pressing drum, C liquid passing into filter from central feed chamber for collection, D  
 642 scrapers for removing filter cake from filter, E feed funnel with Archimedes screw for introducing  
 643 BSG into central feed chamber.

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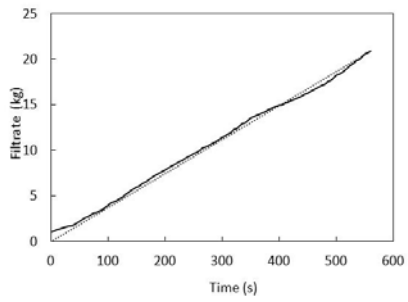
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648 **Fig. 3** Overview of the BSG fractionation process investigated. Gray boxes: Unit operations used;  
649 black boxes: Fractions produced and analysed.

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654 **Fig. 4** Filtration rate from the 100 micron filter immediately after starting and for a 560 s period  
655 during which all of the 120 kg of BSG available was processed. The rate of filtrate produced as  
656 found by fitting a straight line to the data is 0.0373 kg/s.

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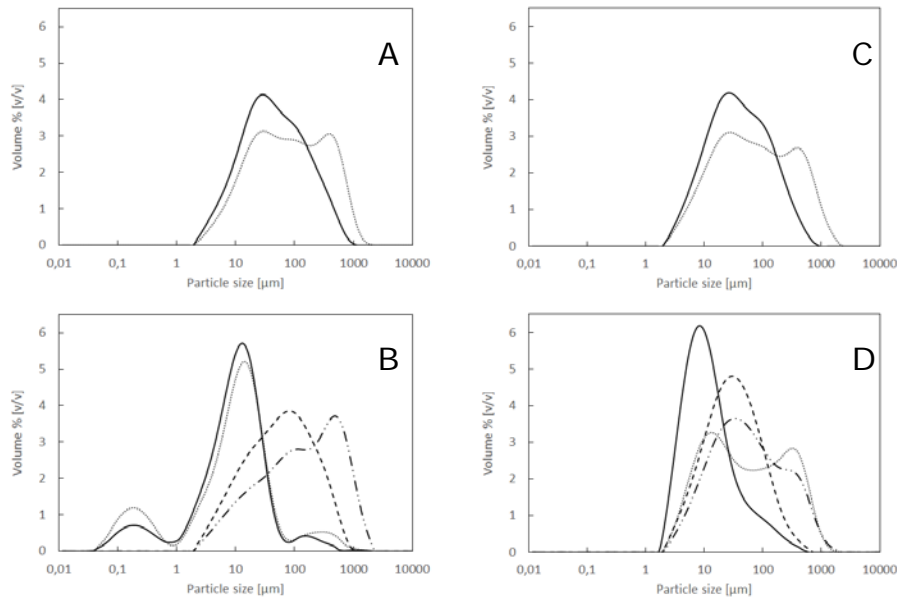
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**Fig. 5** Particle size distribution of the filtrate fractions from the rotary drum press and those from the subsequent centrifugation. Two separate sets of BSG were processed: Batch 1 processed with lab centrifuge shown in A and B; batch 2 processed with disc-stack centrifuge shown in C and D. Legend: (A) — Filtrate from 100 micron filter; •••• filtrate from 300 micron. (B) Lab centrifugation of filtrates from A: — Supernatant derived from 100 micron filtrate; - - - paste derived from 100 micron filtrate; •••• supernatant derived from 300 micron filter; -•••••- paste derived from 300 micron filter. (C) — Filtrate from 100 micron filter; •••• filtrate from 300 micron. (D) Disc-stack centrifugation of filtrates from C: — Supernatant derived from 100 micron filtrate; - - - paste derived from 100 micron filtrate; •••• supernatant derived from 300 micron filter; -•••••- paste derived from 300 micron filter

Table 1. Composition of the 300 micron filtrate at the start, middle and end of processing 102 kg of BSG

<b>Sample</b>	<b>Total nitrogen (% of DW)<sup>a</sup></b>	<b>FAN (mg/L)</b>	<b>Soluble protein (g/L)</b>	<b>Maltose (g/L)</b>	<b>Glucose (g/L)</b>	<b>Specific gravity (L/kg)</b>
<b>start</b>	0.283	84	0.199	27.47	6.48	1.029
<b>middle</b>	0.324	109	0.185	33.42	7.62	1.0364
<b>end</b>	0.334	117	0.197	39.67	9.34	1.0404
<b>Average ± std dev</b>	0.314±0.027	104±17	0.194±008	33.5±6.1	7.82±1.44	1.0353±0.006

a. Dumas method used.

**Table 2.** Composition of fractions produced following processing of BSG or trub with a rotary drum press. The results are representative of five separate experiments (two for trub). The data from all can be found in supplementary data.

<sup>a</sup> average of duplicate samples (values differed from the average by < 8%). <sup>b</sup> Values are averages of 1 sample analysed in duplicate (values differed from the average by < 5%). <sup>c</sup> average and standard deviation of quadruplicate analysis <sup>d</sup>Averages of two samples (values differed from the average by < 12%). <sup>e</sup> Averages of two samples (values differed from the average by < 5%). <sup>f</sup> Averages of two samples, (values differed

Properties measured after filtration			Wort	Byproduct (BSG or Trub)	Liquid fraction, filtrate		Solid fraction, filter cake	
					100µm	300µm	100µm	300µm
Pilsner BSG	Wet matter	Wet mass (kg)	504.77	120.23	25.39	35.05	23.91	35.3
		Percentage of wet mass ending in this fraction <sup>a</sup>	Na	Na	21.22	29.29	19.98	29.50
	Dry matter	Dry mass (kg)	57.43	27.74	2.40	3.62	8.58	12.36
		Dry weight-% <sup>a</sup>	11.38	23.08	9.45	10.33	35.9	35.03
		Percentage of dry mass ending in this fraction	Na	Na	8.90	13.42	31.81	45.87
	Total Protein (% of the dry weight)	Kjeldahl <sup>b</sup>	4.71	16.34	15.78	18.98	17.04	16.56
		Dumas	3.82	20.78	15.25	20.96	18.97	16.84
	Soluble protein (mg/ml; BSA equivalent by Bradford) <sup>c</sup>		0.300±0.016	Na	0.262±0.007	0.261±0.028	Na	Na
	Antioxidants	Total phenolics (mg/100g) as GAE on a dry wt basis <sup>d</sup>	113	102	61	75	148	151
	Soluble sugars (g/L)	Maltose <sup>c</sup>	Na	Na	31.52±0.52	36.44±0.65	Na	Na
		Glucose <sup>c</sup>	Na	Na	5.33±0.37	6.04±0.25	Na	Na
	Dietary fibre (% of DW)	IDF <sup>e</sup>	0	38.380	5.049	9.032	53.832	46.480
		SDFP <sup>e</sup>	1.485	1.772	2.318	1.806	2.495	1.315
		SDFS <sup>e</sup>	5.921	2.196	3.815	3.494	1.019	0.992
Total DF <sup>e</sup>		7.324	42.349	11.182	14.332	57.346	48.787	
Ash (kg) <sup>f</sup>		0.675	0.741	0.0294	0.0449	0.3044	0.4597	
Trub	Wet mass (kg)		Na	27.31	16.25	7.36	0.61	1.52
	% Dry weight <sup>a</sup>		Na	Na	13.7	13.7	26.6	27.1
	Total protein by Dumas % of the dry weight <sup>g</sup>		Na	Na	0.60±0.052	0.61±0.064	6.51±0.074	6.69±0.288

from the average by < 11%). <sup>g</sup> average and standard deviation of triplicate analysis. Nd = not detected. Na = not applicable. IDF = Insoluble dietary fibre; SDFP= Soluble in water, but precipitates in ethanol; SDFS = Soluble in water, not precipitated in ethanol (oligosaccharides). Total DF = total dietary fibre. GAE = gallic acid equivalents.

**Table 3.** Composition of fractions after centrifugation of filtrates from two similar sets of pressed BSG: One using a lab centrifuge (corresponds to data in Table 2) and one using a continuous disc-stack centrifuge. Data from repeat experiments is in supplementary data.

<sup>a</sup> = calculated from centrifugation in duplicate 500 ml batches (values differed from the average by 5 < %). <sup>b</sup> Values are averages of 1 sample analysed in duplicate (values differed from the average by < 5%). <sup>c</sup> Averages of two samples (values differed from the average by < 6%). <sup>d</sup> Averages of two samples (values differed from the average by < 5%). <sup>e</sup> Averages of two samples, (values differed from the average by < 11%)

Properties measured after centrifugation			100 $\mu$ m filtrate			300 $\mu$ m filtrate		
			Filtrate from filter	Supernatant	Paste	Filtrate from filter	Supernatant	Paste
Lab centrifuge	Wet matter	Wet mass (kg)	25.39	18.62	6.77	35.05	22.31	12.74
		% of wet mass in this fraction	20.85±0.65	73.34	26.66	27.08±2.44	63.65	36.35
	Dry matter	Dry mass (kg) <sup>a</sup>	2.4	1.32	1.18	3.62	1.67	2.36
		Dry weight-% <sup>b</sup>	8.09	7.10	17.46	8.67	7.51	18.5
		% of dry mass in this fraction	8.37±0.5	20.35±0.19	18.28±0.19	12.06±1.19	23.59±2.81	37.78±2.32
	Total protein, % of dry weight	Kjeldahl <sup>b</sup>	16.49	4.53	32.19	18.21	4.61	32.53
		Dumas	15.25	3.63	32.55	20.96	Nd	34.20
	Soluble protein (mg/ml; BSA equivalent) <sup>b</sup>		0.262	0.173	0.293	0.261	0.210	0.232
	Antioxidants	Total phenolics (mg GAE /100g) on DW basis <sup>c</sup>	61.34	63.06	114.63	75.5	76.26	114.0
	Dietary fibre (% of DW)	IDF <sup>d</sup>	5.05	0.00	14.42	9.03	0.00	16.80
		SDFP <sup>d</sup>	2.32	2.09	0.70	1.81	1.67	1.61
		SDFS <sup>d</sup>	3.82	5.23	4.49	3.49	4.73	4.81
		Total DF <sup>d</sup>	11.18	7.29	19.61	14.33	6.34	23.22
Ash (kg) <sup>e</sup>		0.0294	0.007	0.022	0.0449	0.002	0.026	
Viscosity (Pa-s) @ shear rate 1s <sup>-1</sup>		0.0831	0.0027	2.251	0.1806	0.0015	17.2	
Continuous disc stack centrifuge	Wet matter	Wet mass (kg)	24.56	18.01	6.54	31.8	20.24	11.55
		% of wet mass in this fraction	Na	73.33	26.6	Na	63.64	36.3
	Dry matter	Dry mass (kg)	1.99	0.92	0.83	2.76	0.97	1.77
		Dry weight-% <sup>b</sup>	9.45	5.08	12.64	10.33	4.81	15.31
		% of dry mass in this fraction <sup>b</sup>	Na	46.23	41.71	Na	46.56	64.13
	Total protein % of dry weight	Kjeldahl <sup>b</sup>	18.58	8.87	33.98	19.98	13.42	34.36

11%) IDF = Insoluble dietary fibre; SDFP= Soluble in water, but precipitates in ethanol; SDFS = Soluble in water, not precipitated in ethanol (oligosaccharides). Total DF = total dietary fibre. GAE = gallic acid equivalents. Nd = not detected. Na = not applicable.