Hydro-mechanical processing of brewer's spent grain as a novel route for separation of protein products with differentiated techno-functional properties.

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Abstract

Hydro-mechanical processing using a colloid mill with a large gap setting leads to the preferential breakup of the residual aleurone and endosperm tissues of brewer's spent grain, forming a protein rich fines material with small particle size around 1-10 µm. This fraction can be separated from the coarser husk fraction by centrifugation, giving a protein product with enhanced techno-functional properties. The fines have good stability in aqueous suspensions, with potential for stabilising other particulate materials in food or drink formulations. The fines particles can stabilise oil-water emulsions, possibly through a Pickering mechanism, which may also support use in food applications. Fines suspensions have strong shear-thinning behaviour, which may be beneficial from a textural or transport perspective. Spray drying of fines suspensions is shown to avoid particle coalescence, which is important for effective resuspension on rehydration. The high surface area of the fines also leads to more efficient digestion by proteases.

Keywords: Brewer's-spent-grain; milling; protein; particles; properties; functionality

Industrial relevance

A novel hydro-mechanical milling process has been investigated for separation of a protein fine fraction from brewer's spent grain having enhanced techno-functional properties. The small particle size of the fines would be a key attribute for formulation in shake or smoothie products, where sensory attributes of the product would not be compromised and the properties of the fines could confer stability against settling. Applications may be found for the fines material as an ingredient in spreads and sauces or infant purees, in-particular where the it might be used to stabilise of products based on oil-water emulsions. The market for protein-rich ingredients for foods and drinks is already established in the fitness and well-being market, as derived from other vegetable or cereal sources such as hemp, pea or rice. This controlled pre-milling step is also shown to lead to greater rate and extent of protease digestion of spent grain, which may be of value for generation of protein and peptide products for well-being and cosmetics applications.

1. Introduction

Spent grain is a major by-product from the brewing industry, which consists of the wet solid material remaining after the mashing process, when the majority of starch and soluble sugars have been extracted from the malt prior to fermentation of the wort liquor. Over 500,000 tonnes of brewer's spent grain (BSG) are produced annually by breweries in the UK alone, where the bi-product is sold primarily as a low-value ruminant feed [Thomas et al 2010]. A range of useful nutritional components are present in the residual non-extracted tissues of the grain, which include cellulose and other insoluble polysaccharides, lipids and protein, as well as a proportion of lignin. The

nutritional value for feed applications is therefore well established, [Aliyu and Bala 2011]. The proximate composition of the BSG used in the work is shown in table 1. However, as-made wet BSG may consist of only 30% solids, even after dewatering, which not only dilutes the deliverable nutritional value but also leads to microbial instability resulting in a low storage life. The product must therefore be shipped immediately from the brewery to the livestock facility for use Mussatto et al 2006].

According to fractionations based on differential extractabilities, the main protein fractions of BSG consists of a range of hordeins, with other fractions including glutelins, also albumins and globulins, as described in previous studies [Celus et al 2006]. The relatively high amount of this protein component raises the possibility of further processing of BSG to separate a protein enriched product, which would have higher value for both feed and also for human nutrition or wellbeing supplements or as cosmetics ingredients [McCarthy et al 2013]. Many investigations have focussed on methods for extracting the proteins present in BSG by application of commercially available protease enzymes, which are designed to reduce protein molecular weight and therefore increase solubility. Crude extraction yields in excess of 70% have been quoted, where extracts consist of lower molecular weight oligomers and peptides, as a result of the hydrolysis of amide linkages between amino acids. [Celus et al 2007; Treimo et al 2008, Robertson et al 2011]. Although the process is simple, typically operating at 50-60°C, further process effort is required to recover the protein concentrate as a final solid, also with the requirement to adjust the pH by alkali addition to neutralise the additional amino acid functionality resulting from hydrolysis. Actual recoverable yield may therefore be lowered and the final product may also have a relatively high mineral content in the form of the amino acid carboxylate counter-ion. Other chemical approaches for protein extraction have also been extensively reported, either based on solubilisation of the BSG proteins in aqueous alkali [Tang et al. 2009] or ethanolic alkali [Cookman and Glatz 2009]. Usually sodium hydroxide is the alkali of choice, with extraction again under relatively mild conditions, for example

79 at 60˚C, over a few hours. The solubilised protein is then precipitated by acid addition, which may
80 cause denaturation, although molecular weight may be preserved [Scopes 1994; Celus et al 2007].
81 The precipitate is separated, for example by centrifugation, and therefore recoverable yields may
82 again be reduced. Also, the purity of the resulting concentrate will depend on the extent of co-
83 extractability of other grain components in the alkaline medium, for example hemicelluloses and
84 lignin [Vieria et al. 2013]
85
86 The possibility of application of physical methods for separation of a protein concentrate from BSG
87 has been much less widely reported, which is however a route that could present an alternative to
88 enzyme or alkali extraction methodologies [Kanauchi and Agata 1997, Schwencke 2006]. Wet roller
89 milling is a process widely used in the brewing industry. This has been used to abrade and disrupt
90 the spent grain material, which can then be sieved to allow separation of a fine protein rich fraction
91 from a coarser protein depleted material [Kishi et al. 1997]. Products are reported which contain
92 significantly higher protein content than the original spent grain, where dewatering is carried out to
93 produce a wet cake, which can be dried to give a stable product. This simple concept appears worthy
94 of a more fundamental investigation, to understand the physical aspects of the process more fully,
95 and to consider if features of the process might be further modified or improved, and also to
96 consider whether any novel properties of the enriched fine protein fraction may point to specific
97 food applications. In support of these aims, a commercially available colloid mill has been used for
98 hydro-mechanical processing of BSG, to generate protein fine fractions under controlled conditions,
99 and also for evaluation as a potential alternative process route. A controlled laboratory
100 centrifugation technique has been used as part of these investigations, to better understand the
101 separation behaviour of the fines and coarse fractions, which may relate to particle size and surface
102 chemical properties. Microscopy techniques have been used to characterise the physical features of
103 the separated particles. Studies of the dispersion and settling behaviour of the protein enriched fines

were also carried out in different media. Other post processing methods have also been applied to the protein enriched fines learn more about potential for scale-up and different applications.

2. Materials and methods

2.1 Grain Materials

A batch of wet spent grain was recovered from the commercial production process at Molson Coors UK, Burton-on-Trent brewery. The original malt had been hammer milled prior to mashing and sugar extraction, where after discharge from the mash tun the spent grain residue was dewatered by filter press to a solids content of around 27 wt%. Individual portions of this material were frozen at -18˚C, for storage in readiness for experimental investigations.

2.2 Colloid milling

A vertical toothed colloid mill was used for processing the BSG material under controlled conditions, operating at a fixed speed of 3000 rpm, fitted with a 7 litre hopper (MZ50 model, FrymaKoruma AG, Switzerland). The gap between outer fixed stator and inner rotor was set at 1 mm, which was considered to be a sufficiently large tolerance to limit the direct cutting of the initial grain particles, whilst generating high hydro-mechanical shear forces sufficient for particle break-up. The rotor and stator had a square-tooth cross-helical profile, with depth reducing from the inlet to outlet end.

A 2.5 l batch of 10 wt% slurry of the spent grain was prepared by appropriate addition of water to the as-received material. The samples were repeatedly passed through the colloid mill, up to 24

cycles in total, each time collecting the full batch of product from the outlet before feeding back into the inlet hopper. Duplicate samples of 45 ml were collected in 50 ml graduated falcon centrifuge tubes at selected numbers of mill passes, from 2 up to the 24 cycle limit, ensuring that no settling on sampling had occurred and that the slurries were representative of the total batch. These were retained for further processing and analysis.

2.3 Sedimentation and centrifugation

The duplicate samples of slurries collected after increasing numbers of mill passes were allowed to stand vertically in the falcon tubes under normal gravity for 1 hour. After standing, the settled volume of the solids layer below any clear layer was measured using the graduated scale on the side of the tubes. The extent of solids settling was then expressed as a proportion of the total sample volume. Both duplicate samples in the falcon tubes were then re-suspended by vigorous shaking using an orbital shaker and were loaded immediately into a laboratory centrifuge (Heraeus 16, Thermo-Fisher, UK). Three experiments were carried out at different centrifuge speeds. For the first experiment the tubes were spun at 28 g for 30 minutes, which was sufficient time at this speed for the solid material to settle to equilibrium. After spinning, the volumes of the total settled solids and also any separate upper layer of fines were measured for one duplicate set of samples, using the graduated scale as before. For the other duplicate set of samples, small amounts of the upper fines layer were removed using a spatula and retained for further analysis. This sampling arrangement avoided disturbance of the quantitivity of measurement of layer volumes of the first duplicate set. For the second experiment, both duplicate sample sets were re-suspended in the tubes by vigorous orbital shaking, then re-loaded into the centrifuge and spun at 447 g for 10 minutes. After centrifugation, the layer volumes of the first duplicate set were measured as before. Further small amounts of fines were taken from the second duplicate set for analysis as before. In the third experiment the operation was repeated in full with centrifugation at 2800 g for 5 minutes, with

156 measurement and sampling as before. The small portions of fines taken from the second set of
157 duplicate tubes were each spread evenly onto plastic petri dishes and allowed to dry under ambient
158 laboratory conditions overnight, before transferring for storage for further analysis. This gentle
159 drying regime was chosen to minimise risk of alterations to the particle structure or properties.
160
161 A larger single batch of fines were produced by subjecting a 2 l quantity of 10 wt% slurry of spent
162 grain to 24 passes through the colloid mill, followed by centrifuging at 2800 g using a floor-standing
163 centrifuge (J21, Beckman UK.). The upper fines layer was separated and stored in the wet state at
164 3°C in preparation for further experiments. The moisture content of a small representative quantity
165 of this as-made fines material was determined by gravimetry, by weighing, drying at 100°C and
166 reweighing.
167
168
169 2.4 Compositional analysis
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171 A number of different BSG derived samples were acquired separately, including both milled fines
172 fractions and fractions generated by methods leading to higher concentrations of protein [Tang et al.
173 2009]. These were required to construct a broad range calibration for a rapid infrared spectroscopic
174 method for protein determination. The nitrogen contents of these calibration samples were initially
175 analysed by an automated Dumas method, with values converted to protein concentration using the
176 relation, % protein = % N x 6.25. The infrared spectroscopy measurements were performed using an
177 attenuated total reflectance (ATR) sampling accessory, requiring low milligram samples (Tensor FTIR,
178 Bruker AG). The method was based on the measurement of the intensity of the amide II absorbance
179 at 1518 cm^{-1}, which is related to the amount of peptide linkages and hence the amount of protein.
180 The measurement of the intensity of the organic C-O absorbance at 1097cm^{-1} provided a relation to
181 the amount of hydroxyl groups present and hence amount of polysaccharide and lignin. The

intensities of both these characteristic frequencies were normalised by comparison with the intensities of frequencies in adjacent non absorbing regions. From the measurement of the calibration samples an empirical relation was derived for total protein content, as shown in equation (1), where constants of A = 106 and B = -0.6 were identified by least-squares fitting. The protein contents of all experimental samples were measured using this rapid technique, with examples of spectra shown in Figure 1.

$$\%\ \text{protein} = \left[\mathrm{A} \times \frac{(\mathrm{I}_{1518} - \mathrm{I}_{1800})}{(\mathrm{I}_{1518} - \mathrm{I}_{1800}) + (\mathrm{I}_{1097} - \mathrm{I}_{899})} + \mathrm{B}\right] \quad (1)$$

Analysis of the amino acid profile of the original BSG and representative fine fraction was carried by an external provider using a standard HPLC chromatographic method (Sciantec Analytical Ltd, UK). Determination of the crude fibre fraction of the original grain was carried out using the neutral detergent fibre extraction protocol [Van Soest et al. 1991]. Application of this protocol was difficult as a result of the small particle size of the fines material and an estimate of fibre content was therefore made by quantitative comparison of the C-O infrared absorbance at 1097 cm^{-1} between the grain and fines spectra, with internal intensity referencing [Marotte et al, 2007]. The lignin content of whole BSG and separated fines material was determined by the acetyl bromide method [Hatfield et al. 1999]. Analysis of the lipid content of grain and fines material was determined by extraction in diethyl ether, followed by ambient drying and weighing of the extract [Thiex et al 2003].

2.5 Microscopy

Images of particles dispersed in water and oil were obtained under transmitted light conditions, at low magnification (x4 objective) and also at high magnification (x100 objective) using an oil-immersion method. Microscopy imaging was performed on water suspensions of the original BSG

208 and BSG which had been subjected to 15 passes through the colloid mill. This was performed under
209 reflected light conditions, with oblique illumination, through a cover slip. These suspensions were
210 stained using an aqueous solution of acid Fuschin dye, for selective coloration of the protein rich
211 component of the material. Dye addition was carried out on a dropwise basis, with examination to
212 determine the optimum dye concentration [Craeyveld et al. 2009; Niemi et al. 2012].
213
214 2.6 Oil and water suspensions and emulsions
215
216 Amounts of wet fines produced from the large 2 l batch were mixed with water in cylindrical glass
217 vials at differing concentrations from 0.5 to 5 wt% dry basis, at a 40 ml volume, accounting for the
218 existing moisture content. The suspensions were shaken vigorously and allowed to stand at ambient
219 temperature, after which the aggregation and settling characteristics of each vial sample were
220 recorded over time by imaging and measurement of the layer heights. As a confirmation of
221 behaviour the procedure was repeated by re-suspending the fines by repeated vigorous shaking. A
222 further batch of the centrifuged fines was repeatedly washed in methanol, followed by re-
223 centrifugation and decantation of the methanol layer, in order to replace all water present in the
224 material by organic solvent. The methanol wetted fines were finally air dried, which avoided any
225 irreversible coalescence of particles. The dried fines were then re-suspended and shaken in 40 ml of
226 sunflower oil at different concentrations in cylindrical glass vials, as above, again with measurement
227 of gravitational settling over time. Again for confirmation the procedure was repeated by re-
228 suspending the fines by repeated vigorous shaking. Microscopy images of representative samples of
229 the oil and water particle dispersions were also obtained under transmitted light conditions.
230
231 Further amounts of the wet fines were made up in 20 mls of water in cylindrical glass vials to achieve
232 final total emulsion concentrations from 1 to 10 wt% dry basis, which were shaken vigorously to aid
233 dispersion. Then 20 mls of sunflower oil were added to each of the vials, which were vigorously

234 shaken again and allowed to stand at ambient temperature. The appearance and stability of the
235 resulting oil and water emulsions were determined after standing as before, by imaging and
236 observation of phase heights at increasing time periods. For confirmation of behaviour the
237 procedure was repeated by re-suspending the emulsions by repeated vigorous shaking
238
239
240 2.7 Rheology of fines suspensions
241
242 Three samples of wet fines from the large 2 l batch preparation were made up to 5, 10 and 14 wt%
243 dry basis by appropriate addition of water. The rheological properties of these suspensions were
244 measured using a rotational viscometer, using a concentric cylinder geometry, with a gap width of 1
245 mm with a matt surfaced inner cylinder (RheolabQC, Anton-Paar Gmbh). An upward and downward
246 shear-rate sweep was applied from 2 to 50 /sec, measured over 100 points in each direction at 0.5
247 sec/point. The temperature for all measurements was stabilised at 20˚C
248
249
250 2.8 Wet-milling and enzyme digestion
251
252 Two slurries of 1 l of the initial spent grain were made up at 10 wt% solids content, adjusted to pH 9,
253 by formulation using a stock of 2M sodium tetraborate/boric acid, to give a final buffer
254 concentration of 0.1 M. This formulation was required to avoid the reduction in pH during digestion,
255 which would otherwise occur due to the creation of free amino acid groups due to the hydrolysis of
256 amide bonds. One batch of slurry was subjected to 24 passes through the colloid mill, as described
257 above. This and the other un-milled batch of slurry were preheated to 60˚C and incubated for 5
258 hours in the presence of a commercial Alcalase 2.5L enzyme product, dosed at a concentration of
259 0.1% on dry weight of grain (Sigma-Aldrich Ltd, UK). Small samples from each batch were taken at

increasing time intervals during digestion. These were immediately chilled to 3°C to minimise enzyme action, then centrifuged to allow collection of the supernatant liquor for analysis of protein concentration. A further 1 l batch of slurry was subjected to the same 24 colloid mill passes but was incubated without enzyme. The separated supernatant liquors from all samples were immediately frozen for storage. Each sample set was re-thawed to 3°C as required and 100 µl aliquots were mixed with a 2 ml quantity of commercial Bradford reagent, then held for 20 minutes for colour development (Biorad Inc). Absorbances were measured at 595 nm and converted to apparent protein concentration using a bovine serum albumin calibration curve, with a vegetable protein factor of 2 applied [Kruger 2002].

2.9 Spray drying of fines suspensions

Fines suspensions at 5% and 10% solids were dried using a Buchi B-190 laboratory spray dryer, fitted with a 0.7 mm spray nozzle, aspirated with dry compressed air. The inlet temperature was set at 120°C and the pump rate and chamber air flow rate was adjusted to ensure the outlet temperature did not exceed 60°C. Representative samples of dried powder were examined by transmission optical microscopy as described above.

3. Results and discussion

3.1 Milling and centrifugation

285 The 10 wt% slurry of the original BSG flowed easily through the colloid mill, gaining a smoother
286 texture at each pass, as observed visually by its behaviour when poured into the feed hopper. The
287 relatively coarse particles of the unmilled slurry settled under normal gravity after 1 hour to leave a
288 clear upper liquid layer, with no obvious stratification within the settled particulate material.
289 However, the visual settling of the slurry reduced as the number of mill passes increased, shown in
290 Figure 2a, with no upper liquid layer observed after 12 passes. Centrifugation of the unmilled slurry
291 resulted in greater settling of the particulate material, which reduced in phase volume to 50 % of the
292 total sample volume after centrifuging at 2800 g, compared to 71% on settling under normal gravity.
293 No visual stratification within this phase was observed for the unmilled material following
294 centrifugation. However, after progressive milling centrifugation resulted in a distinct stratification
295 of the slurry particles into an upper fines fraction and a lower coarser fraction, as illustrated in Figure
296 2c. The proportion of the upper fines fraction increased with increasing number of passes through
297 the mill, from Figure 2b, appearing to reach a constant value after 12 mill passes. Also, the figure
298 shows that proportion of the resulting fines fraction was greater at higher centrifuge speed, reaching
299 a maximum of approximately 28 % of the total volume of the particulate phase after centrifugation
300 at 2800 g, with data averaged over 12 to 24 mill passes. Accounting for gravimetric moisture content
301 of the two particulate layers, this gave a maximum yield of the upper fines fraction at 2800 g of 24%
302 on dry basis with respect to the original BSG. Sedimentation velocities are theoretically linearly
303 proportional to the gravitational field, so the improvement in separation of the finer and coarser
304 particles at higher centrifugal speed may be a result of the increased settling distance, due to the
305 greater compaction of the particulate material. These results indicate that there is advantage in
306 centrifugation at high speed as part of an industrial process.
307
308 Reflected light microscopy images of as-received BSG particles from the hammer-milled malt are
309 shown in Figure 3a. These revealed irregular amorphous particulate structures which have become
310 pink-red stained due to absorption of the Acid Fuschin dye, likely to be associated with the inner

311 protein containing aleurone tissue of the original grain [Jääskeläinen at al. 2013, Aubert et al. 2018].
312 These tissue regions are distinct from the more sheaf-like structures presumed to be from the husk
313 and pericarp tissues, which do not contain protein and remained predominately unstained. Figure 3b
314 shows that on multiple passes through the colloid mill the protein containing tissues were broken
315 down into very small pink-stained fragments, which became debonded from the larger
316 husk/pericarp particles, forming a separated dispersed population, estimated to be around 1 - 10 µm
317 in size. In contrast, the husk/pericarp particles did not appear to be significantly reduced in size,
318 remaining at 1 - 2 mm dimensions, These smaller protein containing particles would therefore show
319 slower sedimentation rate under gravitational force, due to the greater influence of Brownian
320 motion, and would form the upper fine fraction after centrifugation. However, from the settling
321 experiments under normal gravity it appears that milling reduces the sedimentation rate of whole
322 slurry, which may therefore suggest the presence of interactions between the smaller fines particles
323 and the coarser husk/pericarp particles. This appears to be an important feature of the overall
324 process, where milling allows the total slurry to be retained in a homogenous suspended state prior
325 to the application of the high centrifugal force, which then allows differential sedimentation of the
326 fine and coarse fractions.
327
328 Samples of the upper fine fraction were examined under transmitted light microscopy, which
329 revealed the uniform texture of the particles, in Figure 4a, which in this semi-concentrated state in
330 water appeared to partially aggregate to form an open extended physical network. The interactions
331 through this network may assist with stabilisation of the husk/pericarp particles in the whole slurry.
332 Observation at higher magnification shows the irregular shape of the small aleurone and endosperm
333 particles, in Figure 4c, although this image also show evidence of more spherical structures around 1
334 - 5 µm in diameter, which may be associated with protein storage vacuoles which have been
335 liberated from these tissues [Swanson et al. 1998]. These vacuoles are the repositories of proteins as
336 required on germination to support seed growth [Herman and Larkins 1999].

337

338

339 3.2 Composition of the fine fraction

340

341 The protein content of the separated fine material was enhanced compared to the original BSG, as
342 summarised in table 1. The fines protein content appeared to increase slightly with increased milling
343 time, in Figure 2b, although this trend was only detected for the highest centrifugation speed and
344 was within the experimental error. A value of 51 wt% protein content was determined after 24 mill
345 passes for the fines which compared with 27 wt% for the original spent grain, representing almost a
346 2 times enhancement. Based on solid content, the weight yield of the fine fraction is estimated to be
347 20% of total solid material so the protein in the fines represents a yield of around 38% of the total
348 protein in the BSG, with the remaining 62% still incorporated in the particles of the coarser
349 centrifuge fraction. This unrecovered protein may be a result of a limit in the efficiency of separation
350 by differential sedimentation, or alternatively some protein containing tissue may still be intimately
351 associated with the coarser husk/pericarp particles.

352

353 The fines material contained a mix of protein, carbohydrate, lignin and lipid components, as
354 summarised in table 1. While the protein content of the fines was enhanced, the amount of
355 carbohydrate was correspondingly reduced compared to the original BSG, which is consistent with
356 the removal of husk and pericarp tissue from this fraction. The remaining carbohydrates in the fines
357 fraction were presumed to originate from the cell wall material of aleurone and endosperm tissues,
358 carried forward from the original grain. Table 2 summaries the amino acid composition of the
359 original BSG and fines, which indicated that the materials had the same overall profile. This suggests
360 that separation of the protein occurred in a non-selective manner, with the fine fraction derived
361 from all protein containing tissue in the original structure. The analytical data suggested a moderate
362 increase in the ether extractable lipid content of the fines fraction compared to the original spent

grain, which might have some nutritional benefit as a contributor to calorific value [Thomas et al, 2013]. This lipid material would originally be present in oil-bodies distributed within the aleurone cells and different tissues of the embryo, which also appears to be carried forward during centrifugation [Neuberger at al. 2008]. One previous study also confirmed the reduction in non-digestible carbohydrate in the fines, which also indicated that this material contained proportionally less cellulose, which is a component of the more structural husk and related tissue, which was separated from the fines [Kanauchi and Agata 1997].

Table 1: composition of brewer's spent grain materials

	Whole BSG (%-total dry wt.)	BSG-fines (%-total dry wt.)
Carbohydrate	48.5 ±1.1	30.5 ±1.8
Lignin	14.0 ±0.65	5.1 ±0.2
Protein	27.1 ±0.9	51.0 ±1.6
lipid	8.6 ±0.11	12.0 ±0.21

389 Table 2: Amino acid proportions in brewers' spent grain materials

	Whole BSG (%- of total measured)	separated fines (%-of total measured)
alanine	5.0	5.2
arginine	5.8	5.9
aspartine	7.4	8.8
cystine	2.2	1.7
glutamine	21.7	19.4
glycine	4.3	4.8
histine	2.4	2.5
iso-leucine	4.4	4.4
leucine	8.0	8.0
lysine	4.4	4.5
methionine	2.1	2.2
phenyl-alanine	5.8	5.7
proline	9.7	9.2
serine	4.6	4.6
threonine	3.8	4.0
tyrosine	2.7	3.2
valine	5.8	5.7

390

391

392 3.3 Properties of fines suspensions and emulsions

393

394 The micrographs in Figure 3b and 4a-e revealed the relatively small size of the fines particles, around
395 1 - 10µm, which were generated despite the large 1 mm gap size of the mill. This indicates that the
396 grain tissue creating the fines is of low mechanical integrity so can be broken up effectively by the
397 hydro-mechanical shear forces in the mill, without need for direct attrition between fixed and
398 rotating metal surfaces. The observation of settling behaviour of water suspensions of fines under
399 normal gravity revealed that suspension stability increased with solids content, from Figure 5a,
400 where although partial settling occurred at 1 wt% solid content, a single stable dispersed phase was

observed at 3 wt%, after 1 hour standing. In a further set of experiments it was found that a stable suspension could also be formed at a 3 wt% concentration in vegetable oil, also illustrated in Figure 5a. This compatibility with both oil and water media suggests that the fines particles have both hydrophilic and hydrophobic characteristics. This may stem from the natural hydrophobicity of the exposed aliphatic and aromatic functional groups of the protein, together with the hydrophilic character of the residual polysaccharide material, conferred the presence of sugar hydroxyl groups [Saha and Hayashi 2001]. These characteristics were further demonstrated by the observed textures of the fines material suspended in water and oil, in Figures 4a and 4b. These micrographs revealed the appearance of a partially aggregated but open network structure in both media, where particle-particle and particle-liquid interactions are apparently in balance.

The potential dual hydrophobic-hydrophilic nature of the BSG fines suggest that the particles may have the ability to stabilise mixtures of oil and water as an emulsion. This is a property associated with so-called Pickering particles, which is a term describing a mechanism of stabilisation where small particles arrange at the interface between two immiscible liquids, stopping droplets from coalescing together by acting as a physical barrier [Wang et al. 2011; Liu and Tang 2018]. This mechanism would operate in addition to any stabilisation caused by the increased viscosity due to particle addition. In addition, a Pickering type mechanism is more effective when the particles are partially hydrophobic, so are wetted evenly between the two different hydrophobic and hydrophilic liquids and have contact with both phases. The images of mixtures of water and vegetable oil provide further evidence of the emulsion stabilisation effect, in Figure 5b and 5c, where in the absence of the fines the two liquids fully separated immediately on standing, with the boundary between the oil and water layers marked by an arrow due to lack of contrast. However, emulsion stability improved even with 1 wt% added fines, although after 10 minutes standing two phases were observed, where an upper oil majority phase had partly separated as an oil in water emulsion, stabilised by a proportion of the fines particles, with a continuous water phase below containing a

lower settled layer of fines particles. Stability improved at higher fines concentration, where at 3 wt% content and above no gross separation was seen after 10 minutes, where the single phase again consisted of an oil-in-water emulsion. The mixture with 6 wt% added fines showed no gross phase separation after 2 hours standing. A micrograph of a spot of the emulsion in Figure 6 showed droplets of oil in water with the presence of fines particles in both phases, but also with particles at the surface of the individual oil droplets, providing further evidence of a Pickering type mechanism.

3.4 Rheology of fines suspensions

The network structures of suspensions of fines material visualised by microscopy in Figure 4 are a result of a balance between particle-particle interactions promoting aggregation of particles and opposing particle-solvent interactions which encourage particle separation within the liquor medium. At sufficient concentration the extended network fills the entire bulk volume of the concentration, leading to longer term stability against gravitational settling. Following these observations, the ability of the fines particle to form a physical network is expected to lead to distinct physical properties, which may be apparent from measurements of fluid viscosity. The behaviour of suspensions measured by rotational viscometry is illustrated by the graphs in Figure 7, which show on a log-log scale the dependence of viscosity on shear rate for the three suspension concentrations of 5, 10 and 14 wt%. An inverse log-linear relationship is seen for the two lower concentrations and the higher concentration up to a shear rate around 10 /s, which is an indicator of shear-thinning behaviour. This can be modelled according to an empirical power law relation, according to equation (3), where η is the viscosity, K is a consistency constant, $\dot{\gamma}$ is the shear rate and n is the power law index, where for shear thinning behaviour $0 < n < 1$ [Rao 2014]

$$\eta = K\dot{\gamma}^{(n-1)} \quad (3)$$

The modelled functions for the three concentrations are overlayed as continuous lines over the experimental data points, where fitting was achieved by iterative adjustment of parameters K and n, for minimisation of the sum of squared difference between model and experiment, using Microsoft Excel™. The final fitted parameters for the three concentrations were: for 4 % solids, K = 1.6 and n = 0.1; for 10 % solids, K = 5 and n = 0.24; and for 14 % solids, K = 32 and n = 0.1. The low values for n confirm the severe shear thinning behaviour, with the parameter K related to the differences in concentration of particles between the three sample suspensions.

From a material perspective the shear thinning of these suspensions is explained by the presence at low shear rates of the previously observed extended physical network formed by particle-particle interactions, which results in a high resistance to shear. As shear rate increases this weak network breaks down and particles move past each other more easily, possibly becoming stratified into micro domains, which flow past each other with low resistance. This behaviour is not untypical of particulate food suspensions, as used for the formulation of pastes and sauces [Bourne 2002]. Interestingly at the highest concentration of 14 wt%, at high shear rates the fines suspension entered a Newtonian rheological region, where viscosity became independent of shear rate. This is presumed to represent the limit of the ability of the particles to reorganise to reduce flow resistance, which was not encountered at lower concentrations.

3.5 Enzymatic hydrolysis of milled material

Previous published work has shown that pre-milling can enhance the rate and extent of enzyme digestion of the polysaccharide fraction of BSG materials, where the use of different wet and dry mills were evaluated [Niemi et al 2012]. However, in this previous work monomodal size

478 distributions were mostly observed, with all tissues in the grain reduced in size. The requirement to
479 efficiently grind the polysaccharide containing tissue meant that the preferential breakup of the
480 weaker protein containing tissue was not studied. As described earlier, the current study has
481 revealed that the aleurone and endosperm related tissue can be preferentially reduced in size by
482 hydro-mechanical action, which may then be more digestible in the presence of protease, by virtue
483 of the increased surface area and the consequent higher accessibility.
484
485 The effect of pre-processing of BSG slurries through the colloid mill on the subsequent enzymatic
486 solubilisation of protein is shown in Figure 8. In the absence of added enzyme the pre-milling step
487 liberated a small amount of protein into solution, presumably that which was more loosely
488 associated with the tissue structure, where solubilisation would be aided by the mildly alkaline
489 environment. However, limited further release of protein occurred over time, which suggested that
490 natural protease activity in the grain was not significant, which might potentially have originated
491 from the original malt. The solubilisation of protein in pre-milled BSG continued at a faster rate in
492 the presence of added Alcalase enzyme, where the kinetic profile indicated that release continued
493 beyond the timescale of the experiment. This contrasted with the kinetic profile for the experiment
494 with added Alcalase but without pre-milling, where no initial liberation of protein occurred and the
495 overall rate of solubilisation was also slow. Overall, the findings suggested that a hydromechanical or
496 equivalent technique for achieving preferential breakup of aleurone and endosperm tissue may offer
497 an efficient approach to improve the rate and extent of enzymatic solubilisation of protein from BSG
498 material. In this investigation the use of the Bradford reagent for protein assay should not be
499 considered fully quantitative, as detection becomes less sensitive to molecular weights below 3000
500 Da. Other published work suggests that enzyme hydrolysis of spent grain leads to a range of
501 molecular weight peptides so a proportion may be under the threshold for effective dye binding
502 [Celus et al 2007]. However this was not considered to negate the use of the technique for
503 comparative purposes.

3.6 Spray drying of fines suspension

A significant process issue to overcome would be the avoidance of irreversible coalescence of the fines particles on drying, leaving a hard coarse material with limited functionality. Transporting and utilising the product as a wet cake would allow easy re-dispersion and formulation in a new food product, but would increase the risk microbial attack during storage. A post heat treatment or alternatively the operation of a sterile process, combined with suitable air-tight packaging might surmount such difficulties. Alternatively, suspensions of fines at moderate concentrations could be injected through a nozzle system for spray drying, taking advantage of their shear-thinning behaviour. The results of laboratory experiments conducted as part of the current study revealed that the powders produced in this way retained their small dimensions, shown in the micrograph in Figure 4e. The higher magnification image in Figure 4d indicated that the particles became more spherical in nature, possibly a result of capillary forces acting on the individual soft, water-plasticised particles during evaporation.

4. Conclusions

This work has demonstrated that wet milling using a commercial colloid mill leads to the formation of a protein rich fines material with particle size range around 1 - 10 μm, derived from the aleurone and endosperm tissue of the original grain. This fine protein rich fraction can be separated from the coarser husk and pericarp fraction by centrifugation of the milled slurry to give a protein enhanced product with useful technofunctional properties. The fines material has good stability in aqueous suspensions partly as a result of the formation an extended physical network of particle-particle interactions. This may lead to the potential for stabilisation of food or drink formulations. The fines material has dual hydrophilic-hydrophobic character and as a consequence may be useful for

stabilisation of oil-water food formulations, possibly through a Pickering type emulsification mechanism. Also, the high surface area of the fines has been shown to lead to faster and more efficient digestion by proteases, which may be beneficial for the production of protein and peptide isolates, of value in cosmetics and health formulations. The rheology of the aqueous mixtures of the fines material has also been studied, which revealed strong shear-thinning behaviour which can be modelled using an empirical power-law relation, as is often seen in food systems. Shear thinning may be beneficial in food applications and for pumping and flow of process concentrates. Spray drying of fines suspensions was shown to avoid coalescence of particles retaining individual particle identity and conserving technical properties.

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Figure captions

Figure 1. Infrared spectra of selected BSG materials., a = whole BSG (homogenised for sampling by dry milling) ; b = protein rich fines material obtained following colloid milling and centrifugation of BSG ; c = reference high concentration protein isolate obtained by alkali extraction and dialysis.

Figure 2. a: Gravitational settling of 10 wt% slurry of BSG, measured on standing for 1 hour, after increasing number of passes through a colloid mill. b: Dependence of volume proportion of fine fraction of BSG on number of mill passes; after centrifugation at 28 g (●), at 447 g (▲), 2800 g (■) ; protein concentration of fines obtained at 2800 g (♦). c: example of stratification of fine and coarse fraction of milled BSG after centrifugation, with upper fine layer indicated by horizontal lines.

Figure 3. Optical reflectance microscopy images of BSG materials, where pink/red Acid Fuschin stain has been preferentially absorbed by protein containing tissue. a: as-received dry hammer milled BSG, b: after further wet milling in a colloid mill.

Figure 4. Optical transmission microscopy images of suspensions of fine particle fraction separated from BSG. a: fines dispersed in water, b: fines dispersed in vegetable oil, c: high magnification image

of fines particles as created, dispersed in water. Fines particles dispersed in water after spray drying, at high magnification (d), overall suspension characteristics (e).

Figure 5. Images of settling behaviour of fines suspensions. a: 1 wt% in water - A, 3 wt% in water - B, 3 wt% in vegetable oil - C. b: 50:50 mixtures of water and vegetable oil, with 0, 1, 2,3, 6, and 10 wt% total of BSG fines added; (b) after vigorous shaking and standing for 10 minutes. (c) standing for 2 hours.

Figure 6. Optical transmission micrograph of oil droplets in the continuous water phase of an oil in water emulsion containing BSG fines particles, showing fines particles distributed within the water phase and at the oil-water droplet interface.

Figure 7. Log-log flow curve determined by rotational viscometry for BSG fines suspended in water at different concentrations; 5 wt% (▲), 10 wt% (■) 14 wt% (●). Continuous lines through each data set are fits to power law model; 4 wt% - K=1.6, n=0.1; 10 wt% - K=5, n=0.24; 14 wt% - K=32, n=0.1.

Figure 8. Release of protein over time following enzyme digestion of BSG slurry, prepared at 10 wt% concentration, with 0.1 wt% Alcalase on solid, incubation at 60°C, pH 9. With enzyme addition with pre-milling through the colloid mill (■) , with enzyme addition but without pre-milling (▲), with pre-milling but without enzyme addition (●).

Fig 1

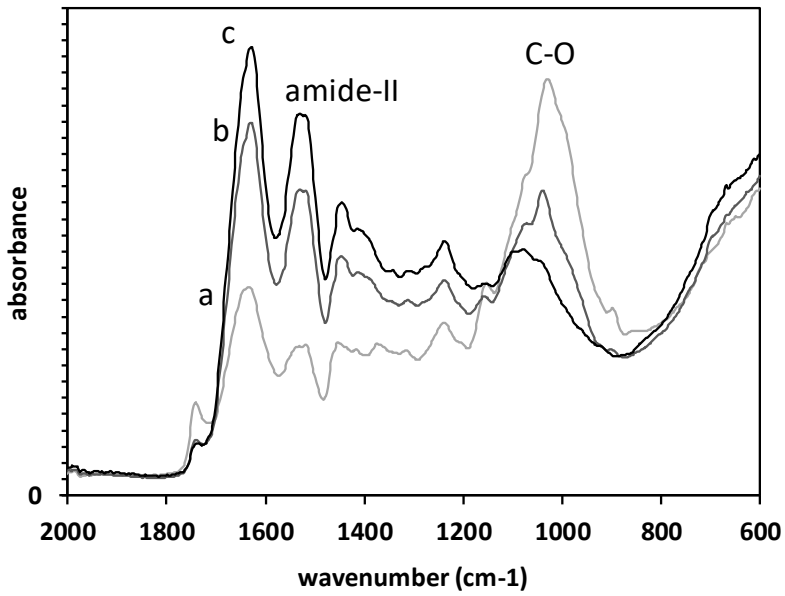

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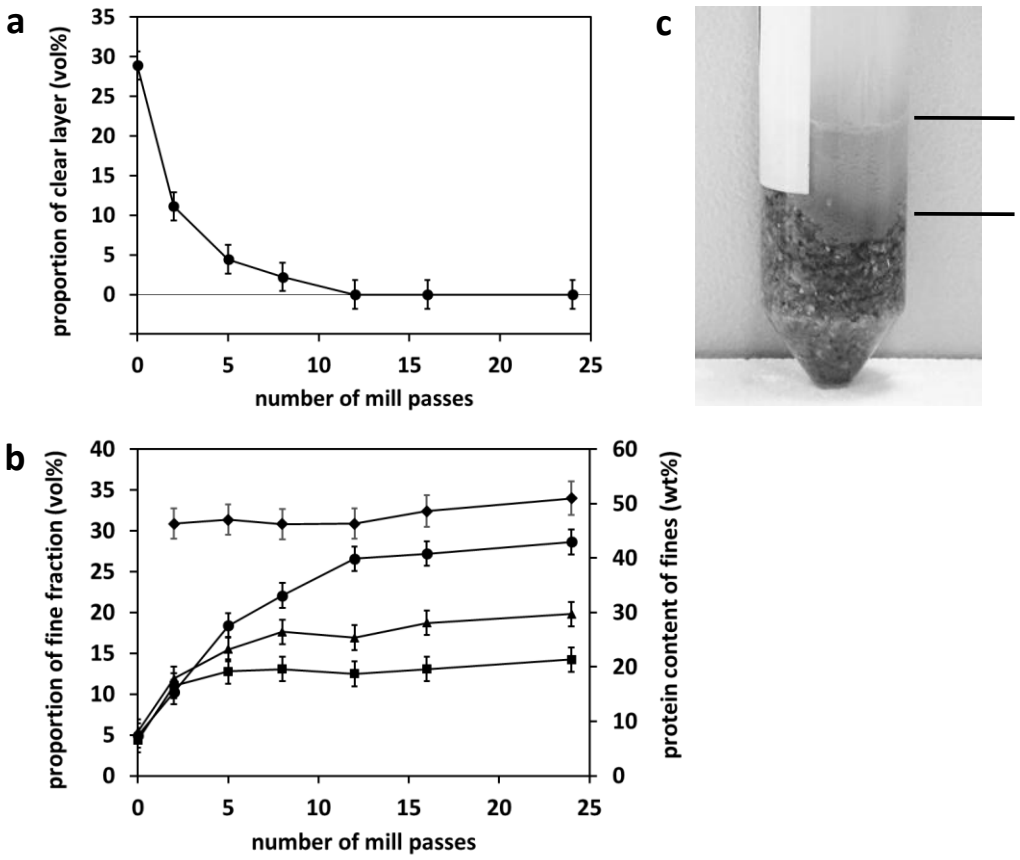
Fig 2



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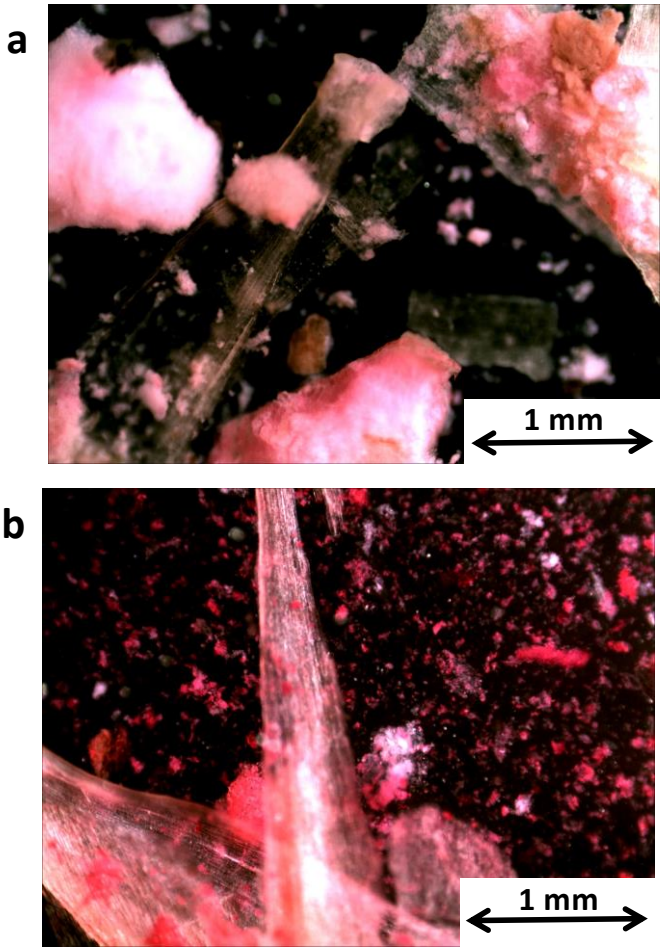

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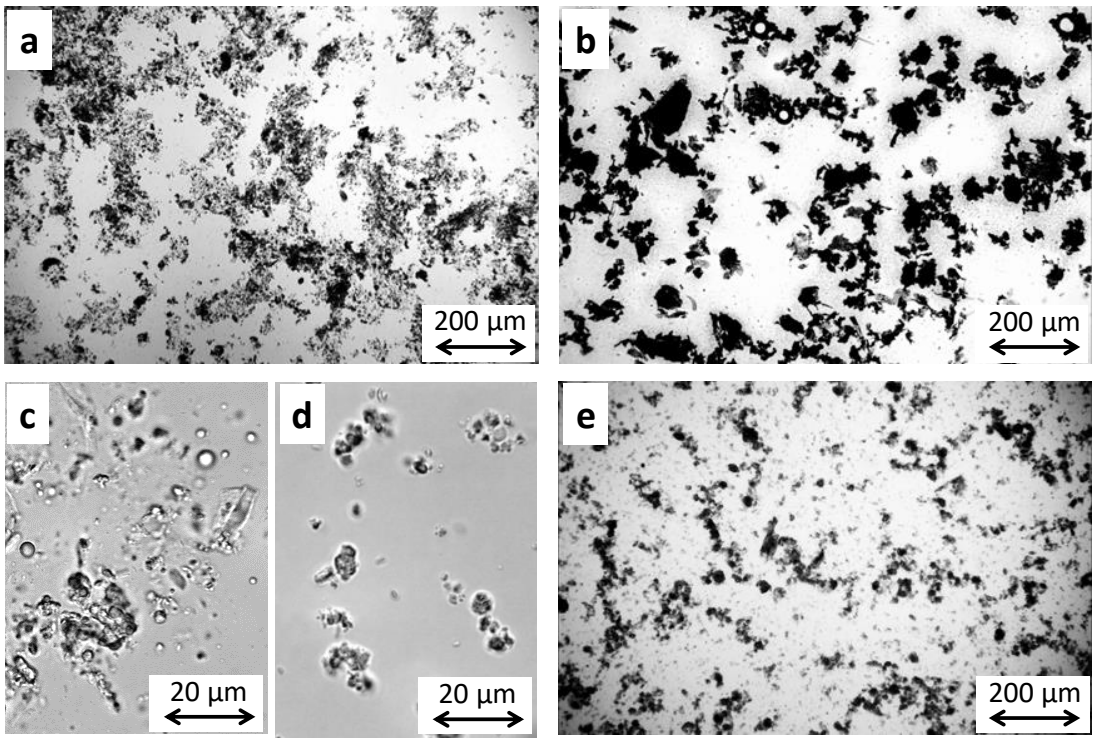

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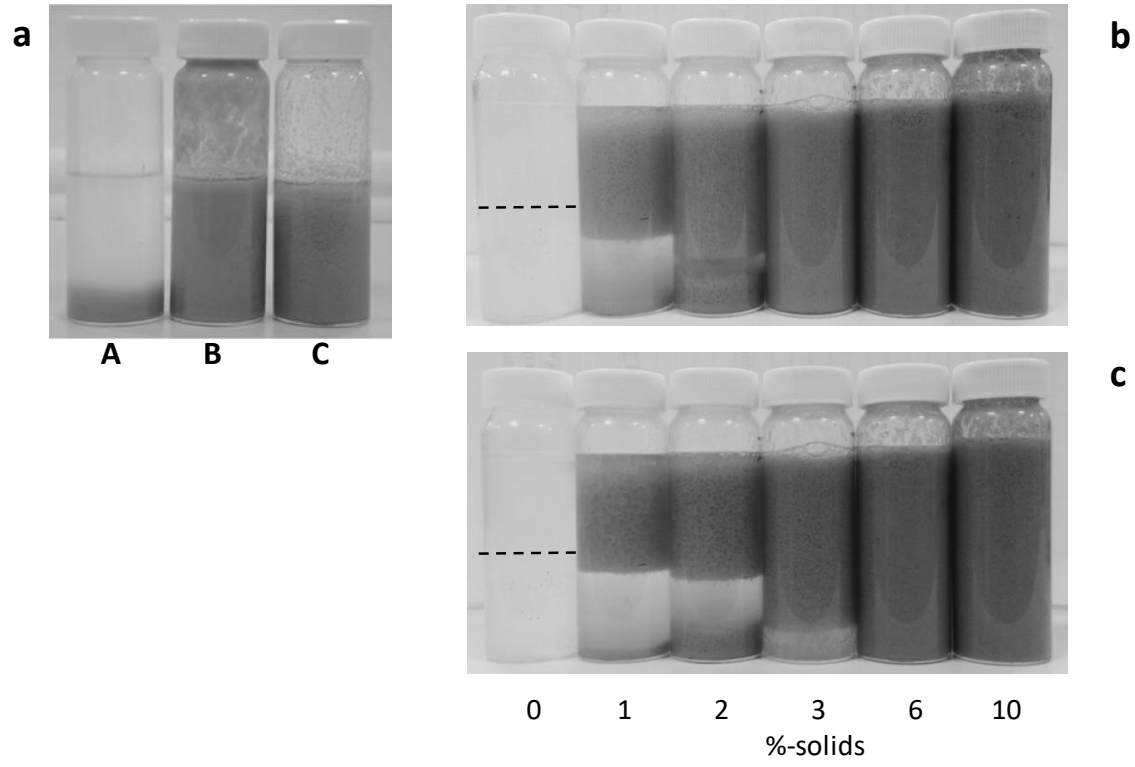

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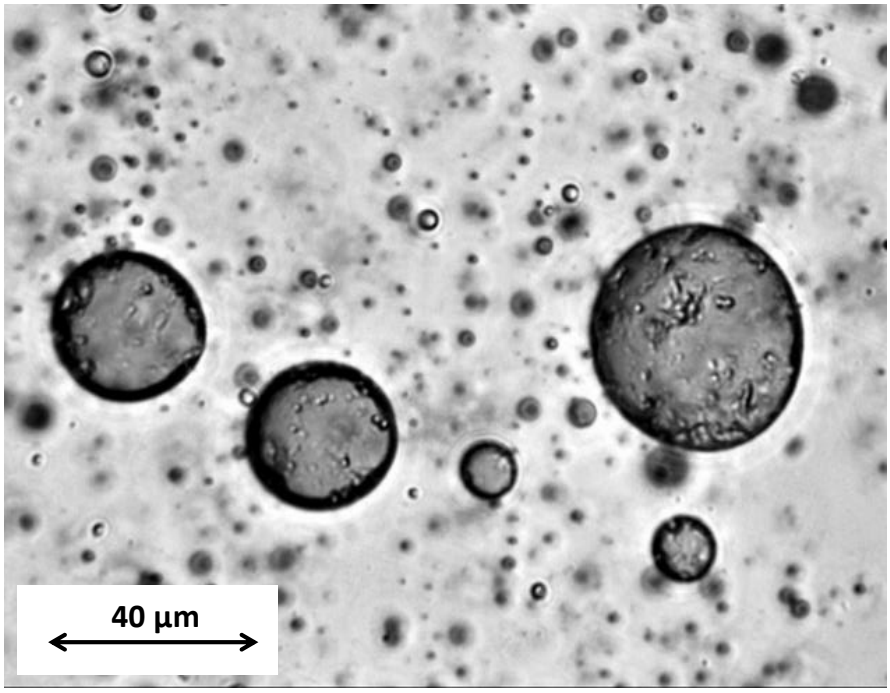

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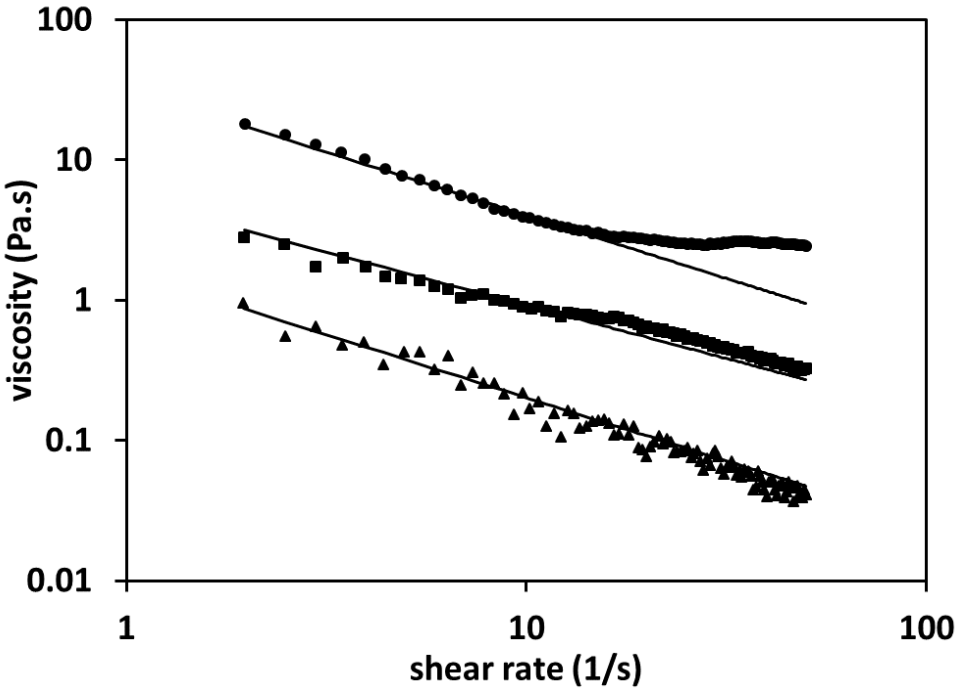

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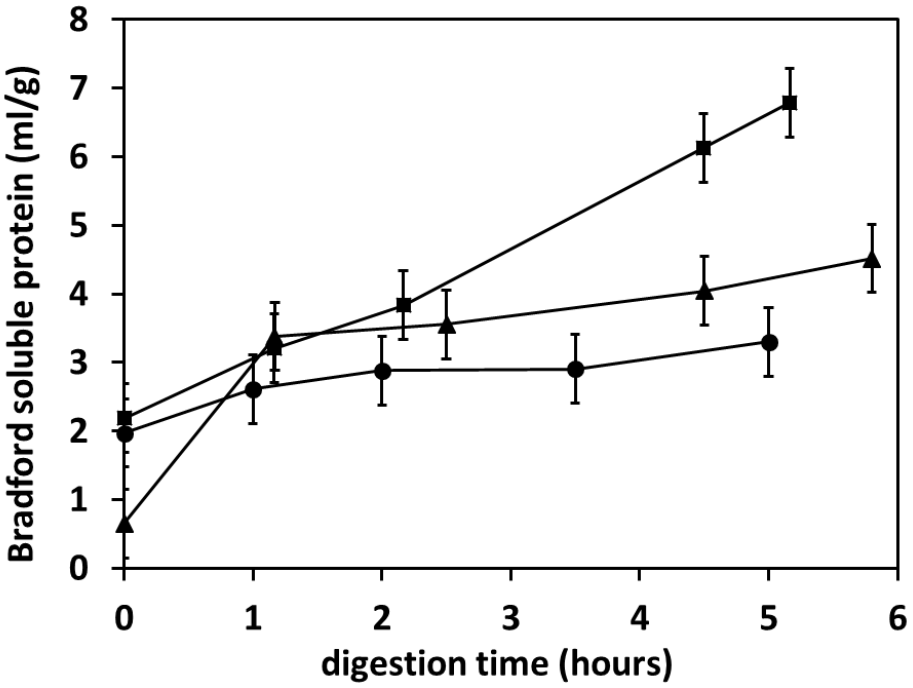
Fig 8



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