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

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ABSTRACT

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Preparation of cheese feeds

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

2.2. Component retention and cheese feed yield

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

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

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

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

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

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

2.4. Cheese feed stability measurements

2.4.1. Rheology

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

2.4.2. Particle size distribution

The particle size distribution (PSD) of the cheese feed samples was determined using a laser diffraction instrument (Mastersizer 2000, Malvern Panalytical, Malvern, UK). The refractive indices of the dispersed phase and the continuous phase were set to 1.49 and 1.33, respectively. Drops of cheese feed were added to the dispersant unit until a final obscuration rate of between 10 and 12% was reached. Particle size was reported as volume-weighted 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 diameter of d_i. (Malvern Instruments, 2015).

2.4.3. Optical characterisation of colloidal stability

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

2.4.4. Microstructure evaluation of cheese feed

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

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

2.5. Raman spectra of retained mass

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

2.6. Statistical analysis

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

3. Results and discussion

3.1. Effect of pH on component retention in cheese

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

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

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

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

3.2. Effect of pH on cheese feed stability

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

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

Additionally, emulsion rheology is often affected by particle size distribution. Increased droplet aggregation or concentration, which is influenced by fat content, is known to increase the viscosity of food emulsions (McClements, 2015). In this study, particle size of the cheese feeds became smaller until pH 6.0 and larger again as the pH was increased up to 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 ± 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 samples, respectively. The highest span (width of distribution) was seen at treatments of pH 6.5 and 6.8 (Fig. 1b), which indicates the heterogeneity of the droplet size distribution. Moreover, these two treatments resulted in the highest particle size values followed by the control sample. A positive correlation was observed between viscosity and particle size values (Pearson correlation coefficient: 0.83). Since the protein content of the feeds was less than 5% (w/w), it could be concluded that the viscosity of the cheese feeds is primarily modulated by the fat content as well as pH-induced changes in protein functionality.

All samples showed a destabilisation by creaming over time regardless of the treatment (Fig. 2). The thickness of the creaming layer (peak thickness) varied primarily depending on the fat content of the feeds. The maximum peak thickness was observed in the control sample while minimum values were found for pH treatments ranging from pH 5.3 to 6.0. The control feed had a similar fat content as the pH 6.8 treated sample, yet the peak thickness was much higher than at pH 6.8. Therefore, it is suggested that another factor affecting the peak height can be the interaction between fat and protein, which influences the density of the dispersed phase and, consequently, the migration rate. The migration rate of the droplets is important for understanding the destabilisation kinetics. It can be affected by various factors such as the 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.
299

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

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

3.4. Raman spectra of retained mass

Fig. 4a illustrates the whole Raman spectral range, where considerable differences were seen in the area under the curve in the S–H stretch region (thiol groups) (2550–2850 cm^{-1}) (Li-Chan, Ismail, Sedman, & van de Voort, 2006). The overall increased intensity with increased pH in the thiol region could be a result of increased thiol activity due to decreased heat stability of beta-lactoglobulin at pH values higher than 6.0 (Wong, 1988). The authors also mentioned that freed thiol groups can cause aggregation of proteins due to conformational changes, which could be linked to the increased fat release into cheese feed at higher emulsification pH values. Fig. 4b shows the region with abundant structural information. The band shows protein amide regions at 1270–1300 (Amide III) and 1655 cm^{-1} (α-helix segments of casein), while CH_2 groups bending at 1440 cm^{-1} (Schulz, 2018). Additionally, the band at 1002 cm^{-1} is characteristic of phenylalanine ring breathing (Smith, Holroyd, Reid, & Gordon, 2017). The Raman intensity of all regions tended to increase with higher pH treatments, where the highest intensities were observed at pH 6.5 and 6.8. Thus, alteration in pH during the emulsification step led to changes in protein structure, which might have influenced the extracted components and colloidal stability.

4. Conclusions

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

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

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

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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.

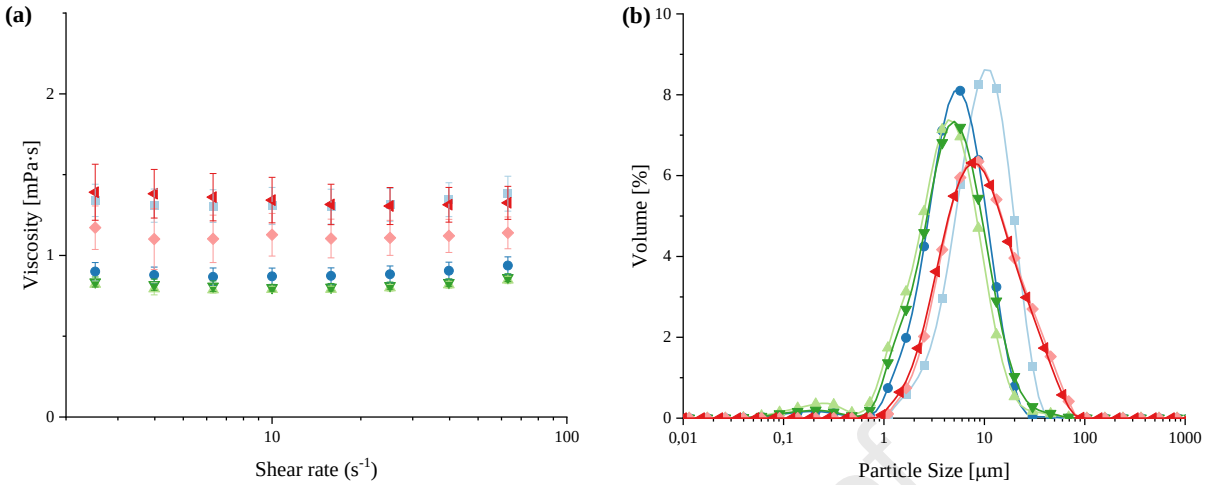

Figure 1

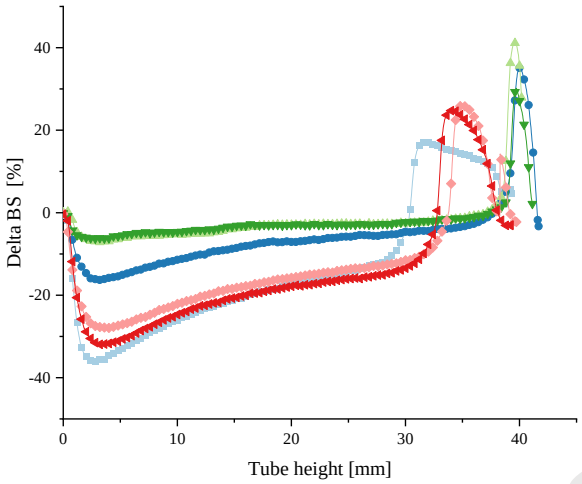

Figure 2

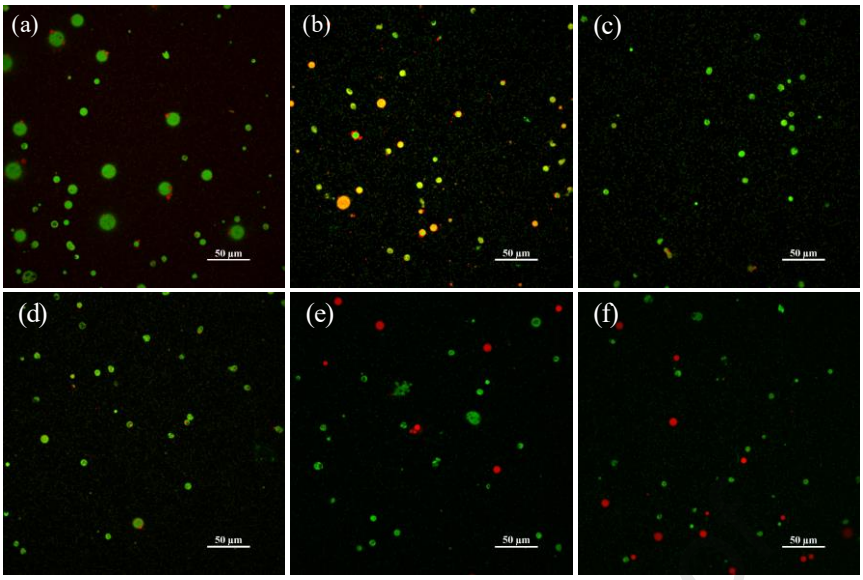

Figure 3

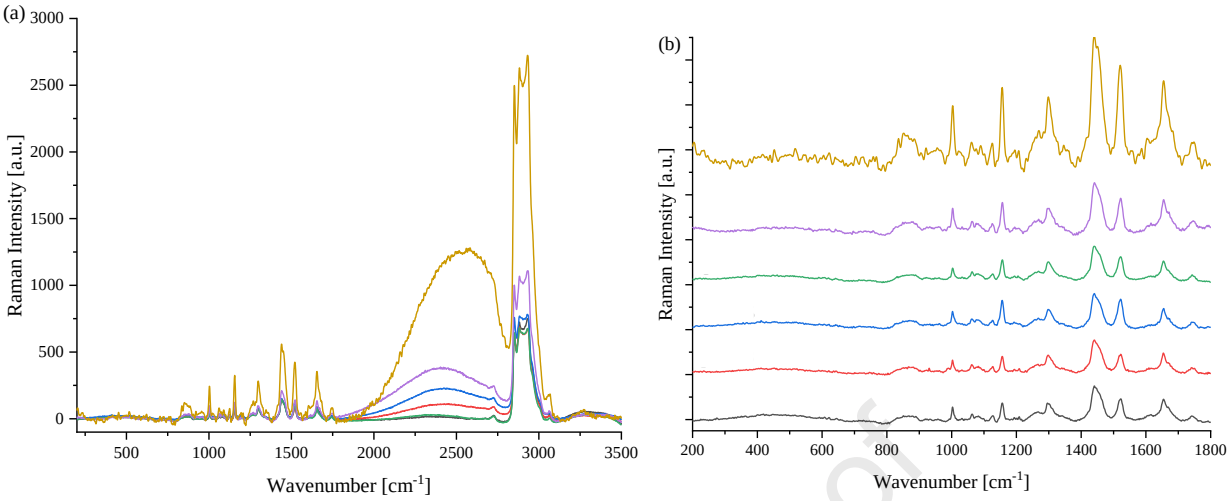

Figure 4

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: