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Colloidal stability of Camembert and Cheddar cheese feeds in the absence of emulsifying salts

Ipek Altay [a], Denise Felix da Silva [b], Rodolphe Marie [c], Jens J. Sloth [a], Mohammad Amin Mohammadifar [a, *]

[a] National Food Institute, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
[b] Lactosan A/S, Nordbakken 2, DK-5750 Ringe, Denmark
[c] Department of Health Technology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

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ABSTRACT

To eliminate emulsifying salts (ES) from cheese powder production, it is essential to understand destabilisation behaviour of cheese feeds without any additives. In this study, cheese feeds of two most used cheeses in cheese powder production were prepared in the absence of emulsifying salts. Colloidal stability of cheese feeds as well as the possible destabilisation causes linked to cheese properties were investigated. Both feeds showed a rapid destabilisation with different separation behaviour. Camembert feed showed better colloidal stability, which was associated with higher component recovery (e.g., fat, protein) and higher viscosity. The presence of colloidal calcium was associated with the poor stability of Cheddar feed, while extensive proteolysis in Camembert cheese affecting protein functionality is suggested to be an important destabilisation factor for Camembert feed.

1. Introduction

Cheese feed is a concentrated emulsion system that is used as a spray dryer feed in cheese powder production. It is made of minced natural cheese, water, and other ingredients such as emulsifying salts (ES), which are mixed and heated to form a homogeneous slurry before spray drying. Therefore, the colloidal stability of cheese feed is of great importance for further processing and final product quality (Felix da Silva, Tziouri, Ipsen, & Hougaard, 2020).

ES are commonly used in the manufacturing of processed cheese for the emulsification of fat in the protein matrix and to form a uniform structure (Buňka et al., 2013; Guinee & O'Kennedy, 2012). In natural cheese, the fat and water phases are supported by a calcium-paracaseinate phosphate network. However, heating natural cheese leads to separation of the oil and water phases in the absence of ES. Commonly used ES are disodium phosphate and trisodium citrate, which improve casein emulsification properties by converting insoluble calcium paracaseinate into sodium paracaseinate (Buňka et al., 2013; Kapoor & Metzger, 2008). This displacement enables hydration and partial dispersion of the calcium-paracaseinate phosphate network, which also establishes hydrophobic interactions with fat. Nevertheless, there is still a lack of knowledge on the interaction between ES and calcium/casein structures (Kapoor & Metzger, 2008). Even though ES play a crucial role in the stability of cheese feed by their ability to chelate Ca^{2+}, increased consumer demand for reducing phosphate and sodium in food products has led to investigations of alternative stabilisation methods (Hougaard, Sijbrandij, Varming, Ardö, & Ipsen, 2015).

A comprehensive understanding of the stabilisation behaviour of cheese feed in the absence of additives is necessary to eliminate ES from the production of cheese powder. The effect of various factors such as homogenisation, pH adjustment, maturation degree, or altering the cheese feed composition (e.g., the addition of buttermilk powder) on the stability of cheese feeds have been previously investigated (Felix da Silva et al., 2021; Felix da Silva, Hirschberg, Ahrné, Hougaard, & Ipsen, 2018). However, these studies only used aged, soft, or low Ca^{2+} cheeses, where different types of cheese were mixed together to provide a better melting. Thus, it is important to create a clearer picture of the destabilisation process by focusing on simple and well-defined systems.

To our knowledge, the chemical characteristics and physical stability of cheese feed made of a single type and batch of cheese and its relevance to the cheese properties have not been studied before. In addition, no previous study was found on the use of mild

* Corresponding author.
E-mail address: moamo@dtu.dk (M.A. Mohammadifar).

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Cheddar cheese in cheese feed production. Therefore, the objective of this study was to investigate the destabilisation behaviour of ES-free cheese feeds made from the most commonly used cheeses in cheese powder production, Camembert and Cheddar, and in parallel to assess the physicochemical characteristics of the cheese samples. The use of these structurally and compositionally different cheeses is expected to help understanding the main possible characteristics affecting the colloidal stability of the feeds. The findings of this study were expected to improve our understanding regarding the effect of cheese composition on the stability of cheese feed and give insight into possible ways to eliminate ES from the production of cheese powder.

2. Materials and methods

2.1. Cheese feed preparation

Two different types of cheese feed were prepared in triplicate, where Cheddar (Sonlac A/S, Middelfart, Denmark, <1 month) or Camembert cheese (Arla Foods Troldhede Dairy, Videbæk, Denmark, <1 month) was used as the main ingredient. To increase process efficiency, the 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). For the preparation of each cheese feed, 500 g of minced cheese was used, and the dry matter content was adjusted to 35% (w/w) with deionised (DI) water.

The minced cheese and DI water were mixed using a Stephan cooker (Stephan UMC 5, Stephan Machinery GmbH, Hameln, Germany) at a blade speed of 1000 rpm for 20 min, where the product was indirectly heated through a heating jacket. The product temperature during mixing was 85 ± 1 °C. Subsequently, the hot mixture was sieved through a 500 μm mesh size, and the permeate is called "cheese feed", while the rest is referred to as "retentate".

2.2. Compositional analysis

For all samples, the total nitrogen content was determined by the Dumas method using a Rapid MAX N exceed® analyser (Elementar Analyse systems GmbH, Hanau, Germany), and a factor of 6.38 was used to calculate the crude protein (Fox, Guinee, Cogan, & McSweeney, 2017). The dry matter content of the samples was determined by oven drying at 105 °C until a constant weight (Bradley & Vanderwarn, 2001). Cheese samples were analysed for pH (Guinee & O'Kennedy, 2009) and insoluble Ca (buffering capacity) by the acid-base titration method (Hassan, Johnson, & Lucey, 2004). The pH of the cheese feed was measured at ~50 °C using a 780 Metrohm pH meter (Metrohm, Herisau, Switzerland).

All samples were analysed for total fat content following the Bligh and Dyer (1959) method with slight modifications using a reduced amount of methanol and chloroform (1:1, v/v). Briefly, 10 g of sample was treated with methanol, chloroform, and water during stirring. The sample was centrifuged at 1400 × *g* for 10 min to separate phases. Finally, the fat content was determined gravimetrically and the results were presented as g fat 100 g^{-1} sample.

The total calcium and phosphorous content of the samples was determined by inductively coupled plasma mass spectrometry (ICP-MS) (8900 ICP-QQQ, Agilent Technologies, Santa Clara, CA, USA) after microwave-assisted digestion (Multiwave 7000, Anton Paar, Graz, Austria) with concentrated nitric acid (SCP Science, Courtaboeuf, France) in quartz vessel tubes (Forghani et al., 2021). Certified stock solutions of calcium and phosphorus were used as external calibration standards for quantification, and rhodium was used as an internal standard (all SCP Science, Courtaboeuf, France). In addition, a certified reference material (DORM-4, NRCC, Ottawa, Canada) was analysed together with the samples.

The levels of soluble nitrogen pH 4.6 (SN4.6) and water-soluble nitrogen (WSN) in cheese were used as indices of the degree of primary proteolysis and primary proteolysis plus casein hydration, respectively (Guinee & O'Kennedy, 2009). A modified method of Guinee and O'Kennedy (2009) was used, where grated cheese was mixed with deionised water at 55 °C at a ratio of 1:2 (w/w) and homogenised at 8000 rpm for 2 min using a high shear disperser (T 25 digital ULTRA-TURRAX® disperser, IKA-Werke GmbH, Staufen, Germany). The mixture was then centrifuged for 20 min at 3000 × *g* at 40 °C and filtered through a glass fibre filter and the permeate was used to determine WSN. Next, the pH of the resulting permeate was adjusted to pH 4.6 using 2 N HCl, then it was centrifuged at 3000 × *g* for 20 min at 4 °C, and filtered through a glass fibre filter again for SN4.6 determination. The nitrogen content of the final filtrate was determined using the method described previously for total nitrogen.

2.3. Dynamic small-amplitude oscillatory rheometry

The thermorheological properties of cheeses were evaluated with a rheometer (DHR-2, TA Instruments, Hullhorst, Germany) using a dynamic small-amplitude oscillatory rheometry technique (Lee, Johnson, & Lucey, 2005). Cheese samples were prepared to have a thickness of 1.5 mm and a diameter of 40 mm, which were conditioned in a fridge at ~4 °C before the measurements. The rheometer was equipped with a 40 mm diameter serrated plate and a serrated platform. An oscillation temperature ramp was performed from 20 °C to 80 °C with a ramp rate of 2 °C min^{-1}. The strain was adjusted to 0.2%, which was in the linear viscoelastic region (LVR) of the samples. During the measurements, the axial force adjustment option was activated and set to 1 N, which avoided the loss of contact between the melting cheese and the serrated plate geometry. The storage modulus (G′), elastic modulus (G″), and loss tangent (tan δ) values were reported.

2.4. Component recovery

The recovery of each component extracted from cheese, including fat, protein, and minerals in cheese feed, was calculated using Eq. (1) (Guinee, O'Kennedy, & Kelly, 2006).

$$\text{Component recovery } \% = \frac{g_{\text{cheese feed}} \times \text{component}\%_{\text{cheese feed}}}{g_{\text{cheese}} \times \text{component}\%_{\text{cheese}}} \quad (1)$$

2.5. Cheese feed viscosity measurements

Viscosity measurements of cheese feed samples were performed in a controlled-stress rheometer (DHR-2, TA Instruments, Hullhorst, Germany) equipped with a Peltier Concentric Cylinder Temperature System geometry, where the temperature was set to 50 °C. The applied shear rate ranged from 1 s^{-1} to 200 s^{-1}, and five equilibrium points were collected to plot each data point.

2.6. Particle size distribution measurements

The particle size distribution (PSD) of the cheese feed samples was determined with a laser diffraction instrument (Mastersizer 2000, Malvern Panalytical, Malvern, UK). The refractive indexes of the dispersed and continuous phases were set to 1.49 and 1.33, respectively. Particle size was reported as volume-weighted mean diameter (Eq. (2)):

$$D[4,3] = \sum n_i d_i^4 \Big/ \sum n_i d_i^3 \quad (2)$$

where n_i is the number of particles with a diameter of d_i.

2.7. Turbiscan stability measurements

The stability of cheese feeds was determined using Turbiscan® Tower (Formulaction, Toulouse, France). The method is based on the static multiple light scattering (S-MLS) principle, which uses an infrared light source at 880 nm. Two synchronous detectors in the system collect backscattered (BS) and transmitted (T) signals by scanning the sample from bottom to top. The Turbiscan stability index (TSI) is used to describe the overall sample stability of the sample, as using only raw BS and T data can be complicated. The equation for TSI calculations is given in Eqs. (3) and (4).

$$N_h = \frac{z_{max} - z_{min}}{\Delta h} \quad (3)$$

$$TSI(t) = \frac{1}{N_h} \sum_{t_i=1}^{t_{max}} \sum_{z_i=z_{min}}^{z_{max}} |BST(t_i, z_i) - BST(t_{i-1}, z_i)| \quad (4)$$

where t_{max} is the time at which the TSI is calculated, z_{min} and z_{max} are the lower and upper height limits, N_h is the number of height positions in the scan zone, Δh is the step of scanning height, and *BST* is the investigated signal. If T is less than 0.2%, the considered signal is BS and vice versa. The sample is considered stable when TSI is low and starts to be unstable as the TSI increases; a TSI value greater than 3 indicates significant or visible destabilisation.

Since cheese feed has a very complex destabilisation behaviour, the TSI index will be used together with ΔBS profile graphs. Immediately after the preparation of the cheese feed, the samples were transferred to Turbiscan® Tower at 70 °C, where the destabilisation kinetics were measured every 2 min for 4 h.

2.8. Confocal microscopy measurements

Confocal laser scanning microscopy (CLSM) was performed to visualise the microstructure of cheese feed samples. The samples were imaged using a 40× lens (Apo LWD water 40x NA 1.15, Nikon, Tokyo, Japan) on a spinning disc confocal microscope, which is composed 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). 0.01% (w/v) Nile red (Sigma-Aldrich Denmark A/S, Søborg, Denmark) and 0.001% (w/v) FCF fast Green (Sigma-Aldrich Denmark A/S) were used to stain fat droplets and proteins, respectively (Felix da Silva et al., 2021). The excitation wavelength for Nile red was 561 nm, and the scanning range of emission wavelength was 607 nm. FCF fast green was excited at 640 nm, and the emission wavelength was 700 nm.

2.9. Statistical analysis

All analyses and experiments 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. Compositional analysis

Table 1 illustrates the results of the compositional analysis of the Cheddar and Camembert cheeses used in the preparation of cheese feeds. According to the values obtained for Cheddar cheese, it can be classified as a high calcium Cheddar cheese type (Ayala-Bribiesca, Lussier, Chabot, Turgeon, & Britten, 2016; Guinee & O'Kennedy, 2009).

As shown in Fig. 1, the pH limits of ~4.1–~5.2 of the buffer curves were chosen to integrate and compare the buffering capacity of two types of cheese (O'Mahony, McSweeney, & Lucey, 2006). Lucey and Fox (1993) mentioned that the solubilisation of CCP results in phosphate ion formation, which can interact with H^+ and lead to a buffering effect. The absence of a buffering peak at pH 5 during back titration with NaOH is in agreement with the results of Hassan et al. (2004). The peak (~pH 6–7) obtained during back titration was reported to be the result from Ca phosphate formation (Hassan et al., 2004). The difference between the forward and backward curves was calculated, where the magnitude of the buffering area is directly proportional to the CCP content of the cheeses (Lucey & Fox, 1993; O'Mahony et al., 2006). The calculated areas for the Cheddar and Camembert cheese were 0.024 ± 0.003 and 0.013 ± 0.000 dB dpH.pH^{-1}, respectively. Cheddar cheese having around two times more buffering area than Camembert cheese indicates the presence of a significantly higher amount of CCP in the Cheddar cheese.

3.2. Dynamic small-amplitude oscillatory rheometry

Fig. 2a and b illustrates the rheograms of the Camembert and Cheddar cheeses as a function of temperature. Both types of cheese showed a decrease in G' value, which indicates a weakening of the structure of the cheese due to the increase in temperature. At the beginning of the rheogram (20 °C), the Cheddar cheese had much higher G' and G'' values in comparison with Camembert cheese. That means Cheddar cheese has a stronger network and a more "solid-like" structure than Camembert cheese (Schenkel, Samudrala, & Hinrichs, 2013). Fox et al. (2017) also highlighted that the relatively high intact casein content in Cheddar-type cheese provides a firmer and more elastic structure than mold-ripened cheeses such as Camembert.

Tan δ value represents the ratio of viscous to elastic properties and is related to the bond relaxation in the cheese matrix. The maximum value of tan δ (tan δ_{max}) indicates the highest bond mobility point and is used as an index of meltability (Schenkel et al., 2013). According to Fig. 2a, tan δ values of Camembert cheese remained less than 1, which means that the structure only weakens and no flow occurs. Furthermore, a negative correlation between

Table 1
Composition of the cheeses.[a]

Composition (wet basis)	Cheddar cheese	Camembert cheese
Dry matter (g 100 g^{-1})	62.2 ± 0.6	45.5 ± 0.0
Fat (g 100 g^{-1})	33.5 ± 0.2	20.8 ± 0.6
Protein (g 100 g^{-1})	24.6 ± 0.1	18.4 ± 0.9
Total Ca (mg 100 g^{-1})	775 ± 15	524 ± 21
Total P (mg 100 g^{-1})	521 ± 9	368 ± 12
SN4.6 (g 100 g^{-1} total N)	10.2 ± 0.3	18.0 ± 0.9
WSN (g 100 g^{-1} total N)	11.4 ± 0.2	20.3 ± 1.1
pH	5.3 ± 0.1	6.3 ± 0.4

[a] Abbreviations are: SN4.6, pH 4.6-soluble N as a percentage of total nitrogen; WSN, water-soluble nitrogen as a percentage of total nitrogen. Data are the mean ± SD.

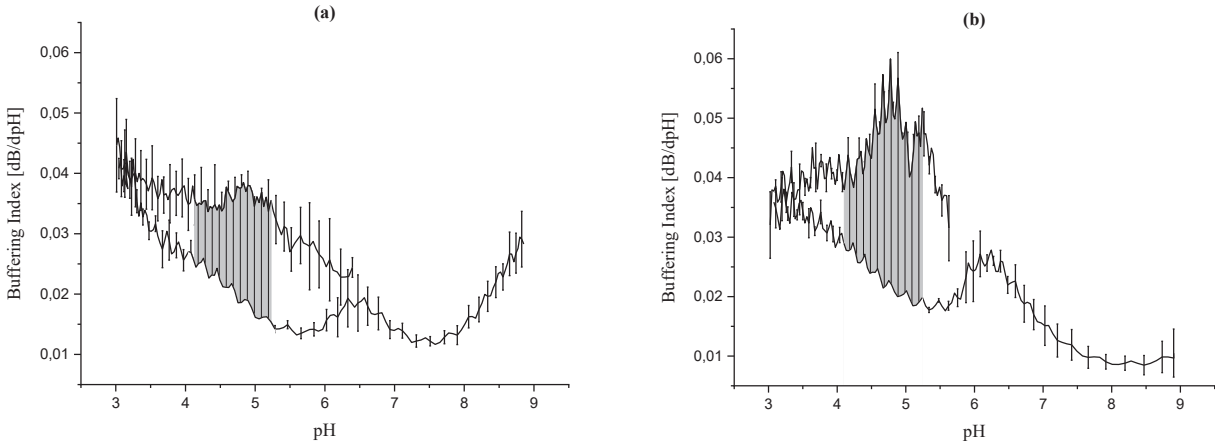

Fig. 1. Buffering curves of Camembert (a) and Cheddar cheese (b). The filled area represents the buffering effect due to colloidal calcium phosphate.

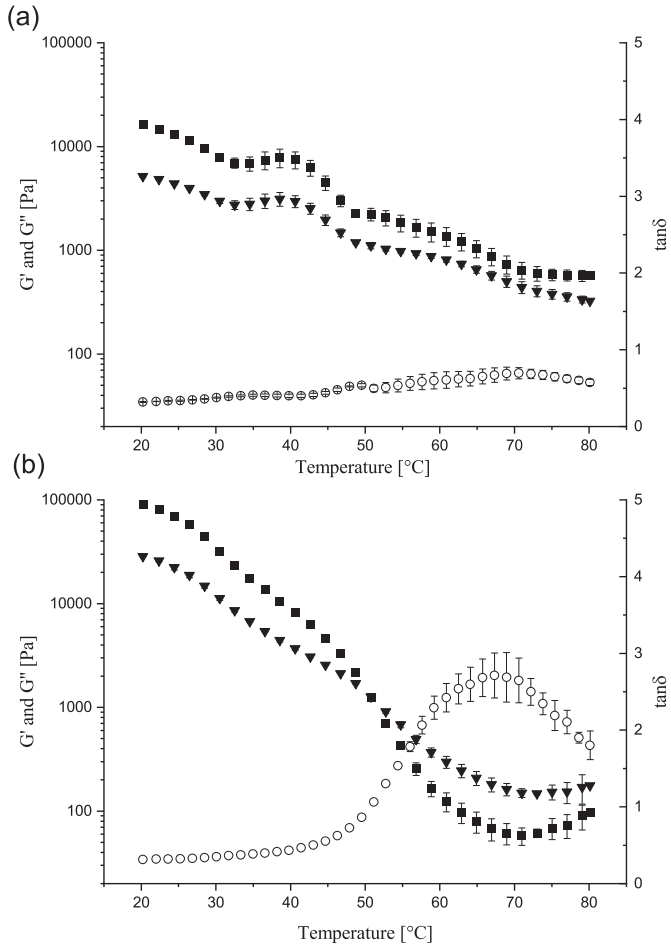

Fig. 2. Viscoelastic moduli (■, G′; ▼, G″) and tan δ (○) values of Camembert (a) and Cheddar (b) cheeses as a function of temperature.

the Ca content of cheese and tan δ_{max} was reported by earlier studies (Fröhlich-Wyder, Guggisberg, & Wechsler, 2009).

Although several authors reported that the total calcium content is directly related to the physical structure of the cheese, insoluble Ca or colloidal calcium (CCP) was also shown to be an important component affecting the texture of the cheese (Johnson & Lucey, 2006). Lucey and Fox (1993) suggested that the casein intact calcium (CCP) plays a crucial role in modulating cheese texture and argued that the total Ca is not the best predictor of the cheese texture. This is because the mineral balance changes during the ripening of cheese, where insoluble Ca becomes solubilised, and consequently, the structure weakens while the meltability increases (Hassan et al., 2004). Thus, it can be said that the total and insoluble Ca measurement results (Table 1 and Fig. 1) support the fact that the Cheddar cheese has a stronger structure than Camembert cheese.

3.3. Cheese component recovery

The component recovery in Camembert feed was significantly higher than in Cheddar feed except for the recovery of dry matter, which did not show a statistically significant difference (Table 2). High component recovery in Camembert feed can be related to structural differences in cheeses due to the total and insoluble Ca content as well as the type of cheese (i.e., production). Moreover, thermo-rheology analyses showed that Camembert cheese has a weaker structure network than Cheddar cheese, which might have assisted in better disintegration and emulsion formation. High P recovery in Camembert feed can be an indication of a higher amount of solubilised CCP compared with Cheddar cheese. The release of phosphate ions via CCP solubilisation was previously reported (Johnson & Lucey, 2006).

3.4. Particle size distribution

The particle size distribution curve of the Camembert feed showed a bimodal distribution with high polydispersity of particles

Table 2
Component recovery and composition of cheese feeds.

Parameter	Cheddar feed	Camembert feed	*p*-value
Composition (wet basis)			
Dry matter (g 100 g^{-1})	27 ± 0.3[a]	31.2 ± 0.3[b]	<0.0001**
Fat (g 100 g^{-1})	19.0 ± 0.4[a]	22.1 ± 1.4[b]	<0.05*
Protein (g 100 g^{-1})	3.5 ± 0.0[a]	8.3 ± 1.3[b]	<0.05*
Total Ca (mg 100 g^{-1})	161 ± 5[a]	195 ± 31[b]	<0.05*
Total P (mg 100 g^{-1})	74 ± 4[a]	144 ± 18[b]	<0.0001**
pH (50 °C)	5.1 ± 0.0[a]	6.4 ± 0.4[b]	<0.05*
Recovery (%)			
Dry matter	43.1 ± 0.7	45.9 ± 4.5	>0.05
Fat	56.1 ± 1.0[a]	71.0 ± 8.2[b]	<0.0001**
Protein	14.1 ± 0.4[a]	30.3 ± 7.3[b]	<0.0001**
Ca	18.9 ± 0.4[a]	19.4 ± 2.2[b]	<0.05*
P	14.0 ± 0.3[a]	26.5 ± 5.9[b]	<0.0001**

Data are the mean ± SD; means within the same row not sharing common superscript letters are statistically different.
* indicates the *p*-value is less than 0.05.
** indicates the *p*-value is less than 0.0001.

(Fig. 3). The D[4,3] values of the Camembert and Cheddar feeds were 48.8 ± 15.1 μm and 11.1 ± 0.5 μm, respectively. It should be noted that D[4,3] value is highly influenced by the presence of big particles, which in this case might correspond to the presence of suspending cheese particles or protein aggregates in the Camembert feed. Moreover, droplet flocculation or coalescence may be another reason for the bimodal distribution (McClements, 2015). The presence of fat clusters as a result of coalescence and of protein aggregates in Camembert feed was also observed in confocal laser microscopy images (Fig. 7c and d). Although small oil droplet size or monomodal system, as observed in Cheddar feed, is generally considered to be in favour of a stable emulsion, there are other factors that play a role in the stability of the system such as emulsifying components (i.e., protein), the oil phase, and the viscosity of the continuous phase (McClements, 2015). In this study, the Camembert feed contained large fat clusters leading to accelerated destabilisation speed while higher solids content of the continuous phase contributed to better stability. Therefore, the colloidal stability of the cheese emulsion systems should be interpreted in combination with other methods (i.e., Turbiscan stability, CLSM) due to its complexity.

3.5. Cheese feed viscosity

The Camembert feed tended to have a shear-thinning behaviour, whereas the Cheddar feed showed a Newtonian trend. Since bimodal distribution can be an indication of flocculation, this shear thinning behaviour might be a result of the deformation and realignment of flocculated droplets along with the shear field (McClements, 2015). According to Fig. 4, Cheddar feed had a lower viscosity than the Camembert feed throughout the shear rate range. The viscosity of Camembert and Cheddar at shear rate 10 was 5.7 ± 1.3 mPa s and 2.1 ± 0.1 mPa s, respectively. Higher viscosity in the continuous phase contributes to a better colloidal stability by retarding droplet movements (McClements, 2015).

Furthermore, presence of protein aggregates and higher solids content in Camembert feed can be another reason for its higher viscosity due to increased particle–particle interactions (McClements, 2015). Earlier studies also reported an increase in viscosity due to the presence of protein aggregates in the system, which was correlated with increased emulsion stability (Dapueto, Troncoso, Mella, & Zúñiga, 2019). Therefore, Camembert feed stability is expected to benefit from its high viscosity as well as having protein aggregates.

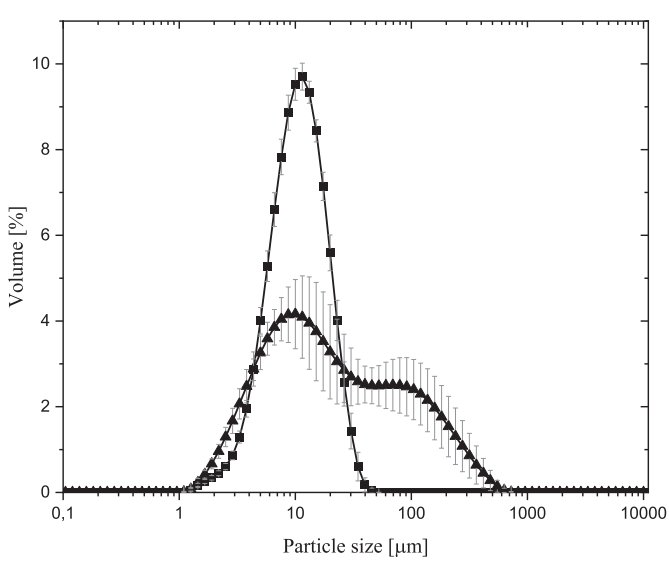

Fig. 3. Particle size distribution of Camembert (▲) and Cheddar (■) feeds.

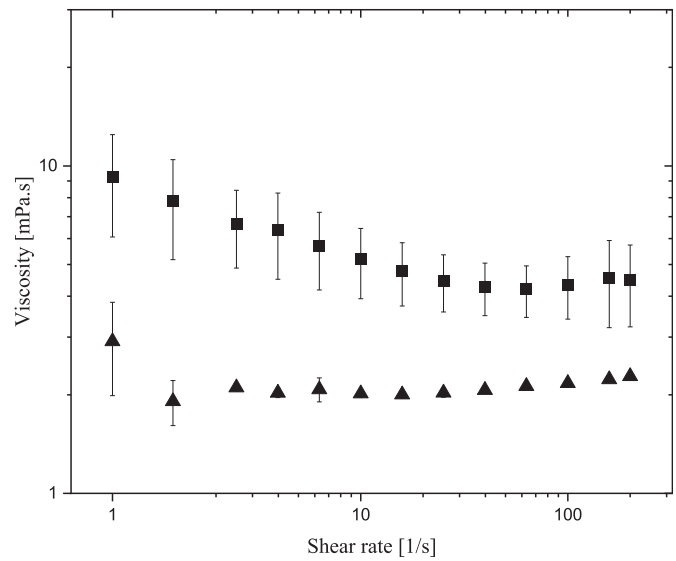

Fig. 4. Apparent viscosity of Camembert (■) and Cheddar (▲) feeds as a function of shear rate.

3.6. Turbiscan stability

Fig. 5a and b shows the ΔBS profiles of cheese feeds as a function of the height of the sample. Cheddar feed showed a clear creaming phenomenon, where the BS signal decreases at the bottom and increases at the top because of coalescence and migration of droplets to the top. Camembert feed had a more complex destabilisation behaviour over time. The negative BS signal at the top represents the clear fat layer formation. In addition, creaming and sedimentation phenomena were detected in BS signals from the bottom to ~38 mm. Creaming is due to the migration of oil droplets, whereas sedimentation can be explained by the presence of disintegrated Camembert cheese particles in the feed. These findings are in line with the particle size and viscosity data since these particles are responsible for the detection of big particles and increasing viscosity.

According to the global TSI change over time (Fig. 6), the Camembert feed seems more stable at the end of 4 h. It is known that the samples reaching a TSI value of 3 means visible destabilisation. Therefore, both Camembert and Cheddar feeds were visibly destabilised at 16.6 ± 1.5 min and 12.2 ± 0.4 min, respectively. As mentioned previously, the TSI value should not be evaluated alone due to the different and complex destabilisation mechanisms of these two cheese feeds. Relatively slower destabilisation of the Camembert feed can be attributed to the higher viscosity of the sample. However, the appearance of a clear fat layer might be an indication of poor emulsifying functionality of Camembert proteins.

According to Table 1, the Camembert cheese used for the production of feed had a higher degree of proteolysis than the Cheddar cheese. Burrington (2000) mentioned that emulsifying properties of proteins decrease as the cheese ages. The proteins become more water-soluble (WSN), and protein–protein interactions weaken over time, which in the longer term leads to poor emulsification. It was also explained that para-casein is hydrolysed to free amino acids and peptides during maturation, lowering the intact casein level (IC). These changes in cheese protein structure were associated with loss in rigidity and a lower degree of fat emulsification (Fox et al., 2017).

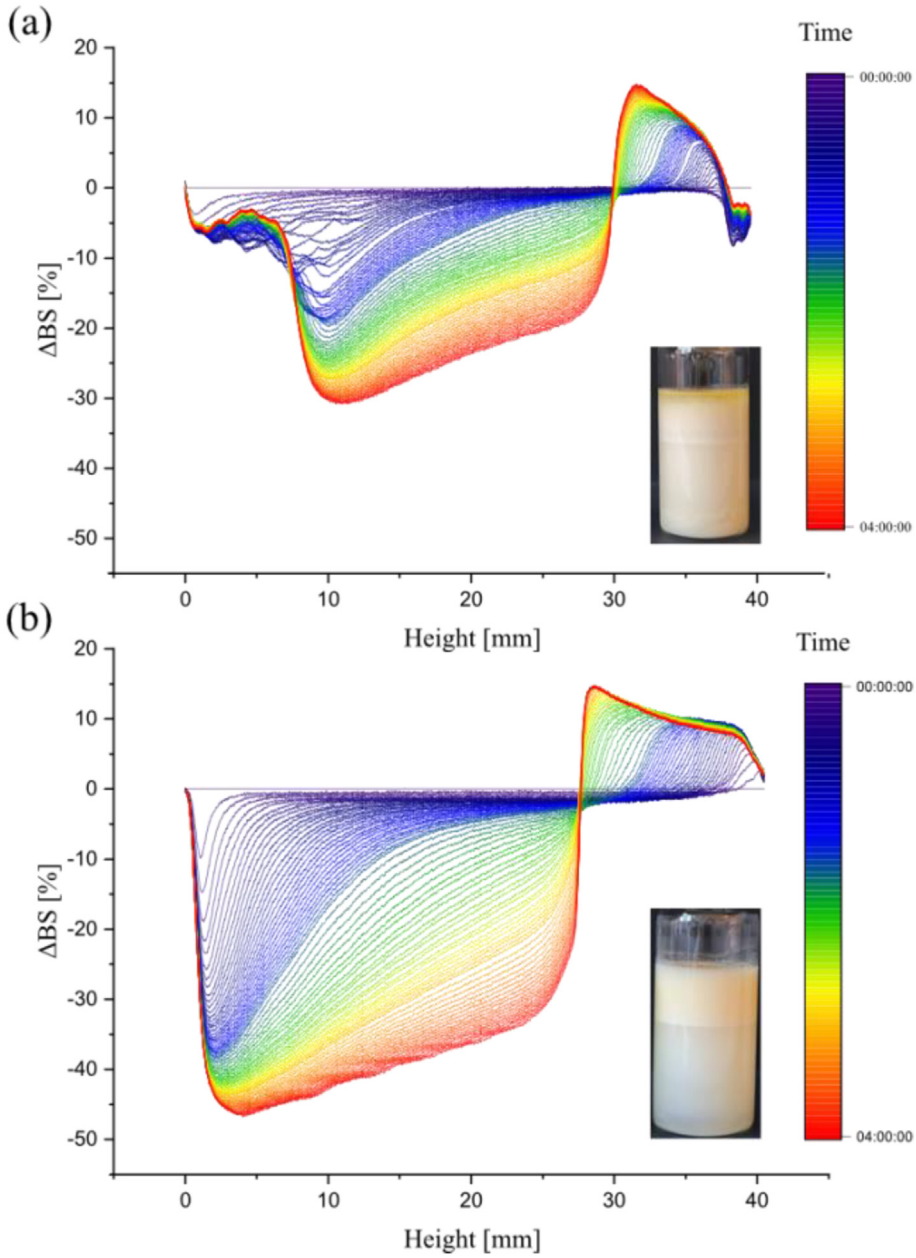

Fig. 5. Delta backscattering graphs and photo of samples after 4 h for Camembert (a) and Cheddar (b) feeds.

A high CCP content also hinders emulsification, as protein solubility is negatively affected by the presence of colloidal calcium (Burrington, 2000). As mentioned above, Cheddar cheese had a higher amount of bound calcium content, which explains the lower solubilised protein (protein recovery) in the cheese feed preparation (Table 2).

3.7. Confocal microscopy measurements

The microstructure of the cheese feed samples was evaluated using CLSM to further support the results on the particle size distribution and rheological properties (Fig. 7). The Camembert feed showed a packed structure of protein aggregates, where the larger fat clusters seem to be trapped in between. On the other hand, Cheddar feed was much less concentrated in comparison with Camembert. The protein aggregates are smaller and no large clusters of fat were detected in the Cheddar feed. However, the interactions between fat and proteins are not clear. The higher concentration of particles and the presence of irregular-shaped protein aggregates in the Camembert feed could help to explain the higher viscosity and the significant difference in the particle size distribution of the cheese feeds.

Additionally, the faster destabilisation kinetics of Cheddar feed may be related to the absence of significant protein–fat interactions in the matrix. It should be noted that pH values of the feeds, besides the degree of proteolysis, could influence the protein – protein interactions. Increased pH is known to increase the electrostatic repulsion between the proteins whereas lowering pH closer to isoelectric point (~pH 4.6) could promote hydrophobic interactions leading to aggregation (Fox & McSweeney, 2003). However, in this

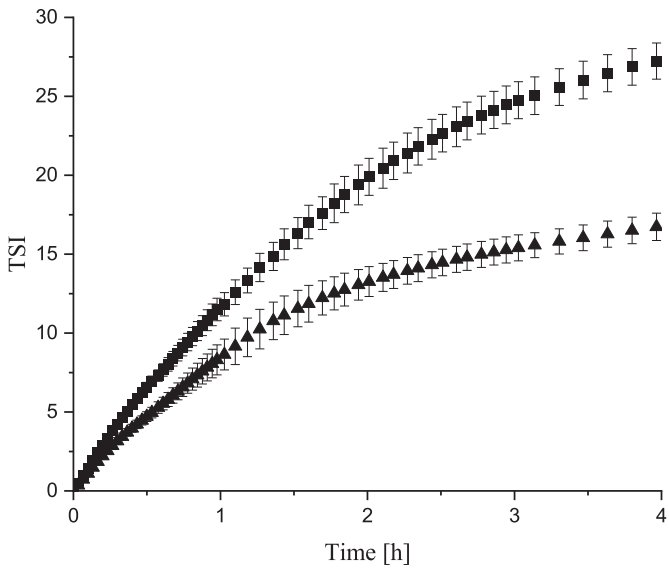

Fig. 6. TSI index of Camembert (▲) and Cheddar (■) feeds over time.

case, although Cheddar feed had a lower pH, it did not show any pronounced aggregation due to its low protein content.

4. Conclusion

In this study, the colloidal stability of cheese feeds made of two different types of cheese was evaluated in the absence of emulsifying salts. The type of cheese had a significant effect on the rheology, microstructure, and colloidal stability of the cheese feeds. Both types of cheese feeds showed relatively fast destabilisation, yet the behaviour of destabilisation seemed to be completely different. Cheese feed made of Cheddar cheese, which had a high CCP, showed inferior colloidal stability and component recovery compared with Camembert feed. This can be related to the fact that CCP hinders the disintegration of casein micelles during the emulsification process. In the case of Camembert feed, poor protein functionality due to extensive proteolysis was considered as the cause of cheese feed instability. This study showed that the characterisation of various types of cheese and understanding of their contribution to cheese feed production are necessary to adopt the appropriate method for the production of ES-free cheese feed with desired quality.

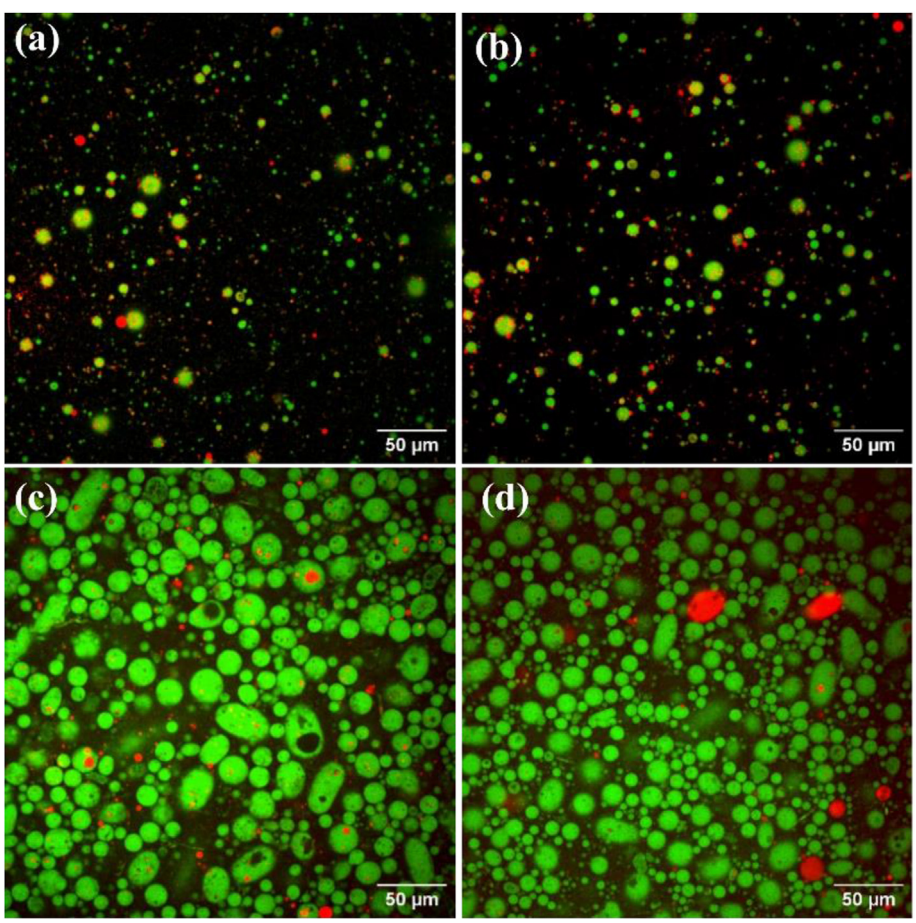

Fig. 7. Confocal laser scanning microscopy images of Cheddar (a, b) and Camembert (c, d) feeds. Green and red colors indicate protein and fat, respectively. The void area appears black.

Author contributions

Ipek Altay: Data Curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft. **Denise Felix da Silva:** Resources; writing – review and editing; supervision. **Rodolphe Marie:** Resources; writing – review and editing. **Jens J. Sloth:** Resources; writing – review and editing; supervision. **Mohammad Amin Mohammadifar:** Conceptualization; funding acquisition; project administration; writing – review and editing; supervision.

Declaration of competing interest

None.

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