



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Nguyen, HTH; Ong, L; Kentish, SE; Gras, SL

Title:

Homogenisation improves the microstructure, syneresis and rheological properties of buffalo yoghurt

Date:

2015-07-01

Citation:

Nguyen, H. T. H., Ong, L., Kentish, S. E. & Gras, S. L. (2015). Homogenisation improves the microstructure, syneresis and rheological properties of buffalo yoghurt. *International Dairy Journal*, 46, pp.78-87. <https://doi.org/10.1016/j.idairyj.2014.08.003>.

Persistent Link:

<https://hdl.handle.net/11343/54983>

27 **1. Introduction**

28 Yoghurt is a semi-solid food with a microstructure consisting of a protein network, in which fat
29 globules are integrated (Lucey & Singh, 1997; Sodini, Remeuf, Haddad, & Corrieu, 2004). This
30 microstructure and the rheological properties of yoghurt are critical to product quality, as they
31 directly link to desirable functional and sensory properties including the smoothness, firmness
32 and flowability (Lee & Lucey, 2003; Rao & Lopes da Silva, 2007). These properties can be
33 controlled by careful selection of the ingredients, such as the milk source and concentration of
34 milk solids, starter culture, additives and processing conditions.

35 In the production of bovine yoghurt, the milk is often subjected to homogenisation at pressures
36 of 100-200 bar at 55-65 °C (Chandan & O'Rell, 2006; Lucey & Singh, 1997). Under these
37 pressures, the integrity of the casein micelles is maintained, while the fat globules are
38 significantly affected. These changes include the **creation** of smaller fat globules with an
39 increased surface area that is partly or entirely coated with adsorbed casein and denatured whey
40 protein (Michalski, Cariou, Michel, & Garnier, 2002; Ong, Dagastine, Kentish, & Gras, 2010;
41 Sameen, Anjum, Huma, & Nawaz, 2010). The adsorbed proteins are incorporated in the gel
42 network during acid induced coagulation and can lead to a number of improvements in yoghurt
43 production, including a better texture, decreased whey separation, increased gel firmness and
44 better integration of fat globules (Tamime & Robinson, 2007).

45 Buffalo yoghurt is typically made with unhomogenised milk (Tamime & Robinson, 2007). This
46 results in a yoghurt that exhibits greater syneresis and a larger hysteresis area than bovine
47 yoghurt (Nguyen, Ong, Lefevre, Kentish, & Gras, 2014b), indicating a reduced ability to recover
48 the original structure after shear-induced structural **breakdown** compared to bovine yoghurt
49 (Folkenberg, Dejmek, Skriver, Guldager, & Ipsen, 2006). The structure of buffalo yoghurt likely
50 contributes to these properties, as the network is more porous and contains larger more
51 numerous fat globules due to the high concentration of fat in buffalo milk (Nguyen et al.,
52 2014b).

53 Lowering the fermentation temperature has been shown to reduce the syneresis of buffalo
54 yoghurt, but only to a limited extent (Nguyen, Ong, Kentish, & Gras, 2014a). Other strategies
55 often employed in dairy processing to reduce syneresis are problematic; buffalo milk powders
56 are not readily available and stabilisers or thickeners can be perceived as detracting from a
57 natural product. Homogenisation may provide a simple unit operation to improve product

58 properties but there is limited information on the application of homogenisation to buffalo milk.
59 While a few studies report on selected properties of buffalo yoghurt made from homogenised
60 milk (Ghadge, 2008; Raju & Pal, 2009; Shiby & Mishra, 2008), they do not provide
61 comparisons between homogenised and unhomogenised products and there is no systematic
62 study of the potential application of homogenisation and the optimisation of this process. The
63 homogenisation of bovine milk has been extensively studied but the significant differences
64 between buffalo milk and bovine milk such as fat content (7.3 ± 1.0 % w/w vs. 4.1 ± 0.4 % w/w)
65 and fat globule size (5.0 ± 0.1 μm vs. 3.5 ± 0.2 μm) (Ménard et al., 2010) could lead to different
66 yoghurt properties in response to homogenisation. As a result, the conditions for optimal
67 processing may differ for two types of milk.

68 In this study, we investigate the potential use of homogenisation of buffalo milk at either 80 bar
69 or 160 bar to reduce the syneresis and improve the rheological properties of buffalo yoghurt.
70 The effect of homogenisation on the microstructure of buffalo yoghurt is also examined.
71 Changes in the viscoelastic properties of the buffalo milk gel and the growth and viability of
72 probiotic bacteria during fermentation and cold storage are investigated to better understand how
73 homogenisation may be applied to buffalo yoghurt production.

74 **2. Materials and methods**

75 **2.1 Yoghurt preparation**

76 Raw buffalo milk was provided by a local dairy farm (Shaw River, Yambuk, Australia). Upon
77 receipt, buffalo milk was divided into three portions (4 L per portion). One portion was used to
78 produce an unhomogenised yoghurt sample and the other two portions of buffalo milk were
79 warmed to 60 °C before homogenisation at 80 bar or 160 bar using a single stage homogeniser
80 (GEA Panda Plus 1000, GEA Niro Soavi, Parma, Italy). Both the homogenised and
81 unhomogenised milk portions were then batch-pasteurised at 85 °C for 30 min using a water
82 bath (Qualtex, Watson Victor Ltd., Perth, Australia) and cooled to 40 °C for yoghurt production
83 following a procedure described previously (Nguyen et al., 2014b). Briefly, the milk was
84 inoculated with 0.062 g L⁻¹ freeze dried direct vat starter culture ABT-5 containing probiotic
85 *Lactobacillus acidophilus* La-5, *Bifidobacterium lactis* Bb-12 and *Streptococcus thermophilus*
86 (CHR-Hansen, Bayswater, Australia), distributed into plastic containers of either 50 mL or 100
87 mL in volume and incubated at 40 °C. Samples in containers 100 mL in volume were used for
88 textural and rheological analyses, while samples in containers 50 mL in volume were used for

89 the analysis of syneresis and microstructure. When the pH of the milk reached pH 4.5, the
90 yoghurt samples were immediately transferred to a cold room (4°C) and stored for 28 d. The
91 yoghurt making was replicated in a second trial on a different day for all treatments using the
92 fresh milk sample collected on a different day with an average composition of $3.8 \pm 0.2\%$ (w/w)
93 protein, $7.2 \pm 0.3\%$ (w/w) fat, $4.9 \pm 0.1\%$ (w/w) lactose and $16.7 \pm 0.2\%$ (w/w) total solids,
94 determined using the methods presented in section 2.2.

95 **2.2 Measurement of pH and determination of fat, protein, lactose and total solids content**

96 Milk fat, protein content and pH were determined using methods described previously (Atwood
97 & Hartmann, 1992; Pesce & Strande, 1973) using a spectrophotometer (Fluostar Optima, BMG
98 labtech, Ortenberg, Germany) and a pH meter (Orion 720A plus, Orion Pacific Pty Ltd.,
99 Wallsend, Australia). The concentration of lactose in milk was determined following the method
100 of Gosling et al. (2009) using an High Performance Liquid Chromatography Shimadzu
101 Prominence system (Rydalmere, Australia) featuring a RID-10A refractive index detector and a
102 Rezex RCM-Monosaccharide Ca^{2+} column measuring 300 x 7.8 mm (Phenomenex, Lane Cove,
103 Australia). Total milk solids content was determined using an oven drying method (ISO/IDF,
104 2010). Three replicate samples were analysed for each treatment and two trials were conducted;
105 the data presented are therefore the mean of six replicates.

106 **2.3 Measurement of size distribution of fat globules**

107 The size distribution of the milk fat globules in buffalo milk was determined by light scattering
108 using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) (Ong et al., 2010). The refractive
109 index of the milk fat and distilled water was set at 1.460 and 1.330, respectively. The milk
110 sample was diluted (1:1 (v:v)) in ethylenediamine tetraacetic acid (EDTA; 50 mM, pH 7)
111 (Merck, Kilsyth, Australia) for 2 h to dissociate the casein micelles. The mixture was then added
112 gradually into the circulating cell of the apparatus containing distilled water and 0.05% (w/v)
113 sodium dodecyl sulphate (SDS; Merck) to dissociate the fat globule aggregates. Three
114 measurements were performed for each treatment in each trial and two trials of yoghurt
115 production were carried out; the results presented are therefore the mean of six measurements.

116 **2.4 Syneresis determination**

117 Syneresis was determined by centrifugation (700 g, 8 °C, 10 min) as previously described by
118 Purwandari, Shah, and Vasiljevic (2007) using a bench-top centrifuge (Eppendorf 5810R, North

119 Ryde, Australia). The syneresis was expressed as a percentage of the weight of the expelled
120 whey over the initial weight of a yoghurt sample. Three replicate samples for each treatment in
121 each trial were analysed at each time point during storage and two trials of yoghurt production
122 were carried out; the results presented are therefore the mean of six measurements.

123 **2.5 Texture analysis**

124 The gel firmness of yoghurt was analysed using a TA.XT-2 texture analyser (Stable
125 Microsystems, Godalming, UK) equipped with a 2 kg load cell and a 10 mm cylindrical probe as
126 described in Nguyen et al. (2014b). Briefly, the texture analyser was set at a contact area of 1
127 mm², a contact force of 5 g, an instrument speed of 1 mm s⁻¹ and a compression distance of 20
128 mm. The maximum force during penetration was defined as the gel firmness. Three replicate
129 samples for each treatment in each trial were analysed at each time point during storage and two
130 trials of yoghurt production were carried out; the results presented are therefore the mean of six
131 measurements.

132 **2.6 Rheological analysis**

133 Rheological properties during fermentation and storage were analysed following methods
134 described in Nguyen et al. (2014b). A controlled strain rheometer (Advanced Rheometrics
135 Expansion System, TA Instruments, New Castle, DE, USA), equipped with a 34 mm diameter
136 cup and a six blade vane 32 mm in diameter and 33 mm in height, was used for the analysis of
137 rheological properties of samples during fermentation. Briefly, the equipment was set at an
138 oscillation frequency of 0.1 Hz, a constant strain of 1% and a temperature of 40 °C. The storage
139 modulus (G') and loss tangent ($\tan \delta$) were measured every 5 min until the milk reached a pH of
140 4.5. Measurement was performed once for each treatment in each trial and two trials of yoghurt
141 production were carried out; the results presented are therefore the mean of two measurements.
142 A controlled stress rheometer (AR-G2, TA Instruments Ltd.) fitted with a cone plate (40 mm
143 diameter/4° angle) was used to monitor rheological properties during storage. Briefly, the
144 measurement included an initial equilibration to 20°C, a pre-shear at 500 s⁻¹ for 60 s, an
145 equilibration for 300 s, a logarithmic increase in shear rate from 0.1 to 100 s⁻¹ in 300 s, a holding
146 time of 5 s at 100 s⁻¹ and a logarithmic decrease in shear rate from 100 to 0.1 s⁻¹ in 300 s. The
147 hysteresis area, flow behaviour index and consistency coefficient were obtained by fitting the
148 data to the modified power law model ($\eta = K \gamma^{n-1}$) where η is the apparent viscosity (Pa s), γ the
149 shear rate (s⁻¹), K the consistency coefficient (Pa sⁿ) and n the flow behaviour index ($R^2 > 0.97$)

150 using a method described previously (Purwandari & Vasiljevic, 2009). The data were also fitted
151 to the Herschel-Bulkley model ($\tau = \tau_o + K \dot{\gamma}^n$) where τ is the shear stress (Pa), τ_o the yield stress
152 (Pa), K the consistency coefficient (Pa sⁿ) and n the flow behaviour index, as previously
153 described by Ciron, Gee, Kelly, and Auty (2012) for the assessment of the yield stress values
154 and a further comparison of the results obtained using different rheological models. The data
155 were analysed using the Rheology Advantage Data Analysis software (Version V5.70, TA
156 [Instruments](#) Ltd.). Three independent samples for each treatment in each trial were analysed at
157 each time point during storage and two trials of yoghurt production were carried out; these
158 results presented are therefore the mean of six measurements.

159 **2.7 Microstructural analysis using confocal laser scanning microscopy and cryo** 160 **scanning electron microscopy**

161 The CLSM analysis was carried out using an inverted confocal laser scanning microscope
162 (Leica TCS SP2; Leica Microsystems, [Mannheim](#), Germany) as described in details in our
163 previous work (Nguyen et al., 2014b). For CLSM observation, yoghurt and milk samples were
164 stained with Nile Red and Fast Green FCF (both supplied by Sigma-Aldrich, St. Louis, MO,
165 USA) for 30 min. CLSM observation was carried out in a dark room and the excitation/
166 emission wavelength of Nile Red and Fast Green FCF were set at 488 nm/500-600 nm and 633
167 nm/650-710 nm respectively. For the milk analysis, the three dimensional (3D) images were
168 reconstructed from a series of two dimensional (2D) layers (38-63 stacks of 2D images in each
169 3D image) and the total number of fat globules in a 1 μm^3 sample volume was analysed using
170 image analysis software (Imaris, Bitplane, South Windsor, CT, USA) as previously described by
171 Ong, Dagastine, Kentish, and Gras (2012). The results were presented as the total number of fat
172 globules per 1 μL of milk (conversion factor of 1 $\mu\text{m}^3 = 10^{-9}$ μL). Three 3D images were
173 obtained for each milk treatment (in an additional trial) for the image analysis and the results
174 presented are the mean of three analyses. For the yoghurt analysis, the cryo-SEM analysis was
175 performed using a field emission scanning electron microscope (Quanta, Fei Company,
176 Hillsboro, OR, USA.), as previously described by Ong et al. (2011). At least two CLSM and
177 cryo SEM images were taken for each yoghurt treatment in each trial. Two trials of yoghurt
178 were carried out and hence a minimum of four images were collected for each treatment. A
179 typical image is presented in each of the figures.

180 **2.8 Microbiological analysis**

181 The enumeration of bacteria during fermentation and storage were performed as previously
182 described (Nguyen et al., 2014b). Two plates were selected for manual counting for each
183 treatment in each trial and two trials of yoghurt were carried out; the results presented are
184 therefore the mean of four counts.

185

186 **2.9 Statistical analysis**

187 Minitab software (V16, Minitab Inc., State College, PA, USA) was used for data analysis. The
188 difference between means was assessed by one way analysis of variance (ANOVA) and
189 Fisher's paired comparison using a significance level of $P = 0.05$.

190 **3. Results and discussion**

191 A single stage homogenisation process was selected to assess the effect of homogenisation on
192 buffalo milk, reflecting the process most commonly applied in the production of bovine yoghurt
193 (Ozer, 2010; Tamime & Robinson, 2007; Varnam & Sutherland, 1994). Two homogenisation
194 pressures of 80 bar or 160 bar were also selected, based on the pressures reported in previous
195 studies for buffalo yoghurt (74.5 bar (Shiby & Mishra, 2008)) and the typical pressures applied
196 for milk homogenisation in bovine yoghurt production (100-200 bar (Lucey & Singh, 1997)).
197 Our hypothesis was that both treatments would improve the quality of buffalo yoghurt, including
198 the microstructure and level of syneresis and that homogenisation at higher pressures would be
199 more effective.

200 **3.1 The effect of homogenisation on the size distribution, surface area and microstructure** 201 **of buffalo milk fat globules**

202 Homogenisation at 80 bar or 160 bar resulted in a significant decrease in the size of fat globules
203 in buffalo milk and a considerable increase in specific surface area ($P < 0.05$) (Table 1). The
204 average volume-weighted mean diameter $D[4,3]$ of fat globules in the milk sample homogenised
205 at 80 bar was reduced more than threefold, while the specific surface area of fat globules in this
206 sample increased by a factor of eight compared to the unhomogenised milk. Increasing the
207 homogenisation pressure from 80 bar to 160 bar led to a further decrease in $D[4,3]$ of the fat

208 globules from 1.6 μm to 1.0 μm and an increase in the specific surface area from 14 $\text{m}^2 \text{g}^{-1}$ fat to
209 20 $\text{m}^2 \text{g}^{-1}$ fat.

210 The size distribution of the fat globules was bimodal in all cases (Figure 1). In the
211 unhomogenised samples, the major peak was at 5.8 μm with a minor peak at 1.0 μm ,
212 corresponding to 93% and 7% of the total volume of fat respectively. For milk homogenised at
213 80 bar, the main peak shifted to 2.2 μm (corresponding to 77% v/v) and the minor peak to 0.2
214 μm (corresponding to 23% v/v). An increase in homogenisation pressure to 160 bar resulted in a
215 further shift of the main peak diameter to 1.5 μm (corresponding to 55% v/v) and an increase in
216 the volume contribution of the minor peak to 45% v/v at 0.2 μm . The size distributions at both
217 pressures were also less expansive and varied within the range of 0.04 μm and 7.6 μm , while the
218 distribution in the unhomogenised samples spanned from 0.6 μm to 17.4 μm .

219 CLSM images confirmed the significant change in fat globule size observed by light scattering
220 and show the appearance of a protein coating and some aggregation of fat (Figure 2). Fat
221 globules were significantly smaller and more numerous in homogenised samples compared to
222 unhomogenised milk (Figures 2c and e vs. 2a) and were approximately 1-2 μm in diameter (in
223 the homogenised samples) compared to ~5 μm diameter (in the control). Smaller fat globules
224 could also be observed in all samples, consistent with the bimodal distribution observed using
225 light scattering (Figure 1). Aggregates or clusters of fat globules were larger and more numerous
226 in samples homogenised at 160 bar than at 80 bar (Figures 2e and f vs. Figures 2c and d).

227 The behaviour of buffalo fat globules during homogenisation is consistent with the behaviour
228 seen for bovine milk (Michalski, 2007; Walstra, Geurts, Noomen, Jellema, & Boekel, 1999),
229 where the combined effects of turbulence and cavitation are known to reduce fat globule size.
230 Homogenisation induced damage to the milk fat globule membrane is also known to result in
231 milk proteins absorbed to the fat globule surface (Cano-Ruiz & Richter, 1997; Michalski et al.,
232 2002; Ong et al., 2010). These proteins include mainly caseins and some denatured whey
233 proteins (Michalski et al., 2002).

234 The efficiency of homogenisation, defined as the decrease in fat globule size, appears less for
235 buffalo milk fat globules examined here compared to bovine milk fat globules, as similar
236 pressures were reported by Walstra (1975) to reduce bovine fat globules to smaller diameters of
237 0.9 μm (50 bar) and 0.5 μm (150 bar) using a single stage homogeniser. When expressed as a

238 percentage reduction in size, however, the changes induced in the larger buffalo fat globules are
239 larger and the process more effective.

240 The clusters of coalesced fat globules or “homogenisation clusters” observed in both
241 homogenised buffalo milk samples are thought to occur due to the instability of the
242 homogenised fat globules (Ogden, Walstra, & Morris, 1976; Walstra, 1975). Our observations
243 are similar to the findings of Doan (1929), who investigated the cluster formation of samples
244 with different fat concentrations ranging from 4.2% to 16.1% by mixing cream with fresh
245 bovine milk followed by a single stage homogenisation at ~240 bar. Samples containing 7.2%
246 bovine milk fat, similar to the fat content of buffalo milk, contained homogenisation clusters
247 while samples containing 4.2% bovine milk fat contained a well dispersed emulsion with little
248 clumping of fat. The larger specific surface area of the buffalo fat globules homogenised at 160
249 bar here (Table 1) may lead to a greater number of fat clusters (Figure 2f vs. Figure 2d).

250 Two stage homogenisation is thought to prevent the formation of homogenisation clusters
251 (Walstra et al., 1999). Such clusters can be prevented if the fat content of milk or cream samples
252 is less than 9% and a two stage homogeniser is used (Walstra et al., 1983). The two stage
253 process is not commonly used in the manufacture of bovine yoghurt (Ozer, 2010; Tamime &
254 Robinson, 2007; Varnam & Sutherland, 1994) but this process could have potential use for high
255 fat products, such as buffalo yoghurt. Our results also support the idea of lower homogenisation
256 pressures inducing fewer homogenisation clusters in buffalo milk. Indeed, two stage
257 homogenisation has been successfully applied to buffalo yoghurt production (Raju & Pal, 2009)
258 but the effect on the microstructure of the milk has not been reported.

259 **3.2 The effect of homogenisation on the changes in pH, storage modulus and loss tangent** 260 **during fermentation of buffalo yoghurt**

261 The fermentation time, defined as the time for milk to reach pH 4.5, was slightly shorter (~15
262 min) in homogenised buffalo milk samples (Figure 3a). This may be due to the better
263 availability of nutrients released from the disrupted MFGM in homogenised samples, which can
264 promote the activity and metabolism of lactic acid bacteria.

265 Homogenisation led to a significant increase in storage modulus of buffalo yoghurt at the end of
266 fermentation ($P < 0.05$) (Figure 3b). This result is consistent with previous studies for both acid

267 and rennet gels made from homogenised bovine milk, where an increase in storage modulus was
268 observed (Michalski et al., 2002; Sohrabvandi, Nematollahi, Mortazavian, & Vafae, 2013).

269 The increase in storage modulus is likely due to changes in the size and specific surface area of
270 the fat globules and the composition of the milk fat globule membrane. In the unhomogenised
271 buffalo samples, the fat globules are covered by the native MFGM, which acts as inactive
272 material that interacts less with milk proteins. These fat globules fill the pores as inert fillers if
273 the size is smaller than the serum pores or they act as structural breakers if the size is bigger than
274 the serum pores (Michalski et al., 2002; Nguyen et al., 2014b), leading to a weaker protein
275 network with a lower storage modulus. In contrast, the protein coating on the surface of the
276 homogenised buffalo fat globules plays an active role as a structure promoter and takes part in
277 the network formation during fermentation, as has been observed for bovine milk acid gels
278 (Xiong & Kinsella, 1991). This leads to a more cross-linked gel network resulting in an
279 increased storage modulus in homogenised samples. Previous reports for bovine milk suggest
280 that at least 40% of the surface area of the native membrane must be damaged and replaced by
281 proteins in order for the storage modulus to be significantly altered (Michalski et al., 2002). The
282 data presented here suggest buffalo milk fat globules behave in a similar way.

283 Some differences were observed in the response of buffalo milk fat globules to different
284 homogenisation pressures compared to previous reports for bovine milk. Here the storage
285 modulus at the end of the fermentation increased by more than 80% for yoghurt homogenised at
286 80 bar compared to the control sample. While the storage modulus of buffalo yoghurt produced
287 from milk homogenised at 160 bar was higher than the control sample, it was lower than the
288 yoghurt produced from milk homogenised at 80 bar at the end of fermentation. The lower
289 storage modulus for buffalo yoghurt made from milk homogenised at an increased pressure is
290 different to reports for acid and rennet gels made from bovine milk (Michalski et al., 2002). The
291 difference in these responses is likely linked to the differences in the properties of buffalo milk
292 including the higher fat content and bigger fat globules.

293 Homogenisation of buffalo milk also led to a more stable yoghurt network that was less easily
294 deformed. The $\tan \delta$, which represents the ratio of the storage modulus (G') to the loss modulus
295 (G''), was lower for homogenised samples (Figure 3c) but there was no significant difference (P
296 > 0.05) between samples treated with the two homogenisation pressures.

297 **3.3 The effect of homogenisation on the syneresis of buffalo yoghurt**

298 Homogenisation significantly reduced the level of syneresis observed for buffalo yoghurt ($P <$
299 0.05) ~ 6 fold, from 12 – 14% (w/w) to 0.8 – 2.1% (w/w) (Figure 4a). No significant difference
300 was found in the syneresis of yoghurt samples treated at different homogenisation pressures ($P >$
301 0.05). Homogenisation at 80 bar is therefore sufficient to reduce whey separation in buffalo
302 yoghurt production.

303 The decrease in syneresis is possibly due to the reduction in the size of fat globules and
304 production of an interactive protein coating. This leads to the formation of a gel network with a
305 greater number of cross-links within homogenised samples. The protein absorbed to the surface
306 of homogenised milk fat globules can also immobilize water (Keogh & O’Kennedy, 1998), an
307 effect that could lead to a more hydrated product with less whey separation.

308 The results presented here indicate that homogenisation could be used in buffalo yoghurt
309 production to reduce whey separation, minimizing the need to fortify buffalo milk with milk
310 powder, stabilizer or emulsifiers. The level of syneresis observed is similar to that obtained for
311 bovine yoghurt fortified with milk powder in our previous work (1-2% w/w, Nguyen et al.
312 (2014b)) but is lower than that observed for homogenised buffalo yoghurt in previous studies
313 (20-31% w/w) (Ghadge, 2008; Raju & Pal, 2009), probably due to the differences in the method
314 for syneresis determination, the processing conditions and the raw materials used for yoghurt
315 production. Interestingly, syneresis has also been reduced in sheep yoghurt by milk
316 homogenisation. These findings suggest homogenisation could be applied to a wide range of
317 animal milk types for effective yoghurt production.

318 **3.4 The effect of homogenisation on the gel firmness of buffalo yoghurt**

319 Homogenisation significantly ($P < 0.05$) increased yoghurt gel firmness (Figure 4b) by
320 approximately 50%, consistent with previous studies using bovine milk (Lucey, 2004), goat milk
321 (Abrahamsen & Holmen, 1981) and sheep milk (Muir & Tamime, 1993). Interestingly, the gel
322 firmness of **buffalo yoghurt produced from homogenised milk** at both pressures in this study was
323 significantly higher than the gel firmness obtained for fortified and homogenised bovine yoghurt
324 in our previous work (Nguyen et al., 2014b); despite the similar total solids content of the two
325 milk preparations (16.7 ± 0.2 vs. $16.8 \pm 0.1\%$ w/w) and the slightly lower protein concentration
326 of the buffalo milk (3.8 ± 0.2 vs. $4.6 \pm 0.3\%$ w/w), suggesting that the fat content or strength of
327 the protein network are the contributing factors.

328 Our results highlight the different properties of buffalo and bovine yoghurt following milk
329 homogenisation. The gel firmness of yoghurt produced from buffalo milk homogenised at 160
330 bar was lower than for samples homogenised at 80 bar ($P < 0.05$) (Figure 4b), consistent with
331 the lower storage modulus for these samples (Figure 3b). This is different to results reported for
332 bovine yoghurt (Michalski et al., 2002), where an increase in gel firmness was obtained with an
333 increase in homogenisation pressure. These differences likely arise from the different
334 composition, as discussed above. Interestingly, when bovine milk-based dessert products with a
335 high fat content similar to buffalo yoghurt were studied (6.5% fat vs. $7.2 \pm 0.3\%$ fat), a low
336 homogenisation pressure of 50 bar increased the gel firmness while a further increase in the
337 homogenisation pressure to 150 bar decreased the gel firmness (Sohrabvandi et al., 2013). These
338 data indicate the importance of the fat content in determining the product properties.

339 Cold storage significantly ($P < 0.05$) increased the gel firmness of yoghurt samples in all
340 treatments and an increase of 0.32-0.38 N was observed over 28 d for homogenised samples,
341 compared to an increase of only 0.18 N for the unhomogenised samples. An increase in gel
342 firmness during cold storage was also observed in previous studies for buffalo, bovine and sheep
343 yoghurt (Domagala, 2009; Nguyen et al., 2014b; Salvador & Fiszman, 2004) but not in goat
344 yoghurt (Domagala, 2009).

345 The increase observed for buffalo and other yoghurts may be due to the further development of
346 the gel network or the production of exopolysaccharide (EPS) by the starter culture bacteria.
347 EPS producing starter cultures can improve textural properties (Duboc & Mollet, 2001;
348 Folkenberg et al., 2006) and EPS concentration has been found to increase during cold storage
349 of yoghurt in a previous study (Amatayakul, Halmos, Sherkat, & Shah, 2006). *Bifidobacterium*
350 *lactis* used here has previously been reported to produce EPS such as L-rhamnopyranose, D-
351 glucopyranose, D-galactosepyranose and D-galactofuranose (Hidalgo-Cantabrana et al., 2013;
352 Leivers et al., 2011), which could interact with the protein network leading to the observed
353 increase in gel firmness. The properties of buffalo yoghurt are likely to differ to goat yoghurt, as
354 goat milk contains a lower concentration of casein and higher concentration of non-protein
355 nitrogen (Domagala, 2009; Park, Juárez, Ramos, & Haenlein, 2007).

356 The firmness of yoghurt also depends on the pH (Walstra et al., 1999). Yoghurt generally has a
357 higher gel firmness at a lower pH, with a preferred pH range of 4.1 and 4.6 (Walstra et al.,
358 1999). A further drop in pH can occur during the cold storage following fermentation, often

359 referred to as post acidification; pH was not monitored during storage in this study but could
360 contribute to the increase in gel firmness observed here and would be worthy of further study.

361 **3.5 The effect of homogenisation on the rheological properties of buffalo yoghurt**

362 Homogenisation appears effective at improving the texture and rheological properties of buffalo
363 yoghurt, as indicated by the significant decrease in the hysteresis area and consistency
364 coefficient and significant increase in the flow behaviour index ($P < 0.05$) (Figure 5). An
365 increase in homogenisation from 80 bar to 160 bar, however, did not have a significant effect (P
366 > 0.05) on each of these rheological properties.

367 The rheological flow curves that measure shear induced structural deformation differed between
368 control and homogenised yoghurt samples (see supplementary Figure A.1 for an example flow
369 curve). A plot of the stress scaled hysteresis area as a function of the shear stress at the
370 maximum shear rate (τ_{100}) obtained from these curves provides further insights into the shear
371 induced structural degradation in these samples (Figure 5a). The shear stress at the maximum
372 shear rate increased following homogenisation, with the exception of data collected for the 80
373 bar sample after 28 d of storage. The stress scaled hysteresis area (Figure 5 a) and the total
374 hysteresis area (Figure 5 b) were significantly reduced following homogenisation, indicating
375 increased stability (Jaros, Heidig, & Rohm, 2007) and an ability to recover the original structure
376 after deformation (Folkenberg et al., 2006). These rheological properties are consistent with the
377 improved texture of homogenised yoghurt samples, including the lower whey separation (Figure
378 4a) and the firmer gel (Figure 4b).

379 The consistency coefficient, defined as the apparent viscosity at shear rate of 1 s^{-1} , was found to
380 be between 10-25 $\text{Pa}\cdot\text{s}^n$ for samples assessed after one day of storage where the data was fitted
381 with the modified power law model ($R^2 \sim 0.97-0.99$) (Figure 5c). The magnitude of these
382 consistency coefficients is within the wide range reported for bovine yoghurt of 0.76-165.03 Pa
383 s^n (Abu-Jdayil & Mohameed, 2002; Purwandari et al., 2007) and buffalo yoghurt of 4.9 - 35.2 Pa
384 s^n (Bezerra, Souza, & Correia, 2012) where the power law or modified power law model has
385 been applied.

386 Alternatively, a yield stress may be determined from the flow curves and the data fitted to the
387 Herschel-Bulkley model. The yield stress, defined as the initial stress or force required to initiate
388 the yoghurt to flow, was $20.7 \pm 11.5 \text{ Pa}$ for the control yoghurts. This yield stress was

389 significantly higher ($P < 0.05$) than those obtained for the samples made from milk homogenised
390 at 80 bar and 160 bar, which were 11.4 ± 2.0 Pa or 10.6 ± 1.9 Pa, respectively (Supplementary
391 Figure A.1).

392 The consistency coefficient determined from the Herschel-Bulkley model for samples assessed
393 after one day of storage was smaller in magnitude ($1-2.5$ Pa.sⁿ) but the trends observed were
394 consistent with those determined using the modified power law model, despite a poorer fit to the
395 data ($R^2 \sim 0.15-0.97$). These values of consistency coefficient are also within the range of 0.1-
396 88.86 Pa sⁿ, previously reported for bovine yoghurt using the Herschel-Bulkley model (Ciron et
397 al., 2012; Paseephol, Small, & Sherkat, 2008; Paskov, Karsheva, & Pentchev, 2010).

398 The homogenised samples also exhibited a significantly higher flow behaviour index compared
399 to the control samples (Figure 5d), indicating these samples are more Newtonian in behaviour.
400 The range of flow behaviour index recorded is within the range of 0.08-0.56 previously reported
401 for bovine yoghurt using the power law model (Abu-Jdayil & Mohameed, 2002; Espírito-Santo
402 et al., 2013). When the data are fitted to the Herschel-Bulkley model the trends are similar but
403 magnitude of the numbers larger ($\sim 0.56-0.89$), consistent with the range reported of 0.26-0.83
404 using this model in the literature (Paseephol et al., 2008; Paskov et al., 2010).

405 The observations recorded here are in agreement with a previous study (Shaker, Abu Jdayil, &
406 Jumah, 2002), where a decrease in consistency coefficient and an increase in the flow behaviour
407 index were observed for yoghurt made from homogenised bovine milk.

408 **3.6 The effect of homogenisation on the microstructure of buffalo yoghurt**

409 Cryo-SEM images of the microstructure of buffalo yoghurt show that the fat globules in the
410 homogenised samples are smaller than in the unhomogenised samples (Figures 6c and e vs. 6a,
411 Figures 6 d and f vs. 6b). Most of these small fat globules are integrated within the protein
412 network, which makes them almost indistinguishable from this network (Figures 6c-f). There are
413 also a few remaining fat globules with size of approximately 2-3 μm observed within the
414 microstructure of the samples homogenised at 80 bar (Figures 6c-d).

415 The protein network produced from homogenised milk shows thicker protein strands compared
416 to the unhomogenised samples, especially after 28 d of storage (Figures 6d and f vs. 6b). The
417 thicker protein strands and the more interconnected gel networks observed in the microstructure

418 of homogenised buffalo yoghurt are consistent with the firmer gel and higher stability of this
419 yoghurt.

420 The CLSM images of the yoghurt microstructure confirm the presence of the smaller fat
421 globules in the homogenised yoghurt samples (Figures 7c and e). The fat globules in the control
422 samples are large and located within the serum pores as inert fillers (Figures 7a-b). In contrast,
423 the fat globules in the homogenised samples are better embedded within the protein network
424 (Figures 7c-e and d-f). Clusters of fat globules could also be observed within the protein
425 aggregates in the microstructure of the homogenised samples (indicated by arrows in Figure 7),
426 similar to observations of the homogenised milk (Figures 2d and f). There was no obvious
427 difference, however, between the microstructure of the yoghurt samples produced from buffalo
428 milk homogenised at different pressures. This suggests that the bulk microstructure is not
429 responsible for the differences observed in storage modulus and gel firmness, although the
430 strength of binding between components in the microstructure may play a role.

431 **3.7 The effect of homogenisation on the growth and viability of bacteria of buffalo yoghurt**

432 Homogenisation had no significant effect on the growth of the bacteria during fermentation
433 (Figure 8) ($P > 0.05$) but led to a decrease in the viability of *Lactobacillus acidophilus* at the end
434 of the storage period ($P < 0.05$) (Figure 8a), particularly for milk homogenised at 80 bar. This is
435 possibly due to the increased activity of the *Streptococcus thermophilus* that may result from the
436 better availability of nutrients within homogenised samples. The greater production of
437 metabolites by *Streptococcus thermophiles* may in turn adversely affect the viability of
438 *Lactobacillus acidophilus*. This result indicates that homogenisation may have a negative effect
439 on the viability of probiotic bacteria during the storage of buffalo yoghurt. An increase in the
440 number of bacteria used for inoculation, the use of a different strain of probiotic bacteria or the
441 use of encapsulation technology may improve bacterial survival during storage.

442 **4. Conclusion**

443 Homogenisation significantly affected the properties of buffalo milk fat globules and improved
444 the properties of buffalo yoghurt. Treatment at either 80 bar or 160 bar decreased the fat globule
445 size, increased the specific surface area of fat and led to a protein coating and formation of fat
446 clusters. These changes affected the properties of yoghurt improving the microstructure and
447 rheological properties; syneresis was reduced approximately five fold and the texture was

448 improved, resulting in a firmer gel, a higher storage modulus with a lower hysteresis area that
449 demonstrates a better capacity to recover upon deformation. The protein microstructure of
450 buffalo yoghurt made from homogenised milk also appeared dense with small fat globules
451 embedded within this network. Our results suggest that homogenisation in the range of 80-160
452 bar can be used to improve the quality of buffalo yoghurt, especially to decrease syneresis and
453 large differences are not observed within this range of pressures. Homogenisation of buffalo
454 milk avoids the need for strategies such as milk fortification or addition of stabilisers and offers
455 a solution more effective than lowering the temperature during fermentation.

456 **Acknowledgements**

457 The authors would like to acknowledge the Australian Government for providing an Australian
458 Postgraduate Award (International) (APA - International) scholarship, the Rural Industries
459 Research and Development Cooperation (RIRDC) for financial support and Shaw River for
460 supplying the buffalo milk used in this study. The authors also thank the Particulate Fluids
461 Processing Centre (PFPC), the Electron Microscopy Unit and the Biological Optical Microscopy
462 Platform of the Bio21 Molecular Science and Technology Institute for access to equipment, Mr
463 Roger Curtain for his help in operating the cryo-SEM and Dr Sandy Clarke (Department of
464 Mathematics and Statistics, University of Melbourne) for her assistance in statistical analysis.
465 Dr Lydia Ong and Associate Professor Sally Gras are supported by The ARC Dairy Innovation
466 Hub.

467 **References**

- 468 Abrahamsen, R. K., & Holmen, T. B. (1981). Goat's milk yoghurt made from non-homogenised
469 and homogenised milks, concentrated by different methods. *Journal of Dairy Research*,
470 48, 457-463.
- 471 Abu-Jdayil, B., & Mohameed, H. (2002). Experimental and modelling studies of the flow
472 properties of concentrated yoghurt as affected by the storage time. *Journal of Food*
473 *Engineering*, 52, 359-365.
- 474 Amatayakul, T., Halmos, A. L., Sherkat, F., & Shah, N. P. (2006). Physical characteristics of
475 yoghurts made using exopolysaccharide-producing starter cultures and varying casein to
476 whey protein ratios. *International Dairy Journal*, 16, 40-51.
- 477 Atwood, C. S., & Hartmann, P. E. (1992). Collection of fore and hind milk from the sow and the
478 changes in milk-composition during suckling. *Journal of Dairy Research*, 59, 287-298.
- 479 Bezerra, M. F., Souza, D. F. S., & Correia, R. T. P. (2012). Acidification kinetics,
480 physicochemical properties and sensory attributes of yoghurts prepared from mixtures of
481 goat and buffalo milks. *International Journal of Dairy Technology*, 65, 437-443.
- 482 Cano-Ruiz, M. E., & Richter, R. L. (1997). Effect of homogenisation pressure on the milk fat
483 globule membrane proteins. *Journal of Dairy Science*, 80, 2732-2739.

- 484 Chandan, R. C., & O'Rell, K. R. (2006). *Manufacturing yoghurt and fermented milks* (1st edn.
485 Chapt. 12). Oxford, UK: Wiley-Blackwell.
- 486 Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2012). Modifying the microstructure
487 of low-fat yoghurt by microfluidisation of milk at different pressures to enhance
488 rheological and sensory properties. *Food Chemistry*, 130, 510-519.
- 489 Doan, F. J. (1929). Some factors affecting the fat clumping produced in milk and cream
490 mixtures when homogenised. *Journal of Dairy Science*, 12, 211-230.
- 491 Domagala, J. (2009). Instrumental texture, syneresis and microstructure of yoghurts prepared
492 from goat, cow and sheep milk. *International Journal of Food Properties*, 12, 605-615.
- 493 Duboc, P., & Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry.
494 *International Dairy Journal*, 11, 759-768.
- 495 Espírito-Santo, A. P., Lagazzo, A., Sousa, A. L. O. P., Perego, P., Converti, A., & Oliveira, M.
496 N. (2013). Rheology, spontaneous whey separation, microstructure and sensorial
497 characteristics of probiotic yoghurts enriched with passion fruit fiber. *Food Research
498 International*, 50, 224-231.
- 499 Folkenberg, D. M., Dejmek, P., Skriver, A., Guldager, H. S., & Ipsen, R. (2006). Sensory and
500 rheological screening of exopolysaccharide producing strains of bacterial yoghurt
501 cultures. *International Dairy Journal*, 16, 111-118.
- 502 Ghadge, P. N. (2008). Effect of fortification on the physico-chemical and sensory properties of
503 buffalo milk yoghurt. *Electronic Journal of Environmental, Agricultural and Food
504 Chemistry*, 7, 2890-2899.
- 505 Gosling, A., Alftren, J., Stevens, G. W., Barber, A. R., Kentish, S. E., & Gras, S. L. (2009).
506 Facile pretreatment of *Bacillus circulans* beta-galactosidase increases the yield of
507 galactosyl oligosaccharides in milk and lactose reaction systems. *Journal of Agricultural
508 and Food Chemistry*, 57, 11570-11574.
- 509 Hidalgo-Cantabrana, C., Sanchez, B., Moine, D., Berger, B., de Los Reyes-Gavilán, C. G.,
510 Sánchez, B., Gueimonde, M., Margolles, A., & Ruas Madiedo, P. (2013). Insights into
511 the ropy phenotype of the exopolysaccharide-producing strain *Bifidobacterium animalis*
512 subsp. *lactis* A1dOxR. *Applied and Environmental Microbiology*, 79, 3870-3874.
- 513 ISO/IDF. (2010). *Milk, cream and evaporated milk – Determination of total solids content.*
514 *International standard ISO 6731-IDF 21*. Brussels, Belgium: International Dairy
515 Federation.
- 516 Jaros, D., Heidig, C., & Rohm, H. (2007). Enzymatic modification through microbial
517 transglutaminase enhances the viscosity of stirred yoghurt. *Journal of Texture Studies*,
518 38, 179-198.
- 519 Keogh, K. M., & O'Kennedy, B. T. (1998). Rheology of stirred yoghurt as affected by added
520 milk fat, protein and hydrocolloids. *Journal of Food Science*, 63, 108-112.
- 521 Lee, W. J., & Lucey, J. A. (2003). Rheological properties, whey separation, and microstructure
522 in set-style yoghurt: Effects of heating temperature and incubation temperature. *Journal
523 of Texture Studies*, 34, 515-536.
- 524 Leivers, S., Hidalgo-Cantabrana, C., Robinson, G., Margolles, A., Ruas-Madiedo, P., & Laws,
525 A. P. (2011). Structure of the high molecular weight exopolysaccharide produced by
526 *Bifidobacterium animalis* subsp. *lactis* IPLA-R1 and sequence analysis of its putative eps
527 cluster. *Carbohydrate Research*, 346, 2710-2717.
- 528 Lucey, J. A., & Singh, H. (1997). Formation and physical properties of acid milk gels: a review.
529 *Food Research International*, 30, 529-542.
- 530 Lucey, J. A. (2004). Cultured dairy products: an overview of their gelation and texture
531 properties. *International Journal of Dairy Technology*, 57, 77-84.
- 532 Ménard, O., Ahmad, S., Rousseau, F., Briard-Bion, V., Gaucheron, F., & Lopez, C. (2010).
533 Buffalo vs. cow milk fat globules: Size distribution, zeta-potential, compositions in total

- 534 fatty acids and in polar lipids from the milk fat globule membrane. *Food Chemistry*, 120,
535 544-551.
- 536 Michalski, M. C., Cariou, R., Michel, F., & Garnier, C. (2002). Native vs. damaged milk fat
537 globules: membrane properties affect the viscoelasticity of milk gels. *Journal of Dairy*
538 *Science*, 85, 2451-2461.
- 539 Michalski, M. C. (2007). On the supposed influence of milk homogenisation on the risk of
540 CVD, diabetes and allergy. *British Journal of Nutrition*, 97, 598-610.
- 541 Muir, D. D., & Tamime, A. Y. (1993). Ovine milk. 3. Effect of seasonal-variations on properties
542 of set and stirred yoghurts. *Milchwissenschaft*, 48, 509-513.
- 543 Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2014a). The effect of fermentation
544 temperature on the microstructure, physicochemical and rheological properties of
545 probiotic buffalo yoghurt. *Food and Bioprocess Technology*, 7, 2538-2548.
- 546 Nguyen, H. T. H., Ong, L., Lefevre, C., Kentish, S. E., & Gras, S. L. (2014b). The
547 microstructure and physicochemical properties of probiotic buffalo yoghurt during
548 fermentation and storage: a comparison with bovine yoghurt. *Food and Bioprocess*
549 *Technology*, 7, 937-953.
- 550 Ogden, L. V., Walstra, P., & Morris, H. A. (1976). Homogenisation-induced clustering of fat
551 globules in cream and model systems. *Journal of Dairy Science*, 59, 1727-1737.
- 552 Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2010). The effect of milk processing on
553 the microstructure of the milk fat globule and rennet induced gel observed using confocal
554 laser scanning microscopy. *Journal of Food Science*, 75, 135-145.
- 555 Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2011). Microstructure of milk gel and
556 cheese curd observed using cryo scanning electron microscopy and confocal microscopy.
557 *Lwt-Food Science and Technology*, 44, 1291-1302.
- 558 Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2012). The effect of pH at renneting on
559 the microstructure, composition and texture of Cheddar cheese. *Food Research*
560 *International*, 48, 119-130.
- 561 Ozer, B. (2010). Strategies for yoghurt manufacturing. In F. Yildiz (Ed.), *Development and*
562 *manufacture of yoghurt and other functional dairy products* (pp. 47-96). Boca Raton,
563 FL, USA: CRC Press, Taylor and Francis Group.
- 564 Park, Y. W., Juárez, M., Ramos, M., & Haenlein, G. F. W. (2007). Physico-chemical
565 characteristics of goat and sheep milk. *Small Ruminant Research*, 68, 88-113.
- 566 Paseephol, T., Small, D. M., & Sherkat, F. (2008). Rheology and texture of set yoghurt as
567 affected by inulin addition. *Journal of Texture Studies*, 39, 617-634.
- 568 Paskov, V., Karsheva, M., & Pentchev, I. (2010). Effect of starter culture and homogenisation
569 on the rheological properties of yoghurt. *Journal of the University of Chemical*
570 *Technology and Metallurgy*, 45, 59-66.
- 571 Pesce, M. A., & Strande, C. S. (1973). New micromethod for determination of protein in
572 cerebrospinal-fluid and urine. *Clinical Chemistry*, 19, 1265-1267.
- 573 Purwandari, U., Shah, N. P., & Vasiljevic, T. (2007). Effects of exopolysaccharide-producing
574 strains of *Streptococcus thermophilus* on technological and rheological properties of set-
575 type yoghurt. *International Dairy Journal*, 17, 1344-1352.
- 576 Purwandari, U., & Vasiljevic, T. (2009). Rheological properties of fermented milk produced by
577 a single exopolysaccharide producing *Streptococcus thermophilus* strain in the presence
578 of added calcium and sucrose. *International Journal of Dairy Technology*, 62, 411-421.
- 579 Raju, P., & Pal, D. (2009). The physicochemical, sensory, and textural properties of misti dahi
580 prepared from reduced fat buffalo milk. *Food and Bioprocess Technology*, 2, 101-108.
- 581 Rao, M. A., & Lopes da Silva, J. A. (2007). Role of rheological behaviour in sensory assessment
582 of foods and swallowing. In M. A. Rao (Ed.), *Rheology of fluid and semisolid foods:*

- 583 *Principles and applications* (pp. 403-426). New York, NY, USA: Springer Science and
584 Bussiness Media.
- 585 Salvador, A., & Fiszman, S. M. (2004). Textural and sensory characteristics of whole and
586 skimmed flavored set-type yoghurt during long storage. *Journal of Dairy Science*, 87,
587 4033-4041.
- 588 Sameen, A., Anjum, F. M., Huma, N., & Nawaz, H. (2010). Chemical composition and sensory
589 evaluation of Mozzarella cheese: influence by milk sources, fat levels, starter cultures
590 and ripening period. *Pakistan Journal of Agricultural Sciences*, 47, 26-31.
- 591 Shaker, R. R., Abu Jdayil, B., & Jumah, R. Y. (2002). Rheological properties of set yoghurt as
592 influenced by incubation temperature and homogenisation. *Journal of Food Quality*, 25,
593 409-418.
- 594 Shibby, V. K., & Mishra, H. N. (2008). Modelling of acidification kinetics and textural properties
595 in dahi (Indian yoghurt) made from buffalo milk using response surface methodology.
596 *International Journal of Dairy Technology*, 61, 284-289.
- 597 Sodini, I., Remeuf, F., Haddad, S., & Corrieu, G. (2004). The relative effect of milk base,
598 starter, and process on yoghurt texture: A review. *Critical Reviews in Food Science and*
599 *Nutrition*, 44, 113-137.
- 600 Sohrabvandi, S., Nematollahi, A., Mortazavian, A. M., & Vafae, R. (2013). Effect of
601 homogenisation pressure and sequence on textural and microstructural properties of milk
602 based creamy dessert. *Journal of Paramedical Sciences*, 4, 1-7.
- 603 Tamime, A. Y., & Robinson, R. K. (2007). *Yoghurt: Science and Technology* (3rd edn., Chapt. 2
604 and 5). Cambridge, UK: Woodhead Publishing.
- 605 Varnam, A. H., & Sutherland, J. P. (1994). *Milk and milk products: Technology, chemistry and*
606 *microbiology* (Chapt. 8). London, UK: Chapman and Hall.
- 607 Walstra, P. (1975). Effect of homogenisation on fat globule size distribution in milk.
608 *Netherlands Milk and Dairy Journal*, 29, 279-294.
- 609 Walstra, P., Geurts, T. J., Noomen, A., Jellema, A., & Boekel, M. A. J. S. (1999). *Dairy*
610 *technology: Principles of milk properties and processes* (Chapt. 8 and 20). New York,
611 NY, USA: Marcel Dekker.
- 612 Xiong, Y. L., & Kinsella, J. E. (1991). Influence of fat globule-membrane composition and fat
613 type on the rheological properties of milk based composite gels. 2. Results.
614 *Milchwissenschaft*, 46, 207-212.
- 615
616
617

618 **List of Figures and Figure legends**

619 **Figure 1.** Size distribution of fat globules in unhomogenised buffalo milk (control, ●) and in
620 buffalo milk homogenised at 80 bar (●) or 160 bar (○) obtained using light scattering. Each data
621 point is the average of six measurements (n=6) and the error bars are the standard deviation of
622 the mean.

623 **Figure 2.** Confocal laser scanning microscopy images of the microstructure of unhomogenised
624 buffalo milk (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f). The
625 images were captured using a 100x objective at 2x digital zoom (the first row) or 4x digital
626 zoom (the second row). The scale bars are 5 μm in length. Arrows in Figures 2d and 2f indicate
627 fat clusters induced by homogenisation. Please refer to the online edition for a colour version of
628 this figure.

629 **Figure 3.** Changes in pH (a), storage modulus (G') (b) and loss tangent ($\tan \delta$) (c) during the
630 fermentation of buffalo yoghurt produced from unhomogenised milk (control, ●), milk
631 homogenised at 80 bar (●) or milk homogenised at 160 bar (○). Each data point is the average
632 of six measurements (n=6) in Figure 3a and the average of two measurements (n=2) in Figures
633 3b and c. The error bars are the standard deviation of the mean.

634 **Figure 4.** Changes in the syneresis (a) and gel firmness (b) of buffalo yoghurt produced from
635 unhomogenised buffalo milk (control, ●), buffalo milk homogenised at 80 bar (●) or 160 bar (○)
636 during cold storage. Each data point is the average of six replicates (n=6). The error bars are the
637 standard deviation of the mean.

638 **Figure 5.** Changes in the stress-scaled hysteresis area (a), hysteresis area (b), consistency
639 coefficient (c) and flow behaviour index (d) of buffalo yoghurt produced from unhomogenised
640 buffalo milk (control, ●), buffalo milk homogenised at 80 bar (●) or 160 bar (○) during cold
641 storage. Each data point is the average of six replicates (n=6) and the error bars are the standard
642 deviation of the mean.

643 **Figure 6.** Microstructure of buffalo yoghurt produced from unhomogenised buffalo milk
644 (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f) at d 1 (upper images)
645 and d 28 (lower images) of storage as observed by cryo-scanning electron microscopy. Images

646 were captured using a solid state detector at 16000x magnification. The scale bars are 5 μm in
647 length.

648 **Figure 7.** Microstructure of buffalo yoghurt produced from unhomogenised buffalo milk
649 (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f) at d 1 (upper images)
650 and d 28 (lower images) of storage, as observed by confocal laser scanning microscopy. Images
651 were captured using a 63x objective. The scale bars are 10 μm in length. Arrows indicate the fat
652 cluster. Please refer to the online edition for a colour version of this figure.

653 **Figure 8.** Growth and viability of *Lactobacillus acidophilus* La-5 (a), *Streptococcus*
654 *thermophilus* (b) and *Bifidobacterium lactis* Bb-12 (c) during the fermentation and storage of
655 buffalo yoghurt produced from unhomogenised buffalo milk (control, ●), buffalo milk
656 homogenised at 80 bar (◐) or 160 bar (○). Each data point is the average of four replicates (n=4)
657 and the error bars are the standard deviation of the mean.

Table 1. Specific surface area and **volume-weighted mean diameter D[4,3]** of fat globules in homogenised and control (unhomogenised) buffalo milk determined using light scattering. Data are the mean \pm the standard deviation of the mean (n=6).

Parameters	Control	Milk homogenised at 80 bar	Milk homogenised at 160 bar
Specific surface area ($\text{m}^2 \text{g}^{-1} \text{fat}$)	1.6 ± 0.0^c	14 ± 0.3^b	20 ± 0.5^a
Diameter D[4,3] (μm)	5.3 ± 0.1^a	1.6 ± 0.0^b	1.0 ± 0.1^c

Significant differences ($P < 0.05$) between homogenised and unhomogenised buffalo milk samples are indicated by different superscripts ^{abc} in the same row.

Figure 1

[Click here to download high resolution image](#)

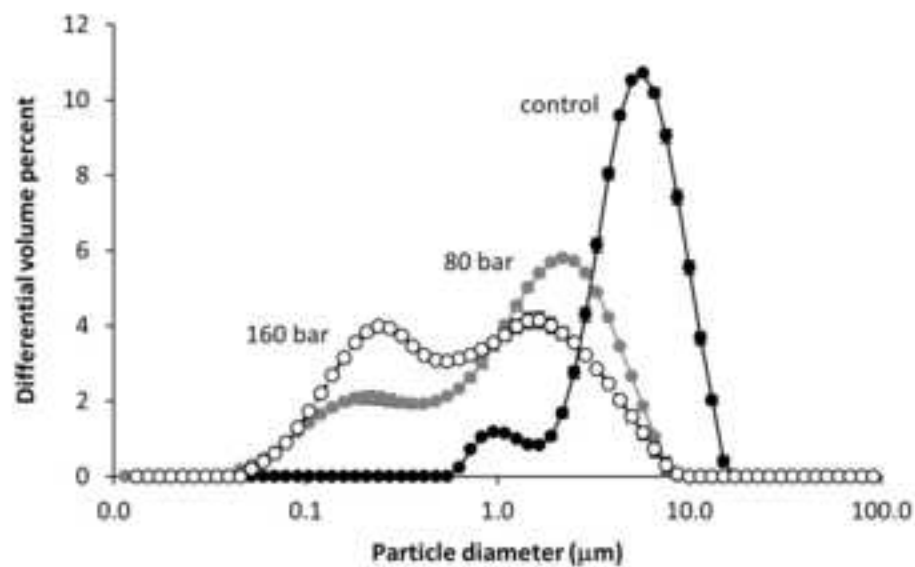


Figure 1. Size distribution of fat globules in unhomogenised buffalo milk (control, ●) and in buffalo milk homogenised at 80 bar (*) or 160 bar (○) obtained using light scattering. Each data point is the average of six measurements (n=6) and the error bars are the standard deviation of the mean.

Figure 2
[Click here to download high resolution image](#)

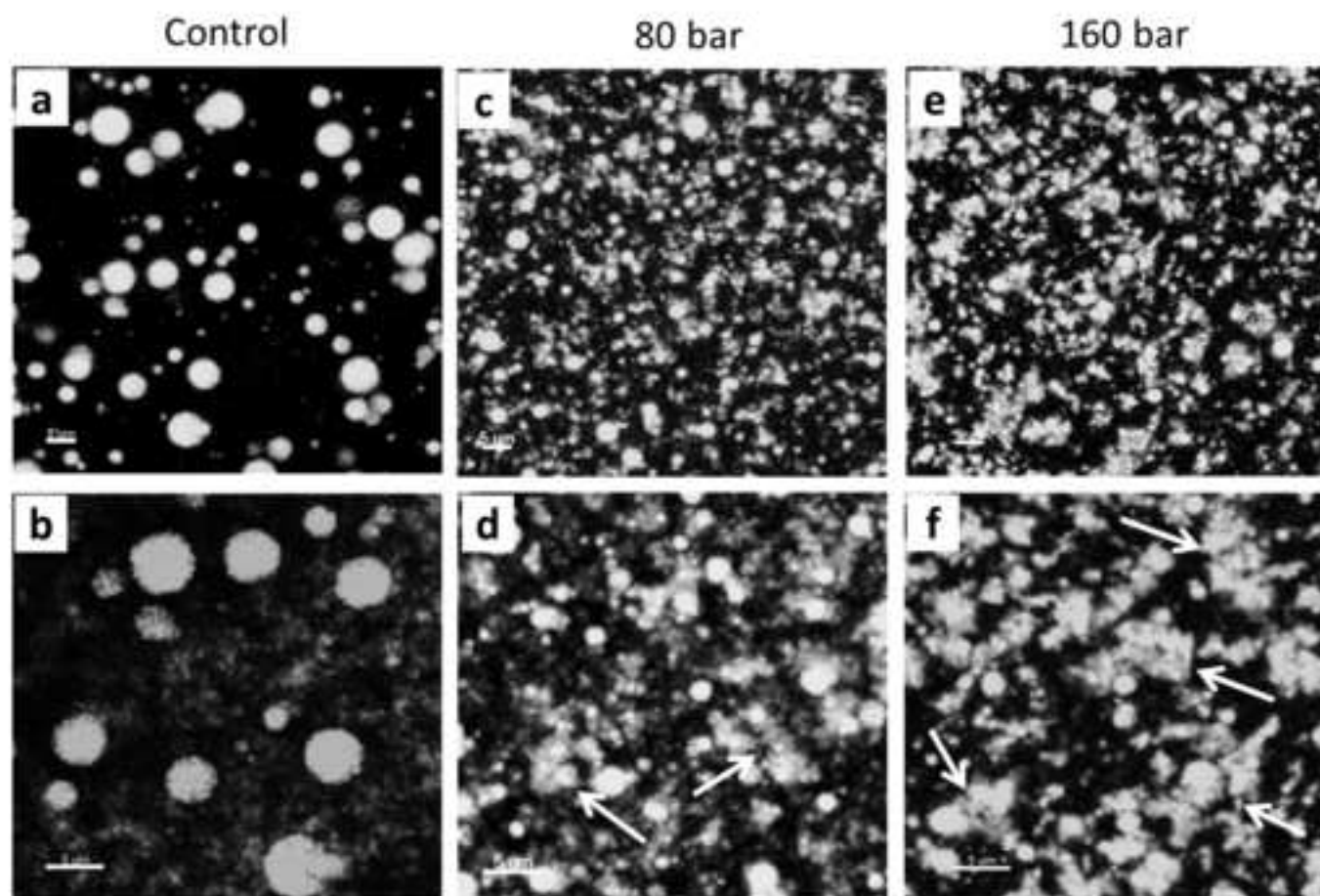


Figure 2. Confocal laser scanning microscopy images of the microstructure of unhomogenised buffalo milk (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f). The images were captured using a 100x objective at 2x digital zoom (the first row) or 4x digital zoom (the second row). The scale bars are 5 μm in length. Arrows in Figures 2d and 2f indicate fat clusters induced by homogenisation. Please refer to the online edition for a color version of this figure.

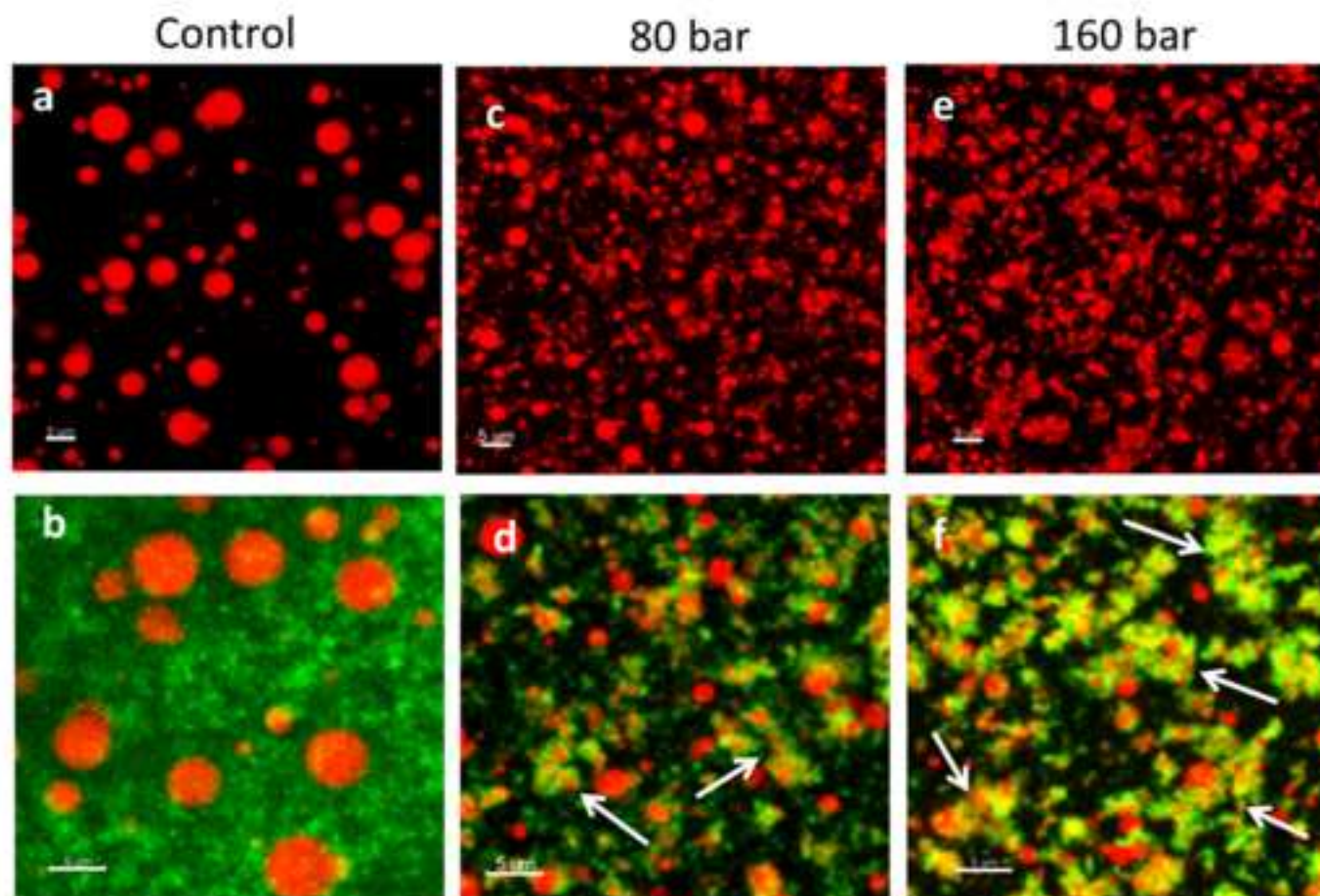


Figure 2 (online version). Confocal laser scanning microscopy images of the microstructure of unhomogenised buffalo milk (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f). Nile Red stained fat appears red, FCF stained protein appears green. The images were captured using a 100x objective at 2x digital zoom (the first and second row) or 4x digital zoom (the third row). The scale bars are 5 μm in length. Arrows in Figures 2d and 2f indicate fat clusters induced by homogenisation.

Figure 3
[Click here to download high resolution image](#)

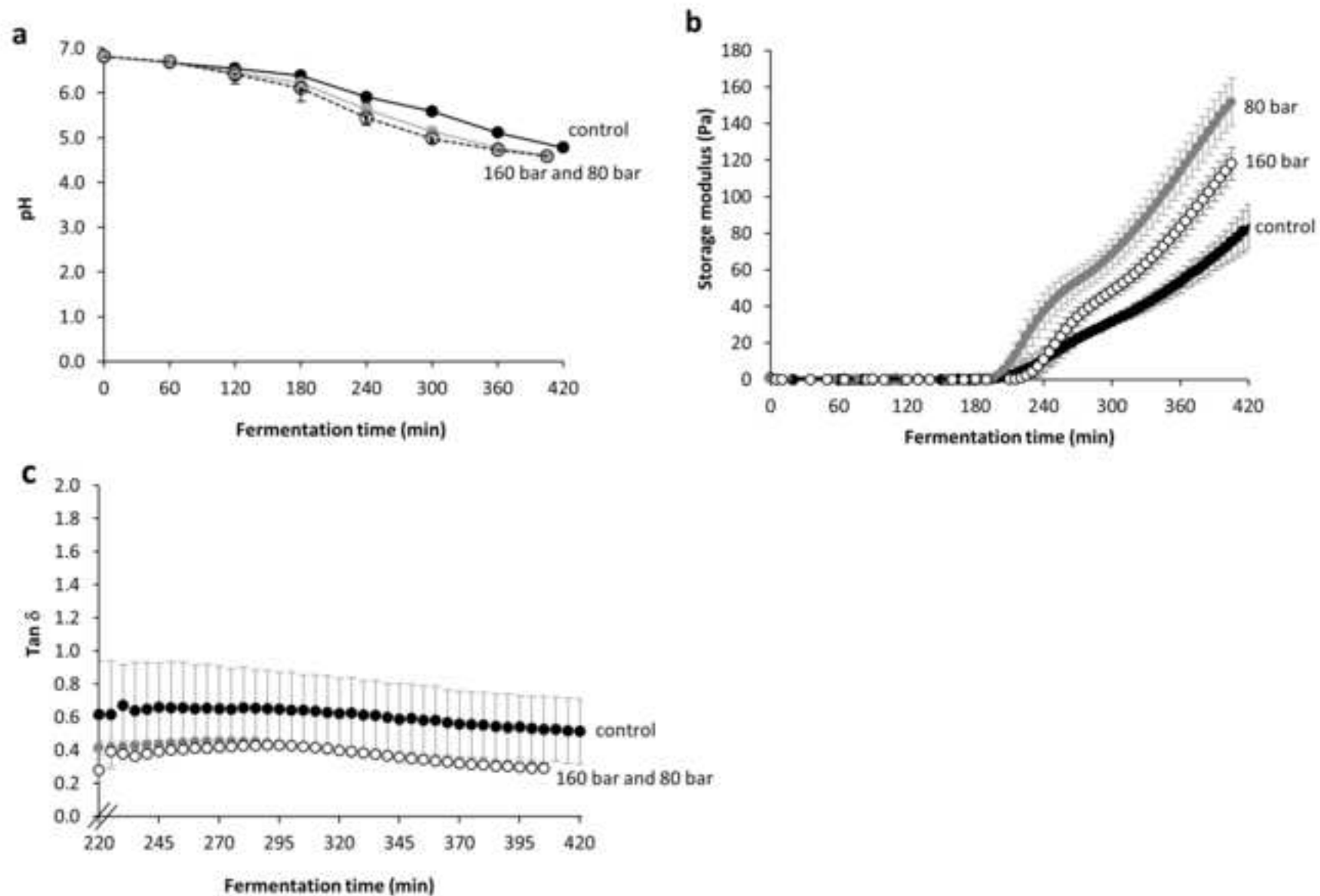


Figure 3. Changes in pH (a), storage modulus (G') (b) and loss tangent ($\tan \delta$) (c) during the fermentation of buffalo yoghurt produced from unhomogenised milk (control, ●), milk homogenised at 80 bar (*) or milk homogenised at 160 bar (○). Each data point is the average of six measurements ($n=6$) in Figure 3a and the average of two measurements ($n=2$) in Figures 3b and c. The error bars are the standard deviation of the mean.

Figure 4
[Click here to download high resolution image](#)

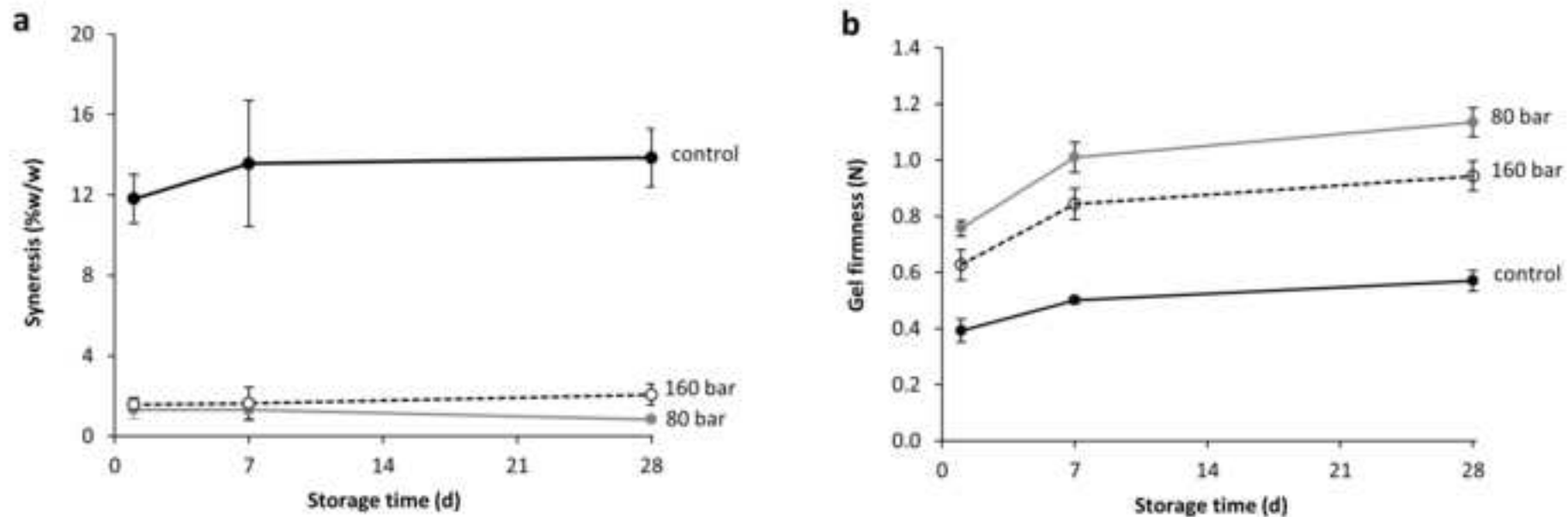


Figure 4. Changes in the syneresis (a) and gel firmness (b) of buffalo yoghurt produced from unhomogenised buffalo milk (control, ●), buffalo milk homogenised at 80 bar (*) or 160 bar (○) during cold storage. Each data point is the average of six replicates (n=6). The error bars are the standard deviation of the mean.

Figure 5
[Click here to download high resolution image](#)

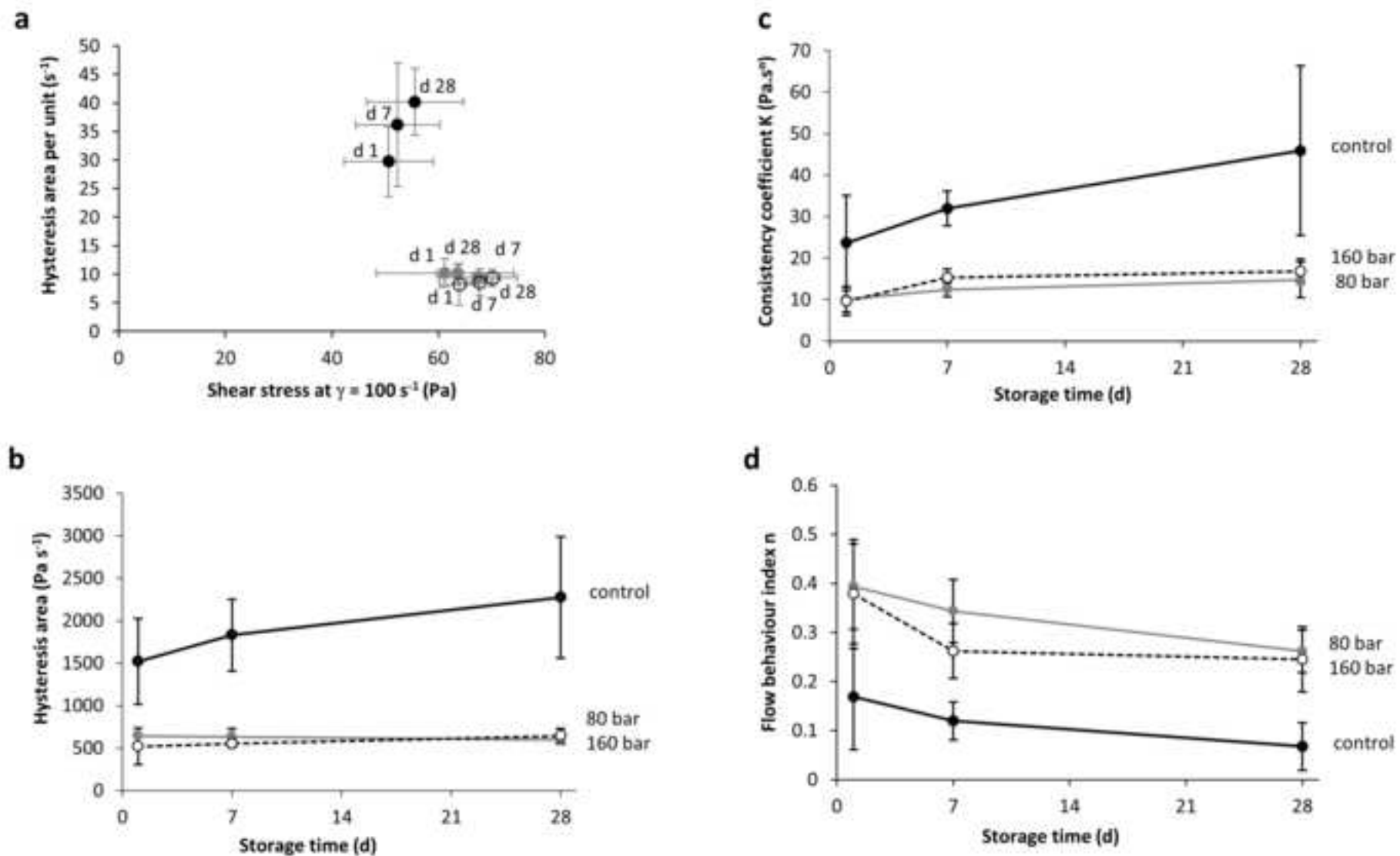


Figure 5. Changes in the stress-scaled hysteresis area (a), hysteresis area (b), consistency coefficient (c) and flow behaviour index (d) of buffalo yoghurt produced from unhomogenised buffalo milk (control, ●), buffalo milk homogenised at 80 bar (*) or 160 bar (○) during cold storage. Each data point is the average of six replicates (n=6) and the error bars are the standard deviation of the mean.

Figure 6

[Click here to download high resolution image](#)

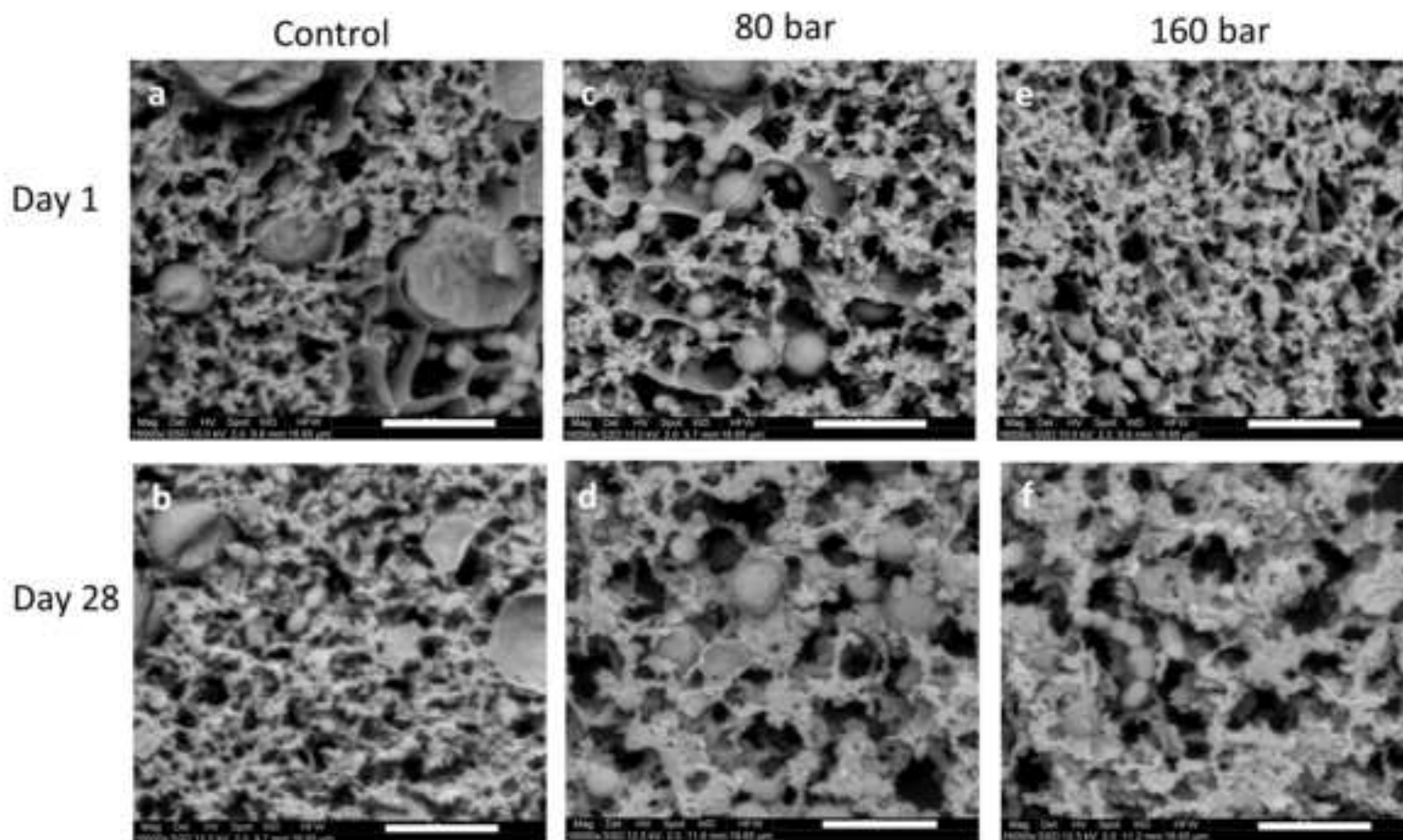


Figure 6. Microstructure of buffalo yoghurt produced from unhomogenised buffalo milk (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f) at d 1 (upper images) and d 28 (lower images) of storage as observed by cryo-scanning electron microscopy. Images were captured using a solid state detector at 16000x magnification. The scale bars are 5 μm in length.

Figure 7
[Click here to download high resolution image](#)

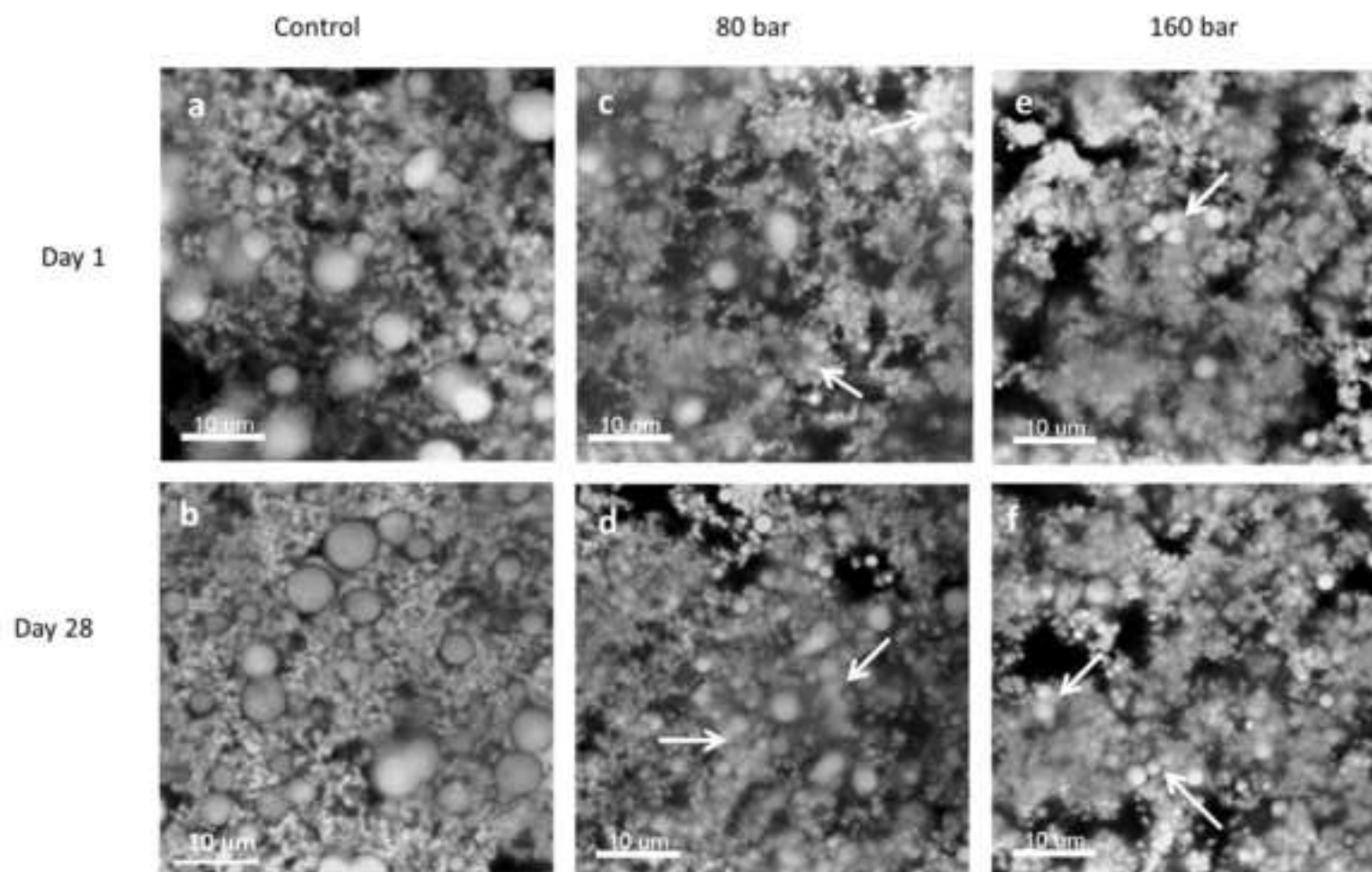


Figure 7. Microstructure of buffalo yoghurt produced from unhomogenised buffalo milk (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f) at d 1 (upper images) and d 28 (lower images) of storage, as observed by confocal laser scanning microscopy. Images were captured using a 63x objective. The scale bars are 10 µm in length. Arrows indicate the fat clusters induced by homogenisation. Please refer to the online edition for a color version of this figure.

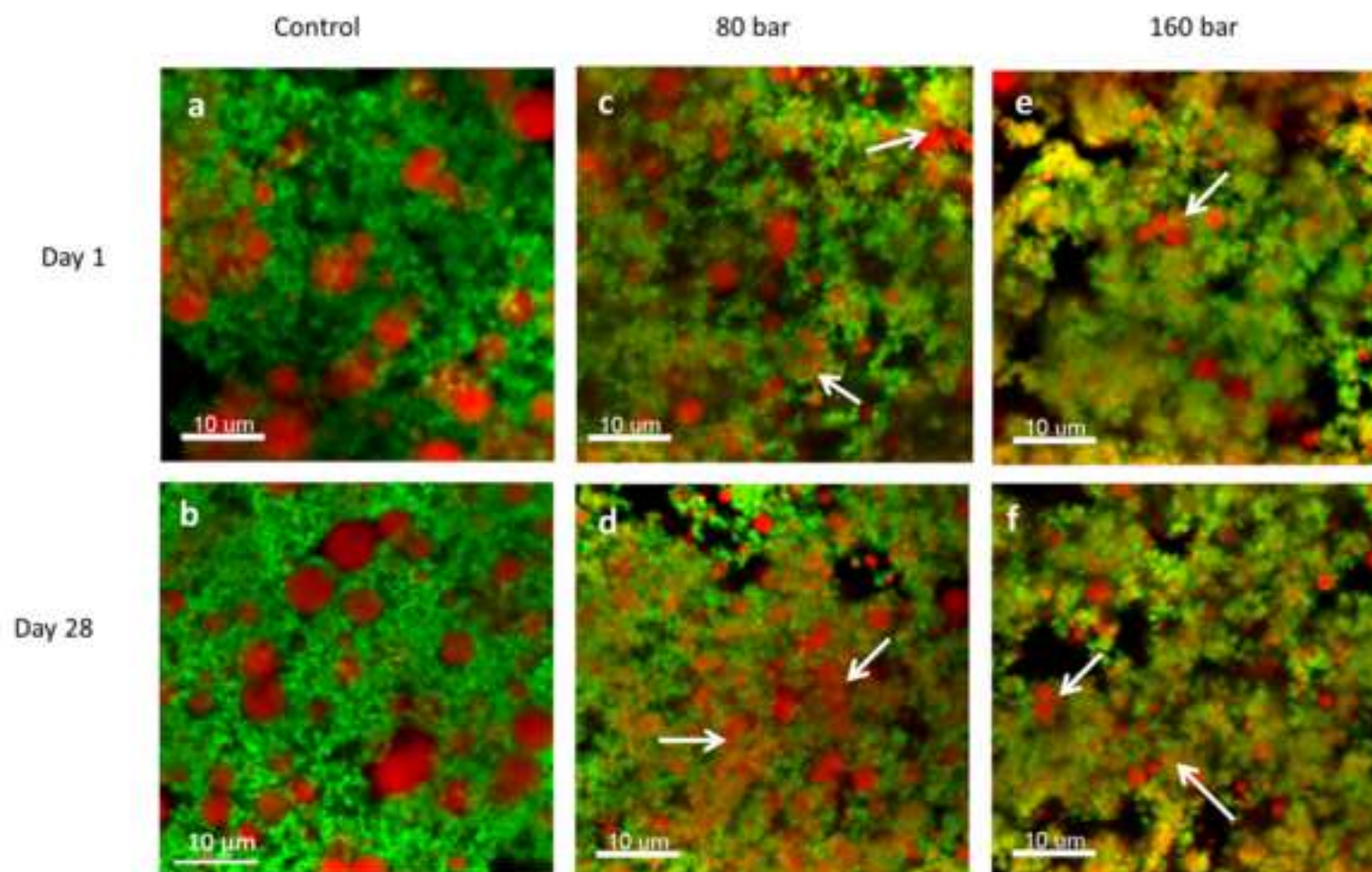


Figure 7 (online version). Microstructure of buffalo yoghurt produced from unhomogenised buffalo milk (control, a-b), buffalo milk homogenised at 80 bar (c-d) or 160 bar (e-f) at d 1 (upper images) and d 28 (lower images) of storage, as observed by CLSM. Nile Red stained fat appears red, FCF stained protein appears green and the black areas are serum pores. Images were captured using a 63x objective. The scale bars are 10 μm in length. Arrows indicate the fat clusters induced by homogenisation.

Figure 8
[Click here to download high resolution image](#)

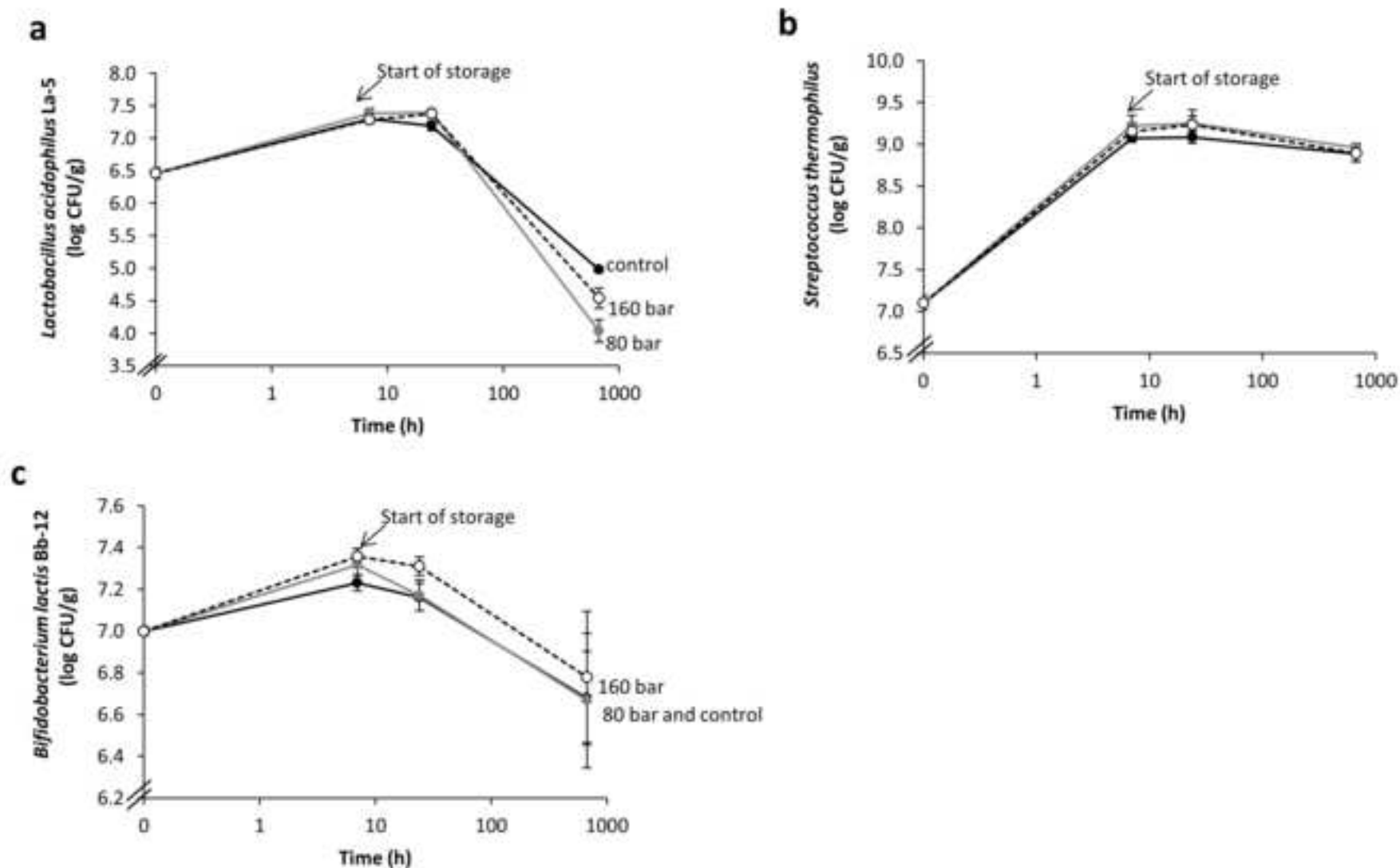
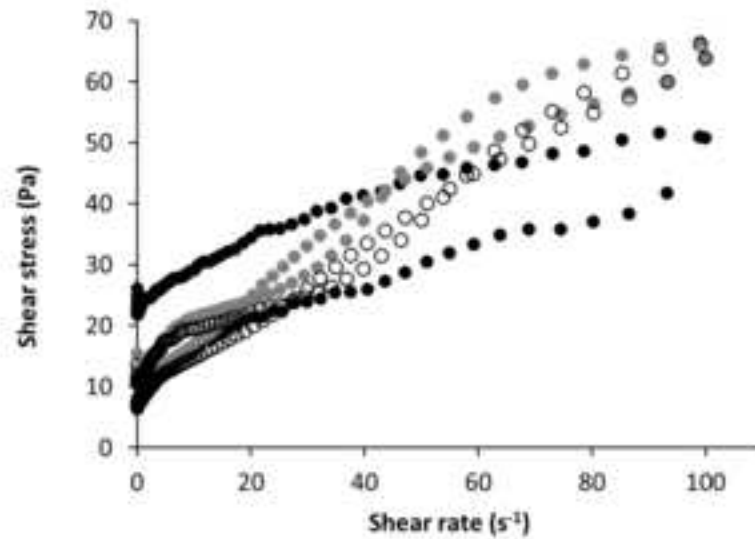


Figure 8. Growth and viability of *Lactobacillus acidophilus* La-5 (a), *Streptococcus thermophilus* (b) and *Bifidobacterium lactis* Bb-12 (c) during the fermentation and storage of buffalo yoghurt produced from unhomogenised buffalo milk (control, ●), buffalo milk homogenised at 80 bar (*) or 160 bar (○). Each data point is the average of four replicates (n=4) and the error bars are the standard deviation of the mean.



Supplementary A1. Flow curves at d 1 of storage of set style buffalo yoghurt produced from unhomogenised buffalo milk (control, ●), buffalo milk homogenised at 80 bar (◐) or 160 bar (○).