



Effect of pH adjustment on production and physicochemical properties of cheese feed made of Irish Cheddar cheese

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1 **Effect of pH adjustment on production and physicochemical properties of cheese feed**
2 **made of Irish Cheddar cheese**

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27 **ABSTRACT**

28

29 The effect of emulsification pH on the composition and colloidal stability of cheese feed (the
30 spray dryer feed of cheese powder) was investigated in the absence of emulsifying salts (ES)
31 to understand the sole effect of emulsification pH and to give insights into ES removal from
32 cheese powder production. Cheese feeds were prepared at six different pH values from 5.1 to
33 6.8. Adjustments of pH during feed production had a significant impact on the mass fraction
34 of several components (i.e., fat, calcium) between feed and retained mass (RM) obtained after
35 sieving. These differences were primarily attributed to electrostatic changes among proteins
36 during the emulsification process. pH-induced alterations in feed composition were
37 associated with a significant change in particle size, viscosity, and colloidal stability.
38 Confocal microscopy images of feeds revealed differences in protein aggregation and fat
39 cluster formations. Raman spectroscopy of RM indicated alterations in protein structure by
40 increased pH.

41

42 1. Introduction

43

44 Cheese powder is a multifunctional ingredient used in a wide range of food products
45 including baked foods, dressings, snacks, soups, and processed cheeses (Felix da Silva,
46 Ahrné, Larsen, Hougaard, & Ipsen, 2018a). The main steps of cheese powder production
47 include mincing the cheese, addition of other ingredients, mixing, emulsifying, and spray
48 drying (Písecký, 2005). Prior to spray drying process, the hot cheese emulsion obtained is
49 sieved to eliminate undissolved cheese mass from the spray dryer feed.

50 Some of the critical factors in the production of cheese powder are the stability and
51 viscosity of the spray dryer feed, which consists of minced natural cheeses, water, and other
52 additives such as emulsifying salts (ES) (Felix da Silva, Larsen, Hougaard, & Ipsen, 2017).
53 ES are ionic compounds consisting of polyvalent anions and monovalent cations (Chen &
54 Liu, 2012). In processed cheese production, phosphate- or citrate-based ES (i.e., trisodium
55 citrate, disodium phosphate) are widely used to adjust the pH and to improve the emulsifying
56 functionality of casein micelles (Deshwal, Gómez-Mascaraque, Fenelon, & Huppertz, 2023).
57 It is suggested that the ES disrupt the micellar structure of casein by calcium chelation and
58 make caseins available for interacting with fat (Guinee, 2003). In cheese powder production,
59 during the melting and emulsification process of the spray dryer feed (cheese feed), ES are
60 used as stabilising agents. However, the growing trend toward a reduction in sodium and
61 phosphate in food production raises the need for alternative ways of stabilising cheese feed
62 (Kelimu, Felix da Silva, Geng, Ipsen, & Hougaard, 2017).

63 pH is known to be a particularly important factor influencing the structure, rheology,
64 and functionality of dairy-based systems (Liu & Guo, 2008). Previous studies investigated the
65 effect of pH on milk, model casein systems, and cheese production, as well as cheese
66 structure (Fröhlich-Wyder, Guggisberg, & Wechsler, 2009; Liu & Guo, 2008; Ong,

67 Dagastine, Kentish, & Gras, 2012; Sinaga, Bansal, & Bhandari, 2017; Vaia, Smiddy, Kelly,
68 & Huppertz, 2006). Different properties such as casein hydration, colloidal calcium, and
69 electrostatic charges of proteins were shown to be affected by the manipulation of pH. These
70 studies have demonstrated that it is challenging to interpret the influence of pH on such
71 complex systems since many other interdependent chemical characteristics are affected
72 simultaneously (Liu & Guo, 2008). Thus, it is important to focus on a simple model system
73 without the influence of other additives such as ES to understand the sole effect of pH on
74 cheese feed preparation.

75 In the production of cheese powder, the pH of the feed is adjusted in the range of 5.8 to
76 6.2 together with the addition of ES to obtain the powder of desired quality (Felix da Silva et
77 al., 2017). Although several studies investigated the influence of different factors on cheese
78 feed quality (Felix da Silva, Hirschberg, Ahrné, Hougaard, & Ipsen, 2018b; Felix da Silva et
79 al., 2017; Kelimu et al., 2017), the fundamental questions regarding the effect of pH increase
80 on cheese feed production and resulting colloidal stability remain unanswered. Therefore, the
81 objective of this study was to determine the sole influence of the emulsification pH on the
82 compound retention (i.e., protein, fat, calcium) in the undissolved/retained mass (RM) of the
83 cheese and the colloidal stability of the resulting cheese feed. The results of this study are
84 expected to give an understanding of the role of pH adjustment in cheese feed preparation
85 and provide insights on how to reduce or eliminate emulsifying salts from cheese powder
86 production.

87

88 **2. Materials and methods**

89

90 *2.1. Preparation of cheese feeds*

91

92 The cheese feeds were prepared in triplicates at six different pH values (i.e., pH 5.1,
 93 pH 5.3, pH 5.5, pH 6.0, pH 6.5, and pH 6.8) using an Irish Cheddar-type cheese (by w/w:
 94 36.7% moisture, 28.8% protein, 31.3% fat, 0.79% calcium, 0.52% phosphate, ~ 3 months
 95 old). Cheeses were minced through 8 mm diameter holes of a stainless-steel plate attachment
 96 of mixing equipment (Bear Varimixer Teddy, Varimixer A/S, Broendby, Denmark). Each
 97 cheese feed was prepared using 500 g of minced cheese and the dry matter content was
 98 adjusted to 35% (w/w) with deionised (DI) water. A Stephan cooker with a heating jacket
 99 (Stephan UMC 5, Stephan Machinery GmbH, Hameln, Germany) was used to mix the
 100 minced cheese and the DI water for 20 min at 85 ± 1 °C with a blade speed of 1,000 rpm. The
 101 pH of the mixture was adjusted using 45% potassium hydroxide during the emulsification
 102 process in Stephan mixer at around 85 °C. The pH was measured using a 780 Metrohm pH
 103 meter (Metrohm, Herisau, Switzerland) during mid-process and in the end of emulsification
 104 to ensure no changes in feed pH occurred through the process. The hot mixture was then
 105 sieved through a stainless-steel sieve of 500 µm mesh size (J. Engelsmann AG,
 106 Ludvigshafen, Germany - DIN ISO: 3310), and the permeate is referred to as "cheese feed",
 107 while the undissolved fraction of cheese is referred to as "retained mass (RM)". Cheese feed
 108 prepared without pH treatment is referred to as "Control", which had a pH of 5.1.

109

110 2.2. Component retention and cheese feed yield

111

112 The retention of each component extracted from cheese, including fat, protein, and
 113 minerals in the RM fraction, was calculated using equation (1) (Guinee, O’Kennedy, &
 114 Kelly, 2006):

$$115 \text{Component retention}\% = \frac{g_{RM} \times \text{component}\%_{RM}}{g_{cheese} \times \text{component}\%_{cheese}} \quad (1)$$

116 The cheese feed yield of each treatment was calculated as shown in equation (2) (Ong
117 et al., 2012):

$$118 \quad \text{Yield\%} = \frac{g_{\text{cheese feed}}}{g_{\text{cheese}} + g_{\text{water}}} \times 100 \quad (2)$$

119

120 2.3. *Compositional analysis*

121

122 2.3.1. *Determination of total solids, crude protein, and fat*

123 For all samples (cheese, RM, and cheese feed), total solids content was determined
124 using a microwave moisture analyser (SMART 6 ProFat, CEM Corporation, Matthews, NC,
125 USA), where approximately 2 g of sample was weighed onto a CEM glass fibre pad (CEM
126 Corporation) and subjected to 105 °C until constant weight. After moisture analysis, the CEM
127 pad (CEM Corporation) containing the sample was analysed for the fat content with an NMR
128 fat analyser (ORACLE, CEM Corporation). Total nitrogen content was measured using the
129 Dumas method with a Rapid MAX N exceed[®] analyser (Elementar Analyse systems GmbH,
130 Hanau, Germany) using a factor of 6.38 to calculate the crude protein.

131

132 2.3.2. *Determination of total Ca and P*

133 The total content of Ca and P in the samples was determined by inductively coupled
134 plasma mass spectrometry (ICP-MS) (8900 ICP-QQQ, Agilent Technologies, Santa Clara,
135 CA, USA). Test positions of 0.3 g for solid and 1 g for liquid samples were placed in quartz
136 vessel tubes and digested in a microwave-assisted digestion system (Multiwave 7000, Anton
137 Paar, Graz, Austria) using concentrated nitric acid (SPS Science, France) (Forghani et al.,
138 2021). Certified calcium and phosphorus stock solutions were used as external calibration
139 standards for quantification, and rhodium was used as the internal standard (all SPS

140 sciences). Moreover, a certified reference material (DORM-4, NRCC, Ottawa, Canada) was
141 analysed along with the samples to verify analytical performance.

142

143 2.4. *Cheese feed stability measurements*

144

145 2.4.1. *Rheology*

146 The apparent viscosity of cheese feeds was measured using a controlled-stress
147 rheometer (DHR-2, TA Instruments, Hullhorst, Germany) equipped with a Peltier Concentric
148 Cylinder Temperature System. A DIN standard concentric cylinder geometry (cup diameter:
149 30.4 mm, bob diameter: 28 mm, bob length: 42.09 mm) was used for the measurements and
150 the temperature was set to 50 °C, which was the temperature of the sample at the time of the
151 measurement. The shear rate ranged from 1 s⁻¹ to 200 s⁻¹, where five equilibrium points were
152 collected for each data point.

153

154 2.4.2. *Particle size distribution*

155 The particle size distribution (PSD) of the cheese feed samples was determined using a
156 laser diffraction instrument (Mastersizer 2000, Malvern Panalytical, Malvern, UK). The
157 refractive indices of the dispersed phase and the continuous phase were set to 1.49 and 1.33,
158 respectively. Drops of cheese feed were added to the dispersant unit until a final obscuration
159 rate of between 10 and 12% was reached. Particle size was reported as volume-weighted
160 mean diameter ($D[4,3] = \sum n_i d_i^4 \div \sum n_i d_i^3$), where n_i is the number of particles with a
161 diameter of d_i . (Malvern Instruments, 2015).

162

163 2.4.3. *Optical characterisation of colloidal stability*

164 The colloidal stability of cheese feeds was evaluated with a Turbiscan[®] Tower
165 (Formulacion, Toulouse, France), which operates with the static multiple light scattering (S-
166 MLS) principles using an infrared light source at 880 nm. The detectors collect backscattered
167 (BS) and transmitted (T) signals during scanning of the sample from bottom to top. The type
168 of destabilisation behaviour (e.g., creaming, sedimentation, coalescence) can be detected by
169 the delta BS graph obtained by the detectors. The peak thickness value indicates the height of
170 the creaming layer (mm) at a fixed sample height during the Turbiscan analysis and can give
171 information regarding the migration kinetics of small particles. The migration rate of the
172 particles was calculated based on the slope of the linear region of a peak thickness-time plot
173 using Towersoft software (Huck-Iriart, Pizones Ruiz-Henestrosa, Candal, & Herrera, 2013).
174 The migration rate is reported as mm h⁻¹. The measurements were performed immediately
175 after the preparation of the cheese feed and the destabilisation kinetics were measured every 2
176 min for 2 h at 70 °C, which was the temperature of the sample at the time of the
177 measurement.

178

179 *2.4.4. Microstructure evaluation of cheese feed*

180 The microstructure of the cheese feeds was evaluated using confocal laser scanning
181 microscopy (CLSM). The samples were imaged using a 40× lens (Nikon Apo LWD water
182 40× NA 1.15) on a spinning disc confocal microscope, which consists of an inverted
183 microscope (Nikon Ti2) equipped with a laser source (405 / 488 / 561 / 640 nm), a confocal
184 spinning disc module (Yokogawa CSU-W1, 50 µm pinholes), a quad-band emission filter
185 (440 / 521 / 607 / 700 nm) and an sCMOS camera (Photometrics Prime95B). Nile red
186 (0.01%, w/v; Sigma-Aldrich Denmark A/S, Søborg, Denmark) and FCF fast Green (0.001%,
187 w/v; Sigma-Aldrich Denmark A/S) were used to stain fat clusters and proteins, respectively
188 (Felix da Silva et al., 2021). The excitation wavelengths for FCF fast green and Nile red were

189 640 nm and 561 nm, respectively, while the emission wavelengths were 700 nm and 607 nm,
190 respectively.

191

192 2.5. *Raman spectra of retained mass*

193

194 The Raman spectroscopy measurements of RM samples were performed using DXR 3
195 Raman microscope (Thermo Fischer Scientific, Waltham, MA, USA) in the range 200 to
196 3500 cm^{-1} . A laser with an excitation wavelength of 532 nm was used, where laser power was
197 8 mW. The aperture was 25 μm , where samples were imaged using a 10 \times objective (Olympus
198 MPlan 10 \times /0.25 BD). The grating was set to 900 lines mm^{-1} to record Raman spectra over the
199 focalised area. All Raman spectra were collected and treated with OMNIC spectra software
200 (Thermo Fischer Scientific).

201

202 2.6. *Statistical analysis*

203

204 All analyses were performed in triplicate measurements, and the results are expressed
205 as mean \pm standard deviation. One-way analysis of variance (ANOVA) and Tukey's paired
206 comparison test were applied to evaluate the differences between means. A significance level
207 of $\alpha = 0.05$ was used for all analyses.

208

209 3. **Results and discussion**

210

211 3.1. *Effect of pH on component retention in cheese*

212

213 The highest solid retention in RM was observed in the cheese feeds prepared at pH
214 values from 5.3 to 6.0, whereas a further increase in the emulsification pH led to a decrease
215 (Table 1). Although control treatment resulted in a higher amount of proteins passing to the
216 cheese feed, more than 90% of proteins from the cheese remained in the RM for all
217 treatments. The most pronounced change due to the pH increase was observed in fat
218 retention, which appeared to have the dominant influence on overall solids retained. Ca
219 retention was increased as the emulsification pH was increased, whereas P retention did not
220 show a statistically significant difference with the different treatments. This can be an
221 indication of decreased solubilisation of colloidal calcium (CCP) with increasing pH, which
222 affects the protein hydration and cheese disintegration during the mixing process (Guinee &
223 O’Kennedy, 2012; Joshi, Muthukumarappan, & Dave, 2004). Thus, the lower component
224 retention and the higher yield (Table 1) for the control treatment could be explained by the
225 higher solubility of CCP due to the lower pH of the emulsification.

226 Liu and Guo (2008) reported that casein micelles in a pH range of 6 to 12 became
227 more negatively charged and further repelled each other by stronger electrostatic repulsion as
228 the pH increased. Consequently, the casein structure became looser and the casein size
229 increased with increasing pH (Liu & Guo, 2008). Likewise, an increase in net negative
230 charge due to increased pH also seemed to influence the compound retention in RM
231 regarding the cheese feeds prepared at higher pH values, especially after pH 6.0. Moreover,
232 adjusting the emulsification pH to higher values can cause calcium phosphate precipitation
233 (Lucey & Fox, 1993). It was previously reported that increasing the pH of milk changes the
234 ionisation state of HPO_4^{2-} to PO_4^{3-} , which has a greater calcium affinity. Consequently, the
235 concentration of ionic calcium and inorganic phosphate in the aqueous phase of milk was
236 decreased and calcium phosphate salt precipitation was observed (Ahmad, Piot, Rousseau,
237 Grongnet, & Gaucheron, 2009). In addition, earlier studies suggested that alkaline-

238 precipitated calcium phosphate might become a part of CCP nanoclusters resulting in growth
239 in number or size (Vaia et al., 2006).

240 These results show that changing the pH during cheese feed preparation leads to
241 alterations in the amount of extracted compounds from cheese, which is an important factor
242 regarding the colloidal stability of the feed. These changes seem to be affected by different
243 factors in different ranges of pH treatment. The increased electrostatic charges can be the
244 main responsible for alterations at higher pH values, whereas amount of CCP nanoclusters
245 (newly formed or retained) could be another significant factor affecting the protein
246 functionality during emulsification. In addition, considerable changes in the fat release into
247 cheese feed may be an indication of pH-induced changes in fat-protein interactions. At pH
248 values from 5.3 to 6.0, the CCP that were precipitated or retained can be the reason keeping
249 the (para)casein structure intact and resulting in reduced fat release from cheese into the feed
250 during emulsification. Despite the increased calcium phosphate precipitation at increased pH,
251 the combined effect of increased net electric charge at pH 6.5 and 6.8 as well as freed thiol
252 groups (Fig. 4a) could have led to a loosened structure at higher emulsification values and
253 thereby, increased fat release.

254

255 3.2. *Effect of pH on cheese feed stability*

256

257 All cheese feeds showed Newtonian fluid behaviour, which means the viscosity of the
258 samples were not affected by the applied shear rate (Steffe, 1996). The viscosity values of
259 cheese feeds ranged from 0.8 to 1.6 mPa s (Fig. 1a). A decrease in viscosity was observed
260 when the emulsification pH was increased up to 6.0 and a further increase in pH led to an
261 increase in viscosity, where the viscosity value at pH 6.8 was as high as the control sample.

262 The viscosity results can be correlated with the total solid content, where a higher amount of
263 solids led to a higher viscosity due to increased intermolecular interactions.

264 Additionally, emulsion rheology is often affected by particle size distribution.
265 Increased droplet aggregation or concentration, which is influenced by fat content, is known
266 to increase the viscosity of food emulsions (McClements, 2015). In this study, particle size of
267 the cheese feeds became smaller until pH 6.0 and larger again as the pH was increased up to
268 6.8 (Fig. 1b). The D [4,3] values of the samples were 10.2 ± 0.4 , 5.7 ± 0.5 , 5.0 ± 0.4 , $5.8 \pm$
269 0.7 , 12.3 ± 1.1 , and 13.4 ± 2.5 μm for control, pH 5.3, pH 5.5, pH 6.0, pH 6.5, and pH 6.8
270 samples, respectively. The highest span (width of distribution) was seen at treatments of pH
271 6.5 and 6.8 (Fig. 1b), which indicates the heterogeneity of the droplet size distribution.
272 Moreover, these two treatments resulted in the highest particle size values followed by the
273 control sample. A positive correlation was observed between viscosity and particle size
274 values (Pearson correlation coefficient: 0.83). Since the protein content of the feeds was less
275 than 5% (w/w), it could be concluded that the viscosity of the cheese feeds is primarily
276 modulated by the fat content as well as pH-induced changes in protein functionality.

277 All samples showed a destabilisation by creaming over time regardless of the treatment
278 (Fig. 2). The thickness of the creaming layer (peak thickness) varied primarily depending on
279 the fat content of the feeds. The maximum peak thickness was observed in the control sample
280 while minimum values were found for pH treatments ranging from pH 5.3 to 6.0. The control
281 feed had a similar fat content as the pH 6.8 treated sample, yet the peak thickness was much
282 higher than at pH 6.8. Therefore, it is suggested that another factor affecting the peak height
283 can be the interaction between fat and protein, which influences the density of the dispersed
284 phase and, consequently, the migration rate. The migration rate of the droplets is important
285 for understanding the destabilisation kinetics. It can be affected by various factors such as the
286 viscosity of the continuous phase, fat-to-protein ratio, droplet size, and differences in

287 continuous and dispersed phase density (McClements, 2015; Tomas, Paquet, Courthaudon, &
288 Lorient, 1994). A higher viscosity slows down the droplet movement whereas a bigger
289 droplet size and phase density difference increase the speed of destabilisation.

290 The results showed that the migration rate of the droplets exerted a tendency to
291 increase with increasing the emulsification pH (Table 2). Even though the control sample had
292 similar viscosity and fat-to-protein ratio (Table 2) as with pH 6.8, it had a slower migration
293 rate. This can be explained by the combined impact of the increased electrostatic charges at
294 higher pH and consequent loosened casein structure leading to the higher fat content in
295 cheese feed (see Section 3.1) (Liu & Guo, 2008). Furthermore, samples having smaller
296 droplet sizes showed a similar or faster migration rate in comparison with the control sample.
297 Therefore, it is concluded that the combined effect of cheese feed viscosity, particle size, and
298 protein functionality have a profound influence on the destabilisation kinetics in cheese feeds.

300 3.3. *Microstructure of cheese feeds*

301
302 The microstructure of cheese feeds showed noteworthy differences depending on the
303 preparation pH (Fig. 3). The control sample had the highest amount of protein aggregates,
304 which were partially surrounded by clusters of small fat globules. The presence of bigger
305 aggregates can be related to the lower pH of the control sample compared with other
306 treatments. It is well-known that aggregation of proteins is more likely when the pH is closer
307 to the isoelectric point of caseins at pH 4.6 due to charge neutralisation (Tomas et al., 1994).
308 The sample produced at pH 5.3 exhibited a more profound interaction between fat clusters
309 and proteins, which can be in favour of the emulsification properties of caseins. At pH 5.5
310 and 6.0, the fat droplets were rather small, whereas larger free fat clusters were observed at
311 higher pH treatments. This is in agreement with the particle size results where pH 6.5 and 6.8

312 treatments showed the highest span and their PSD graphs were shifted to the right, indicating
313 the presence of bigger droplets. Moreover, the presence of free fat clusters leads to faster
314 destabilisation, which was shown in Table 2.

315

316 3.4. Raman spectra of retained mass

317

318 Fig. 4a illustrates the whole Raman spectral range, where considerable differences
319 were seen in the area under the curve in the S–H stretch region (thiol groups) (2550–2850 cm⁻¹)
320 (Li-Chan, Ismail, Sedman, & van de Voort, 2006). The overall increased intensity with
321 increased pH in the thiol region could be a result of increased thiol activity due to decreased
322 heat stability of beta-lactoglobulin at pH values higher than 6.0 (Wong, 1988). The authors
323 also mentioned that freed thiol groups can cause aggregation of proteins due to
324 conformational changes, which could be linked to the increased fat release into cheese feed at
325 higher emulsification pH values. Fig. 4b shows the region with abundant structural
326 information. The band shows protein amide regions at 1270–1300 (Amide III) and 1655 cm⁻¹
327 (α -helix segments of casein), while CH₂ groups bending at 1440 cm⁻¹ (Schulz, 2018).
328 Additionally, the band at 1002 cm⁻¹ is characteristic of phenylalanine ring breathing (Smith,
329 Holroyd, Reid, & Gordon, 2017). The Raman intensity of all regions tended to increase with
330 higher pH treatments, where the highest intensities were observed at pH 6.5 and 6.8. Thus,
331 alteration in pH during the emulsification step led to changes in protein structure, which
332 might have influenced the extracted components and colloidal stability.

333

334 4. Conclusions

335

336 For the first time, a systematic study on cheese feed preparation for an improved
337 understanding of the behaviour of ES-free cheese feed over a wide range of emulsification
338 pH values has been conducted. Interfering additives such as ES were excluded from the study
339 for a better interpretation of the sole impact of emulsification pH during the feed production.

340 Adjustment of emulsification pH during the preparation of ES-free cheese feeds
341 significantly affected the compounds recovered from cheese into the cheese feed including fat
342 and calcium. Additionally, the interactions between fat and protein during the emulsification
343 process are significantly affected by adjustments in pH. The differences were mainly
344 associated with electrostatic alterations among the proteins as well as the formation of CCP
345 nanoclusters throughout the emulsification process. An increase in emulsification pH
346 significantly influenced the viscosity, particle size, and colloidal stability of the resulting
347 cheese feed. Moreover, Raman spectroscopy of RM samples showed that emulsification pH
348 adjustments altered the protein structure including the sulphhydryl groups. It should be noted
349 that increasing the emulsification pH in the absence of ES led to a reduction in feed yield,
350 thus limiting the viability of this approach in production.

351 The findings of this study contribute to a better understanding of the role of pH during
352 the emulsification process of cheese feed and thereby can give insights into new approaches
353 to eliminate the need for ES in cheese powder production.

354

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356

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364

365 **References**

366

367 Ahmad, S., Piot, M., Rousseau, F., Grongnet, J. F., & Gaucheron, F. (2009). Physico-
368 chemical changes in casein micelles of buffalo and cow milks as a function of
369 alkalisation. *Dairy Science & Technology*, *89*, 387–403.

370 Chen, L., & Liu, H. (2012). Effect of emulsifying salts on the physicochemical properties of
371 processed cheese made from Mozzarella. *Journal of Dairy Science*, *95*, 4823–4830.

372 Deshwal, G. K., Gómez-Mascaraque, L. G., Fenelon, M., & Huppertz, T. (2023). A review
373 on the effect of calcium sequestering salts on casein micelles: from model milk
374 protein systems to processed cheese. *Molecules*, *28*, Article 2085.

375 Felix da Silva, D., Ahrné, L., Larsen, F. H., Hougaard, A. B., & Ipsen, R. (2018). Physical
376 and functional properties of cheese powders affected by sweet whey powder addition
377 before or after spray drying. *Powder Technology*, *323*, 139–148.

378 Felix da Silva, D., Hirschberg, C., Ahrné, L., Hougaard, A. B., & Ipsen, R. (2018). Cheese
379 feed to powder: Effects of cheese age, added dairy ingredients and spray drying
380 temperature on properties of cheese powders. *Journal of Food Engineering*, *237*, 215–
381 225.

382 Felix da Silva, D., Larsen, F. H., Hougaard, A. B., & Ipsen, R. (2017). The influence of raw
383 material, added emulsifying salt and spray drying on cheese powder structure and
384 hydration properties. *International Dairy Journal*, *74*, 27–38.

- 385 Felix da Silva, D., Wang, H., Czaja, T. P., van den Berg, F., Kirkensgaard, J. J. K., Ipsen, R.,
386 et al. (2021). Effects of homogenization and pH adjustment of cheese feed without
387 emulsifying salt on the physical properties of high fat cheese powder. *Powder*
388 *Technology*, 378, 227–236.
- 389 Forghani, B., Sørensen, A.-D. M., Fredeus, G., Skaaning, K., Johannesson, J., Sloth, J. J., et
390 al. (2021). Biochemical characterization and storage stability of process waters from
391 industrial shrimp production. *ACS Omega*, 6, 30960–30970.
- 392 Fröhlich-Wyder, M.-T., Guggisberg, D., & Wechsler, D. (2009). Influence of low calcium
393 and low pH on melting characteristics of model Raclette cheese. *Dairy Science &*
394 *Technology*, 89, 463–483.
- 395 Guinee, T. P. (2003). Role of protein in cheese and cheese products. In P. F. Fox & P. L. H.
396 McSweeney (Eds.), *Advanced dairy chemistry. Vol. 1. Proteins* (3rd edn., pp. 1083–
397 1174). Boston, MA, USA: Springer US.
- 398 Guinee, T. P., & O’Kennedy, B. T. (2012). Reducing the level of added disodium phosphate
399 alters the chemical and physical properties of processed cheese. *Dairy Science &*
400 *Technology*, 92, 469–486.
- 401 Guinee, T. P., O’Kennedy, B. T., & Kelly, P. M. (2006). Effect of milk protein
402 standardization using different methods on the composition and yields of cheddar
403 cheese. *Journal of Dairy Science*, 89, 468–482.
- 404 Huck-Iriart, C., Pizones Ruiz-Henestrosa, V. M., Candal, R. J., & Herrera, M. L. (2013).
405 Effect of Aqueous phase composition on stability of sodium caseinate/sunflower oil
406 emulsions. *Food and Bioprocess Technology*, 6, 2406–2418.
- 407 Joshi, N. S., Muthukumarappan, K., & Dave, R. I. (2004). Effect of calcium on
408 microstructure and meltability of part skim mozzarella cheese. *Journal of Dairy*
409 *Science*, 87, 1975–1985.

- 410 Kelimu, A., Felix da Silva, D., Geng, X., Ipsen, R., & Hougaard, A. B. (2017). Effects of
411 different dairy ingredients on the rheological behaviour and stability of hot cheese
412 emulsions. *International Dairy Journal*, *71*, 35–42.
- 413 Li-Chan, E. C. Y., Ismail, A. A., Sedman, J., & van de Voort, F. R. (2006). Vibrational
414 spectroscopy of food and food products. In J. M. Chalmers & P. R. Griffiths (Eds.),
415 *Handbook of vibrational spectroscopy* (1st edn.). Cambridge, UK: Wiley.
- 416 Liu, Y., & Guo, R. (2008). pH-dependent structures and properties of casein micelles.
417 *Biophysical Chemistry*, *136*, 67–73.
- 418 Lucey, J. A., & Fox, P. F. (1993). Importance of calcium and phosphate in cheese
419 manufacture: A review. *Journal of Dairy Science*, *76*, 1714–1724.
- 420 Malvern Instruments. (2015). *A basic guide to particle characterization*. Retrieved February
421 17, 2023, from [https://www.malvernpanalytical.com/en/learn/knowledge-](https://www.malvernpanalytical.com/en/learn/knowledge-center/whitepapers/wp120620basicguidepartchar)
422 [center/whitepapers/wp120620basicguidepartchar](https://www.malvernpanalytical.com/en/learn/knowledge-center/whitepapers/wp120620basicguidepartchar)
- 423 McClements, D. J. (2015). *Food emulsions: Principles, practices, and techniques* (3rd ed.).
424 Boca Raton, FL, USA: CRC Press.
- 425 Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2012). The effect of pH at renneting
426 on the microstructure, composition and texture of Cheddar cheese. *Food Research*
427 *International*, *48*, 119–130.
- 428 Písecký, J. (2005). Spray drying in the cheese industry. *International Dairy Journal*, *15*, 531–
429 536.
- 430 Schulz, H. (2018). Spectroscopic technique: Raman spectroscopy. In D-W Sun (Ed.),
431 *Modern techniques for food authentication* (pp. 139–191). London, UK: Academic
432 Press.

- 433 Sinaga, H., Bansal, N., & Bhandari, B. (2017). Effects of milk pH alteration on casein micelle
434 size and gelation properties of milk. *International Journal of Food Properties*, 20,
435 179–197.
- 436 Smith, G. P. S., Holroyd, S. E., Reid, D. C. W., & Gordon, K. C. (2017). Raman imaging
437 processed cheese and its components. *Journal of Raman Spectroscopy*, 48, 374–383.
- 438 Steffe, J. F. (1996). *Rheological methods in food process engineering*. East Lansing, MI,
439 USA: Freeman Press.
- 440 Tomas, A., Paquet, D., Courthaudon, J.-L., & Lorient, D. (1994). Effect of fat and protein
441 contents on droplet size and surface protein coverage in dairy emulsions. *Journal of*
442 *Dairy Science*, 77, 413–417.
- 443 Vaia, B., Smiddy, M. A., Kelly, A. L., & Huppertz, T. (2006). Solvent-mediated disruption of
444 bovine casein micelles at alkaline pH. *Journal of Agricultural and Food Chemistry*,
445 54, 8288–8293.
- 446 Wong, N. P. (Ed.). (1988). *Fundamentals of dairy chemistry* (3rd edn.). New York, NY,
447 USA: Van Nostrand Reinhold Co.
- 448

Table 1

Cheese feed yield and retention of components from cheese in the retained mass fraction after sieving. ^a

| Treatment | Retention in the retained mass fraction (%) | | | | | Feed yield (%) |
|------------------|---|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|
| | Solids | Protein | Fat | Ca | P | |
| Control (pH 5.1) | 73.3 ± 3.3 ^c | 92.2 ± 0.1 ^b | 59.9 ± 7.1 ^d | 87.6 ± 1.6 ^b | 96.7 ± 1.0 ^a | 48.5 ± 1.7 ^a |
| pH 5.3 | 89.8 ± 0.9 ^a | 93.4 ± 0.4 ^a | 92.1 ± 2.2 ^{ab} | 88.3 ± 0.9 ^b | 96.0 ± 1.0 ^a | 37.0 ± 2.5 ^b |
| pH 5.5 | 92.1 ± 1.2 ^a | 93.4 ± 0.6 ^a | 96.9 ± 1.5 ^a | 94.1 ± 2.5 ^{ab} | 98.9 ± 0.9 ^a | 37.7 ± 3.0 ^b |
| pH 6.0 | 91.7 ± 0.9 ^a | 93.7 ± 0.4 ^a | 96.2 ± 1.5 ^a | 96.9 ± 0.8 ^a | 98.4 ± 0.8 ^a | 38.2 ± 2.3 ^b |
| pH 6.5 | 82.9 ± 2.8 ^b | 93.2 ± 0.4 ^{ab} | 78.7 ± 5.9 ^{bc} | 96.9 ± 0.5 ^a | 99.3 ± 1.5 ^a | 42.0 ± 1.2 ^b |
| pH 6.8 | 80.8 ± 3.7 ^b | 93.5 ± 0.6 ^a | 73.8 ± 7.5 ^c | 98.7 ± 1.0 ^a | 97.5 ± 1.6 ^a | 41.4 ± 2.3 ^b |

^a Values are the mean ± standard deviation (n = 3); different superscript letters within the same column represent significant differences ($p < 0.05$).

Table 2

Fat-to-protein ratio of feeds, migration rate in Turbiscan at 70 °C, and coefficient of determination of the slope. ^a

| Feed | Fat/protein (w/w) | Migration rate (mm h ⁻¹) | R ² |
|---------|-------------------------|---|----------------|
| Control | 5.8 ± 0.9 ^a | 9.0 ± 0.5 ^a | 0.998 |
| pH 5.3 | 1.4 ± 0.5 ^c | 8.6 ± 0.4 ^a | 0.992 |
| pH 5.5 | 0.5 ± 0.2 ^c | 10.3 ± 0.3 ^b | 0.995 |
| pH 6.0 | 0.7 ± 0.2 ^c | 11.4 ± 0.1 ^c | 0.998 |
| pH 6.5 | 3.5 ± 1.0 ^b | 12.3 ± 0.2 ^d | 0.999 |
| pH 6.8 | 4.6 ± 1.1 ^{ab} | 12.0 ± 0.3 ^{cd} | 0.999 |

^a Migration rate is the slope of the linear region of a peak thickness-time plot. Values are the mean ± standard deviation (n = 3); different superscript letters within the same column represent significant differences ($p < 0.05$).

Figure legends

Fig. 1. Apparent viscosity graphs (a) and particle size distribution results (b) of cheese feeds: ■, control (pH 5.1); ●, pH 5.3; ▲, pH 5.5; ▼, pH 6.0; ◆, pH 6.5; ◀, pH 6.8.

Fig. 2. Delta backscattering (BS) graphs of cheese feeds at the end of 2 h in Turbiscan at 70 °C: ■, control (pH 5.1); ●, pH 5.3; ▲, pH 5.5; ▼, pH 6.0; ◆, pH 6.5; ◀, pH 6.8.

Fig. 3. Confocal laser scanning images of cheese feeds prepared at pH 5.1(control) (a), pH 5.3 (b), pH 5.5 (c), pH 6.0 (d), pH 6.5 (e), and pH 6.8 (e). Green and red colours represent protein and fat clusters, respectively.

Fig. 4. Average Raman spectra of retained mass fraction from different treatments in wavenumber ranges are (a) 200 to 3500 cm^{-1} and (b) 200 to 1800 cm^{-1} : —, control (pH 5.1); —, pH 5.3; —, pH 5.5; —, pH 6.0; —, pH 6.5; —, pH 6.8.

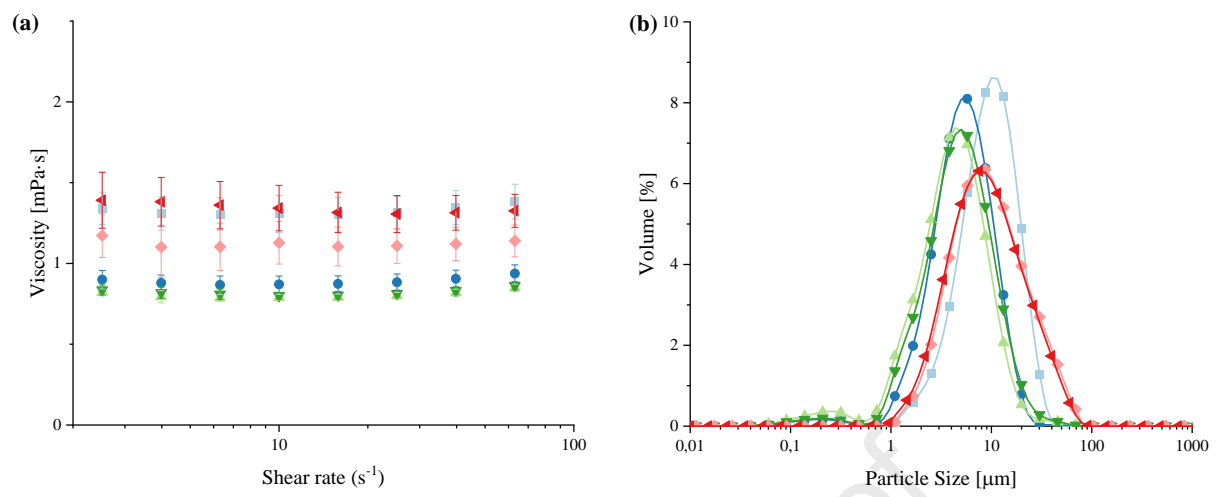


Figure 1

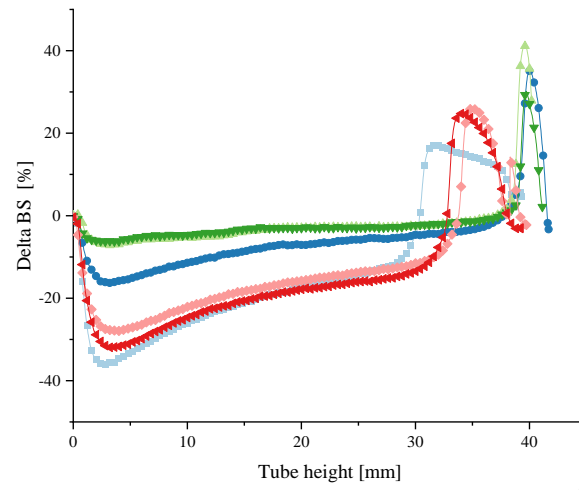


Figure 2

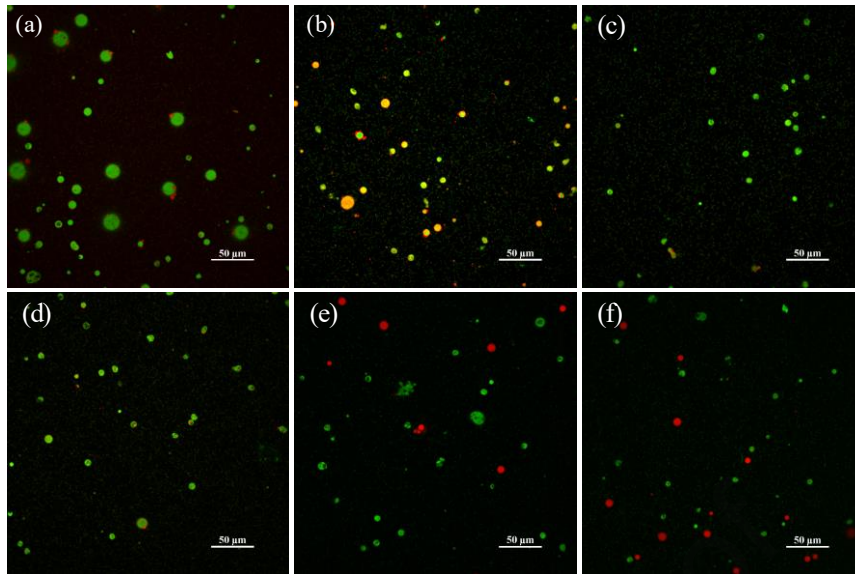


Figure 3

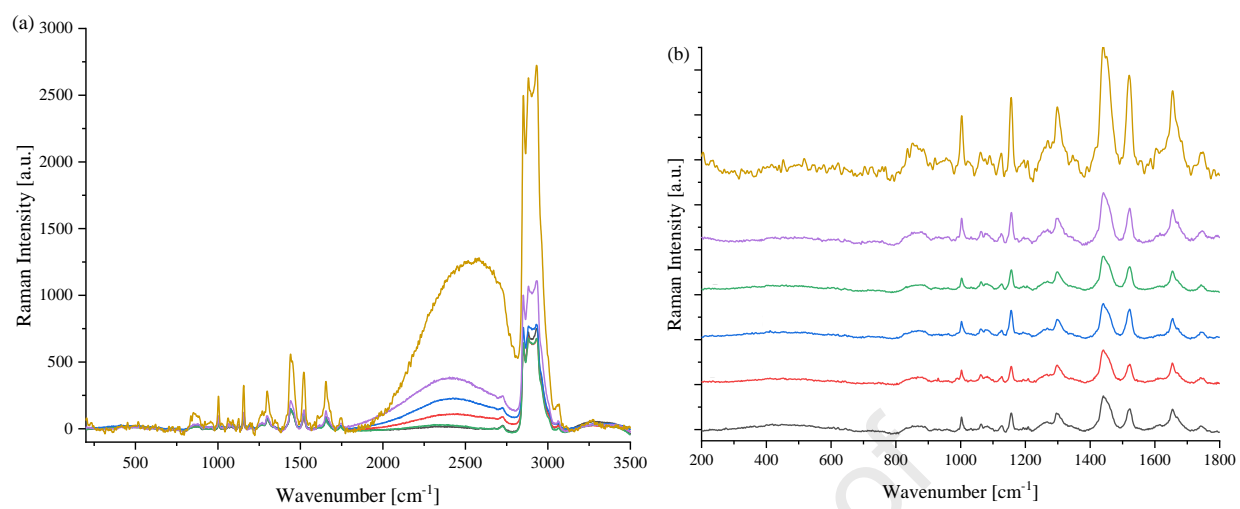


Figure 4

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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