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Effect of Ohmic Heating on Colloidal Stability of Cheese Powder Dispersions

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Abstract— Cheese powder is manufactured by mixing melted cheeses with water and emulsifying salts (ES) to form a homogeneous and stable emulsion, denoted cheese feed, until and during spray drying. The presence of emulsifying salts influences several critical events, including pH adjustment, calcium chelation from the casein micelle by exchanging sodium ions, consequent solubilization of proteins, and subsequent fat emulsification. However, demands for reduction of sodium in foods makes production of cheese powder without emulsifying salts desirable, hence new strategies for stabilization of cheese feed are needed. The advantages of ohmic heating (OH) as heat treatment are considered an attractive option for the dairy industry and so, the present study aimed at understanding the effect of this technology over the use of conventional heating (CH) methods on the stability of cheese emulsions. Cheese powder dispersions with no ES were prepared and processed with different conditions: electricity (OH or CH), temperature (85ºC or 60ºC), and number of homogenizations. OH had a great impact on calcium diffusivity from the casein micelle, which can be related to disturbances on charge organization within protein structure. Overall, ohmic heating is an exciting opportunity to manipulate calcium partitioning between colloidal and serum phase in cheese powder dispersions, and so it could be used to modulate protein functionality by affecting casein micelle structural integrity. However, results indicated that ohmic heating alone is not sufficient to reach the desired quality characteristics. Therefore, charge distribution of the proteins should also be adjusted to an optimum value by other treatments.

Keywords— ohmic heating, cheese emulsions, stability, calcium solubilization

I. Introduction

Cheese powders are dehydrated cheese products with major economic importance due to their widespread use as flavouring agents and nutritional supplements in ready meals, sauces, creams, soups, bakery products, pasta dishes, among others [1, 2]. Several factors favour the use of cheese powders over natural cheese, including their convenience, as they can be easily incorporated into food formulations, and do not require size-reduction, their longer shelf-life, due to lower water activity, and their diversity of flavours [3]. Nowadays, over 100 varieties of cheese powder can be found in the market and can be produced using a wide range of cheeses, including Cheddar, Danbo, Camembert, Gouda, and Emmental [4, 5].

The manufacture of cheese powder essentially involves the four main steps below [1, 3, 6, 7]:

i. **Determination of the blending formulation.** Comminuted natural cheese, water, emulsifying salts, and, optionally, different additives, including dairy ingredients, starch, flavours enhancers, among others.

ii. **Processing of the blend.** Heat treatment of the blend, while constantly stirring. The blend is processed until the emulsion formed, named cheese feed, is homogeneous in colour and consistency and free of lumps and non-hydrated material.

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iii. **Homogenization.** Ensures homogeneity and promotes a finer dispersion of fat droplets, leading to a smoother and creamier hot blend.

iv. **Spray drying of the cheese feed.** The design of the drier and operation conditions influences the physical and the flavour characteristics of the cheese powder.

The cheese emulsion that results from the processing is required to be homogeneous, uniform, stable (without protein precipitation and cream separation) and pumpable, until and during spray drying [8, 9]. With this purpose, emulsifying salts (ES) are added to the mixture, due to their ability to influence several critical events [7, 10, 11, 12, 13]:

pH adjustment: the use of ES usually increases the pH of the cheese, which contributes to an enhanced dissociation and calcium-sequestering ability of the ES, and an increased negative charge on the paracaseinate.

Calcium Sequestration: this involves the ability of ES to chelate calcium from the casein matrix by exchanging sodium ions, which results in the conversion of insoluble calcium paracaseinate into soluble sodium paracaseinate. The effectiveness of ES in binding calcium depends on the valency, type of ionic species forming the emulsifying salt, pH, ionic strength, temperature, among other effects.

Casein Hydration: the sequestered complexes disrupt the major molecular forces that cross-link the various monomers of casein in the network. This disruption, in conjunction with heating and mixing, leads to hydration and partial dispersion of the proteins, which can help emulsification by coating the surfaces of dispersed free fat globules.

Fat Emulsification: Within the matrix, sodium paraca-

seinate acts as an emulsifier due to having both hydrophobic and hydrophilic regions in their peptide chain, which they position in the oil phase and aqueous phase, respectively. This property allows them to lower the interfacial tension and the global free energy of the system. The increase in viscosity as a result of water binding by the paracaseinate also enhances emulsion stability by restricting the mobility of the emulsified fat particles.

Nonetheless, dairy producers are under increasing pressure from health conscious consumers to reduce or remove salt in their products, and there is also an increasing interest in production and application of natural food ingredients, which can lead to "clean labelling" of the final food products. Hence, new strategies for stabilisation of cheese feed without ES are needed [9].

Optimization of processing parameters may improve emulsion stability during production of processed cheese, and so, similar effects could be expected on the stability of cheese feed [14]. The heat treatment step present in cheese powder production, responsible for melting, is one of the processing conditions that has a major effect on the calcium equilibrium, which can interfere with the casein conformation and aggregation state. Usually, high temperatures lead to a decrease in diffusible calcium from the micelle, due to its precipitation [15], which has a negative effect in protein solubilization and fat emulsification. Therefore, new heating systems should be tested to evaluate their effect on emulsion stability.

Ohmic heating (OH) is considered an emerging processing technology that is able to overcome the problems that arise from the use of conventional heating. These last methods, despite being the most used to ensure microbial safety, can lead to overheating of foods, which consequently causes loss of nutritional compounds and sensory changes [16]. Since OH is a process wherein electric current passes through materials, the increase in temperature is due to internal energy transformation (from electrical to thermal), leading to a rapid and uniform heating of the food, when compared to the traditional methods [17, 18]. Besides ensuring nutrient retention capacity and sensory attributes, this technology is easier to control, presents a great variety of designs, and is more energy efficient and environmental friendly. Hence, the use of this new technology as a heat treatment is an attractive option for the dairy industry [19].

The overall aim of the present study was to evaluate the effect of ohmic heating on colloidal stability of cheese powder dispersions. The use of electricity and process parameters, such as frequency and electric field, may influence casein micelles and protein conformational structures [20], as well as the equilibrium between the soluble and insoluble calcium. Hence, as OH influences ionized materials, we hypothesize that its usage may increase the diffusibility of calcium, even at high temperatures, present in the micelle, solubilizing the proteins, which can then emulsify the fat droplets, allowing the manufacture of a stable emulsion without addition of emulsifying salts. Due to being more convenient and ready-to-use, the experiments were done on cheese powder dispersions to mimic the cheese feed formed during production. The samples were then processed using OH and conventional heating and the stability was characterized through different parameters, such as particle size distribution, rheology, and physical stability, and the microstructure was evaluated by confocal laser scanning microscopy. The calcium, protein and fat content of the sediment and serum phases after processing were analysed. No other study has been reported regarding the use of this emerging technology on cheese feed stability, making this investigation a first step to obtain a knowledge platform that provides understanding on the possible effects of OH on cheese feed, and in the future, in powder properties and functionality.

II. Experimental Procedure

a. Materials

Cheese powder of medium-maturated Camembert and similar types of cheese without emulsifying salts (38.10% fat, 47.98% protein, 0.67% calcium, 8.15% moisture) was provided by Lactosan A/S, Ringe, Denmark.

b. Experimental methods

1. Cheese powder dispersion preparation

Cheese powder at a concentration of 17.5% (w/w) was added to deionized water and mixed for 2 hours on a stirring plate. The sample was then placed at 4ºC overnight to establish a good hydration. The preparation procedure for the five samples prepared is displayed in Table 1. The effect of different parameters on the stability of cheese powder dispersions were studied in this experiment: electricity, through the use of ohmic heating and conventional heating, temperature, some samples were processed at 85ºC and others at 60ºC, and homogenization, a homogenization step was added prior to heat treatment in two samples. For each condition, two replicas were produced.

2. Heat treatment

Ohmic heating (OH)

An ohmic heater (BCH ltd., Lancashire, United Kingdom) with an ohmic unit consisting of a holding cell made of W500 grade polyethylene-polypropylene with variable size adjustment and mountings for temperature loggers (K-type) was used. A maximal supply at 230 voltage using alternating current (60 Hz, sinusoidal) was installed with the ohmic heater and a titanium electrode with high corrosion resistance in chloride environments [21] was used. The distance between the electrodes was set at 8 cm apart and the width of the chamber was 9.5 cm. After the system was closed, 250 mL of sample were added to the ohmic heater. The voltage gradient used was 15 V/cm. The voltage gradient (E) is the ratio of applied voltage (V) to the distance between electrodes (L). The applied voltage was the percentage of the total voltage supply (V_{max} = 230 V). The voltage gradient was set by changing the applied voltage (V) at fixed L for all experimental setups. Voltage gradient was calculated as $E = \frac{V}{L} = \frac{\%V_{max}}{L}$. The final temperature to reach was 85ºC. The time required for the samples to come up to the mentioned temperature was recorded. For the samples OH + H it took 18.5 ± 0.5 s and for the samples H + OH + H it required 38.5 ± 1.5 s to reach the temperature (mean ± SD, where n=2). These differences in time are due to the decrease in temperature of the sample after one

Table 1: Procedures used (+) in the preparation of all the five samples. The procedures are showed in order of occurrence from left to right.

Sample ID	Heating plate - 60ºC	Homogenization (H)	Heating plate - 60ºC	Ohmic Heating (OH) - 85ºC	Oil Bath (OB) - 85ºC	Homogenization (H)
OH + H	+			+		+
H + OH + H	+	+		+		+
OB + H	+				+	+
60 + H	+		+			+
H + 60 + H	+	+	+			+

homogenization, which increases the come up time to reach 85ºC. After a holding time of 5 min, samples were rapidly transferred to the high pressure homogenizer.

Conventional Heating (CH)

Samples were subjected to two types of conventional heating:

i. Oil bath

Samples in an Erlenmeyer flask were immersed in an oil bath at 85ºC, where the temperature was controlled through a t-type thermocouple, for a holding time of 5 min. Oil bath was used instead of a heating plate so that the come up time would be similar to the one reached when using OH. OB + H samples reached 85ºC after 69 ± 1 s (mean ± SD, where n=2).

ii. Heating plate

Samples that were not subjected to either ohmic heating or oil bath, continued being heated up at 60ºC in the heating plate, so that the exposure time to temperature would be the same.

3. Homogenization

To disrupt the fat globules, the samples were run in a Rannie High Pressure Laboratory Homogenizer Model MINI-LAB (APV, Izmir, Turkey) at 400 bars. To evaluate the effect of homogenization, some samples were subjected to this procedure before and after heat treatment.

4. Centrifugation

Subsequently to homogenization, the stability, rheological properties, and particle size distribution of the cheese powder dispersions were analysed. The remaining sample was centrifuged in a 4-16Ks centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) equipped with rotor and insertions for 50 mL centrifuge tubes with conical bottom. Centrifugation was performed at 40ºC, with a speed of 5000 x *g* during 60 mins. The accelerated instability provided by centrifugation was observed as separation into three phases. One can assume that fat was primarily found in the top layer, precipitated protein in the bottom phase, leaving an opaque watery phase in between upon phase separation, where soluble protein can be found.

5. Freeze-drying

The three phases obtained after centrifugation were then separated and freeze-dried (Thermo Fisher Scientific Inc., Massachusetts, USA). In this process, also known as lyophilization, the water in the form of ice under low pressure is removed from a material by sublimation. Freezing water in the material prior to lyophilization inhibits chemical, biochemical, and microbiological reactions, which allows the retention of its quality [22]. The fat, protein, and mineral content were then analysed in all the three phases.

c. Analytical methods

1. Chemical analysis of cheese powder dispersions

i. Protein content

Total protein content was analysed according to the Dumas method by using rapid MAX N exceed (Elementar Americas, Inc., Ronkonkoma, New York, USA). The basis of the Dumas method is the conversion of all nitrogen forms in the sample to nitrogen oxides through combustion at high temperatures in an oxygen atmosphere, reduction of these forms to nitrogen gas (N_2), and subsequent measurement by use of a thermal conductivity detector [23]. Dry samples were placed in iron tips. The system was calibrated daily before analysis by running the following sequence: three blanks (empty crucibles), five aspartic acid standards and two run-in samples. Protein content (%) was calculated from the nitrogen content of the material, using a nitrogen conversion factor (NFC) of 6.38 [24], which is the most appropriate value for cheese. The value NFC cannot be identical for all sources of food proteins, as it is related to the amino acid composition and presence of side groups covalently bound to some amino acids of the protein chain [25]. Protein content was analyzed in the sediment and soluble phases. For the fat phase, the protein content was calculated by subtracting the values obtained from the total protein content.

ii. Fat content

The measurement was done through the Rapid Nuclear Magnetic Resonance (NMR) Fat Analyzer (CEM Corporation, Matthews, NC, USA) that is provided with a technology that completely isolates the detection of the proton signal in fat molecules from all other compositional proton sources. The method “Dairy Powders”, already present in the equipment, was used for the analysis. The fat content was measured in all three phases.

iii. Calcium content

Calcium content was measured using an Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). This technique uses an argon plasma, the ICP, to convert the sample into ions, which are then separated by MS by their mass-to-charge ratio. The detector counts the number of selected ions per second which allows the instrument to determine the con-

centration of the chosen element [26]. Calcium content was measured only in the sediment phase, and the value obtained obtained was subtracted from the total calcium content to obtain the soluble phase value. It was considered that the amount of calcium in the fat phase was minimal.

2. Particle size measurements

The measurement of particle size distribution of oil droplet in cheese feed samples was based on the Static light scattering method and done through Mastersizer 2000 (Malvern Instruments Co. Ltd., Worcestershire, UK). Particles pass through a focused laser beam and scatter light at an angle that is inversely proportional to their size. The angular intensity of the scattered light is then measured by a series of photosensitive detectors. The map of the scattering intensity versus angle is the primary source of information used to calculate the particle size, accurately predicted by the Mie scattering model. Samples were placed at the feed hopper at room temperature (sample temperature 40±5°C). The applied refractive index of the dispersed phase for all samples was set to 1.469 and for the continuous phase (water) was 1.330. The statistical dimension of size was expressed through the $d_{(0.5)}$ value, which means that 50% of the total amount of sample is smaller than this size, respectively. To infer about the uniformity and size consistency of samples, the Span value was reported. The closer to 0, the more uniform the sample is. Particle mean diameter was expressed as volume-weighted mean diameter $D_{[4,3]}$.

3. Rheological measurements

The flow behaviour of the cheese feed samples was measured using the Discovery Hybrid Rheometer 2 (DHR-2, TA Instruments, New Castle, USA) using a rotor conical/DIN SST SMART-SWAP (diameter 28 mm) and a fixed lower concentric cylinder cup. For each measurement, approximately 25 mL of cheese feed was placed into the interior cup and the temperature was precisely controlled at 40 °C. Flow curves for each sample were measured over 10 min with shear rate continually increasing from 0.1 to 200 s^{-1}.

4. Physical stability

Turbiscan Tower (Formulaction, Toulouse, France) was used to investigate the stability of the cheese feed. This scanning method is based on Static multiple light scattering principle (S-MLS), where an infrared light source with a wavelength of 880 nm illuminates the sample, and 2 sensors collect the backscattering (BS) and transmission (T) signals. The stability is measured by the amount of light that is backscattered and sent back to the detector. Samples were prepared on a 20 mL glass vial. The BS and T signals were acquired repeatedly over 2 hours every 2 minutes at the whole sample height and the temperature of the equipment was set to 40ºC.

5. Confocal laser scanning microscopy

The emulsions were dyed with Nile red [27] (Sigma-Aldrich Denmark A/S, Søborg, Denmark), dyeing the lipid phase. The dye was excited at 561 nm by a filter at 607 nm. FCF fast green (Sigma-Aldrich Denmark A/S, Søborg, Denmark) was used to dye the proteins and was excited at 640 nm by a filter of 700 nm [28]. The sample was imaged using a 40x lens (Nikon Apo LWD water 40x NA 1.15) on a spinning disc confocal microscope constituted by an inverted microscope (Nikon Ti2) equipped with a laser source (405 / 488 / 561 / 640 nm), a confocal spinning disc module (Yokogawa CSU-W1, 50um pinholes), a quad-band emission filter (440 / 521 / 607 / 700 nm) and an sCMOS camera (Photometrics Prime95B).

III. Results and Discussion

a. Influence of processing conditions on particle size

Various statistical parameters used to understand the particle size of emulsions are expressed in Table 2, and a particle size distribution diagram for all 5 samples is presented in Figure 1. A monomodal particle size distribution was observed for the sample OH + H, whereas bimodal particle size distributions were noticed for the other samples. Independently of the type of heat treatment used, ohmic heating or oil bath, a high temperature of 85ºC led to the highest particle sizes, with a $D_{[4,3]}$ of 8.83 μm for the OH + H sample and a value of 6.46 μm for the OB + H sample. The great majority of whey proteins are removed during cheese production, however, there is still some that remain, especially β-lactoglobulin since it is the main whey protein present in milk. At a temperature of 85ºC these proteins denature and become associated with casein micelles, through interaction with κ-casein, via hydrophobic interactions and disulphide bonds, which can justify the increase in particle size with the increase in temperature [29]. However, the homogenization of the sample prior to ohmic heating (sample H + OH + H) decreased the particle size to one similar to the one obtained by the sample treated at 60ºC with only one homogenization (60 + H). At 60ºC, the use of a second homogenization showed a decrease in particle size (3.19 ± 0.29 μm). These two last results indicate that a homogenization step prior to processing is positively correlated with particle size reduction, through disruption of protein aggregates and fat droplets. The presence of non-conductive materials, such as fat droplets, may be a disadvantage when it comes to the use of ohmic heating [30]. This additional homogenization may help to promote a more uniform heating generation, by creating a more homogeneous sample, and so treatment can be more successful. In terms of uniformity, which is correlated with the Span value showed in Table 2, samples heated at 85ºC demonstrated a better size consistency compared to the ones heated at a lower temperature. The $d_{(0.5)}$ value for the 60 + H and H + 60 + H samples are lower compared to the samples heated at higher temperatures, especially for the last sample, which $d_{(0.5)}$ value is 0.56 ± 0.11 μm which means that the sample presents a high number of small particles, once 50% of them are smaller than 0.56 μm, and then a few bigger particles that occupy the same volume as the small ones. This can be seen through the bimodal particle size distribution diagram where the two peaks have the same height. These samples having a lower particle size also corroborates the effect of higher temperatures on the aggregation of proteins.

Table 2: Average particle sizes of cheese dispersions. Samples processed at high temperatures, independently of the heat treatment used, demonstrated particles with a bigger size. In both cases, homogenization was able to reduce the particle size. The sample H + 60 + H was the most polydispersed. Values are mean ± SD (n=2).

Sample ID	Span	$d_{(0.5)}$ (µm)	$D_{[4,3]}$ (µm)
OH + H	2.01 ± 0.10	6.66 ± 0.01	8.83 ± 0.31
H + OH +H	3.07 ± 0.38	4.31 ± 0.66	5.94 ± 1.73
OB + H	2.16 ± 0.02	6.67 ± 0.22	6.46 ± 0.65
60 + H	4.96 ± 0.04	2.84 ± 0.19	5.49 ± 0.42
H + 60 + H	15.72 ± 2.52	0.56 ± 0.11	3.19 ± 0.29

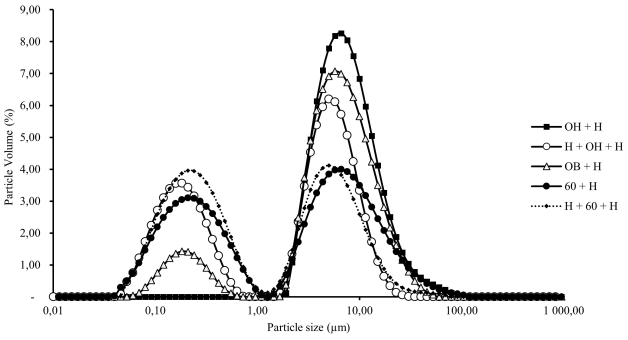

Fig. 1: Diagram of the Particle size distribution obtained. A monomodal particle size distribution was observed for the sample OH + H, whereas bimodal particle size distributions were noticed for the other samples. The plots represented correspond to only one of the replicas produced.

b. Rheological properties of cheese powder dispersions

Viscosity is a measure of the resistance of a fluid towards being deformed when under shear stress and is defined as the ratio of the shear stress to shear rate [31]. The viscosity properties of emulsions before spray drying influences the atomization of the feed and so the characteristics of cheese powder [32]. Data for fluids are often presented in the form of viscosity – shear rate diagrams, as Figure 2 presents for the 5 samples produced. It is possible to observe that the viscosity is independent of time and shear rate for all samples, meaning they all present a Newtonian behaviour [33].

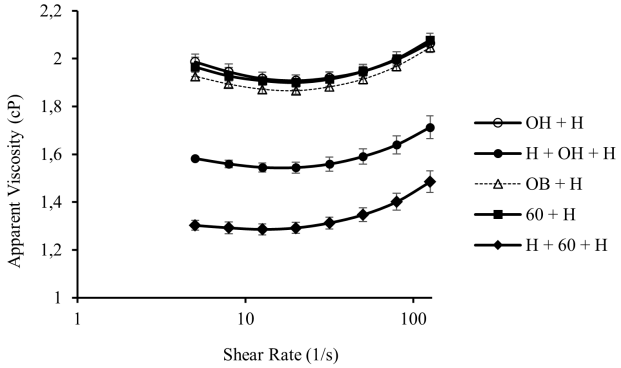

Fig. 2: Apparent viscosity variation of the different cheese powder dispersions as a function of shear rate. All samples demonstrated a Newtonian behaviour once the viscosity did not change as a function of shear rate over time. Samples with a second step of homogenization exhibited a lower viscosity value. The results correspond to the average of the two replicas.

A similar apparent viscosity value was obtained for the 60 + H, OB + H and OH + H samples. The rheogram for the samples with double homogenization shifted downwards, with a maximum shift for the sample treated at 60ºC. The viscosity of cheese powder dispersions was thus decreased by an homogenization step prior to heat treatment. Usually, the smaller the particles, the greater the surface area available for interaction, and so the higher the viscosity [34]. However, this was not observed in the results obtained, as the sample with smaller particles presented the lowest viscosity. Lower viscosities are usually related with unstable samples, which is proven by the fact that the presence of ES leads to an increase in apparent viscosity, when compared to samples with lower amount [32, 9]. Differences in the rheological behaviour can be assumed to be due to particle interactions and structural changes [35] and so, the presence of ES has a positive effect on protein hydration and formation of a continuous protein network structure, which causes a degree in viscosity and fat emulsification [32]. The overlapping results obtained for the other samples indicates that neither the high temperature, nor the type of heat treatment had a significant effect on the flow behaviour of the emulsions.

An adequate viscosity is also required for spray drying, as a too viscous sample can cause problems in pumping and droplet formation during atomization. The high viscosity also has high implications on the powder properties, once it results in a solubility decrease, an increase in particle size and less free flowing powder [9]. Urgu et al., 2018 [32] considered an emulsion too viscous to feed to drier when its apparent viscosity showed results from 5000 to 500 centipoise (cP) . Therefore, all samples produced in this experiment retain the appropriate viscosity for spray drying.

c. Influence of different processing conditions on the stability of cheese powder dispersions

Figure 3 displays the two stability behaviours observed. Samples OB + H and 60 + H presented a stability similar to the sample OH + H (Figure 3**A**), data not shown, while both samples homogenized twice (H + OH + H and H + 60 + H) demonstrated the behaviour showed in Figure 3**B**.

To produce cheese powder, it is a major criterion that the intermediate cheese emulsion does not show any type of instability [32]. However, all samples displayed changes in stability over time, through sedimentation and/or creaming. Samples H + OH + H and H + 60 + H demonstrated a higher sedimentation, seen by the intensity of the BS signal captured (around 11%), when compared to the other samples (around 6%). This could be related to an excess level of insoluble protein that ends up precipitating. However, the opposite behaviour for creaming is observed. We hypothesize that the low creaming layer may be related to the presence of smaller fat droplets that take longer to cream, or to the interaction between fat globules and proteins. The width of the sedimentation layer should also be taken into consideration. As seen in Figure 3, the OH + H, OB + H, and 60 + H present a higher sedimentation layer, which may indicate differences in particle densities, compared to the other two samples, that present a more compact layer.

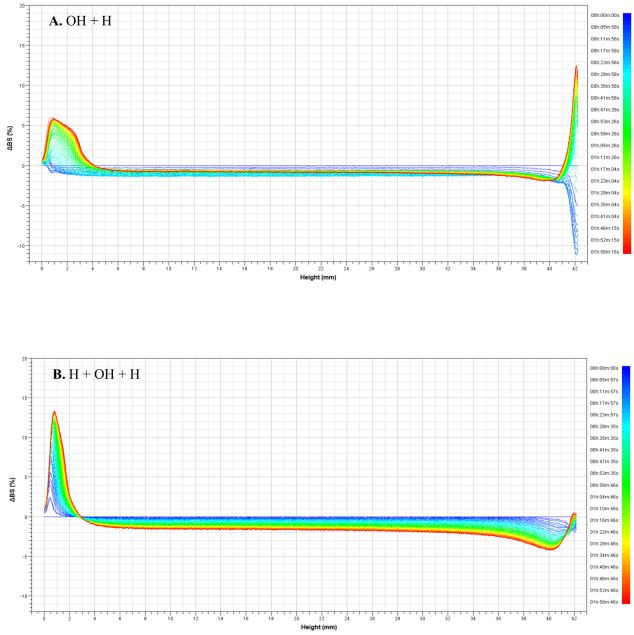

Fig. 3: Physical stability (percentage of backscattering versus vial height). Sedimentation of proteins is associated with an increase in BS% at the bottom of the vial (left part of the graph), while an increase at the top is usually related to the presence of fat droplets in a process called creaming (right part of the graph). Samples with a second step of homogenization exhibited more sedimentation, whereas the other samples presented more creaming.

d. Microstructure of cheese disperisons evaluated by CLSM

Confocal Laser Scanning Microscopy was used to confirm the effects of the different processing conditions used on particle size, rheological properties, and stability behaviour, through the visualization of the microstructure of the samples, Figure 4. The CLSM images for the H + OH + H and H + 60 + H samples exhibited evenly distributed protein particles (green), and small fat droplets (red), which confirms the positive effect of homogenization. For the H + OH + H sample it can also be observed that the surface of the protein particles are coated by fat droplets in a thin layer (yellowish), which may indicate the interaction between fat and proteins, hypothesized in the previous Subsection c for the stability results obtained.

Both these samples demonstrated a better microstructure when compared to all the others, where the CLSM images revealed irregular protein aggregates, void area (black) which is mainly water and fat clusters (orange), where protein and fat droplets were disconnected from each other with little indication of emulsification. These irregularities in sizes corroborate the differences in particle densities, leading to a higher sedimentation layer, observed in Subsection c.

IV. Protein, fat and calcium balance

The cheese powder used for the production of the present cheese powder dispersions does not contain emulsifying salts, which are known to have the ability to sequester calcium from the casein matrix, through the exchange by sodium ions, leading to casein solubilization, and further fat emulsification [7, 36, 11]. It is possible to correlate the emulsification stability of a cheese feed with the powder properties, and so, a good quality cheese powder presents a low amount of free fat and a high protein solubility, which means that the optimal processing condition needs to be able to diffuse the calcium from the casein micelles, solubilizing it, and provide a high amount of fat in the soluble phase, as it would indicate interaction with soluble protein.

For this purpose, the differences in intact and soluble protein, calcium balance and fat content after the different heat treatments were calculated based on the dry matter (DM) content, Table 3. After centrifugation, three layers were obtained, and one can assume that fat was primarily found in the top layer, precipitated protein in the bottom phase, leaving an opaque watery phase in between upon phase separation, where soluble protein can be found.

Heating has a great effect on calcium equilibrium and its interaction with caseins, and it is agreed that high temperatures lead to a decrease in diffusible calcium, due to precipitation of calcium phosphate. Therefore, it is expected that samples treated at a temperature of 85ºC demonstrate a lower amount of soluble calcium, once this mineral is forced inside the micelle, as caseins are effective stabilizers of CCP [15]. Under our experimental conditions, the sample heated at 85ºC in an oil bath (conventional heating) corroborated this theory, as it showed a value of 22.95 ± 9.29 % of soluble calcium. However, this does not apply to both samples treated with OH (54.23 ± 5.50 % and 45.27 ± 9.18 % of soluble calcium in H + OH + H and OH + H, respectively), which presented values of soluble calcium closer to the one obtained by the sample treated at a lower temperature (60 + H - 48.05 ± 16.23 % of soluble calcium). Since both OB and OH samples were treated at 85ºC and there is a significant difference in the results obtained, it is possible to infer the positive impact that the ohmic heating technology has in the shift in calcium balance, enabling it to dissolve from the micelle and migrate to the soluble phase. It can be suggested that the moderate electric field may have imposed, even subtly, disturbances on the charge organization within protein structure and its orientation in the direction of the applied electric field [37], which facilitated the diffusion of calcium.

In this study, soluble protein was defined as the one that did not sediment after centrifugation at 5000 x *g* for 1 hour. All samples demonstrated a similar trend regarding the protein content, with a higher percentage in the soluble phase, followed by the sediment, and then the top layer, which is mainly constituted by fat. Regarding the fat content, all samples displayed a higher amount in the soluble phase compared to the other layers. It is difficult to correlate the results between samples, once many parameters were changed. However, the effect of ohmic heating can be evaluated by comparing the OH + H sample with the OB + H sample, as both were treated at 85ºC for 5 min with one homogenization step, varying only the heat process used. By looking at the results of the protein and fat content and the calcium balance all together, the great impact of OH in all these variables is undeniable. As said before, the ideal process would lead to an increase in the diffusible calcium, which was already discussed that the OH technology had a positive impact, and would increase the amount of soluble protein (58.50 ± 1.47 % for OH + H, compared to 52.06 ± 1.85 % for OB + H) and soluble fat (52.15 ± 1.57 % for OH + H, compared to

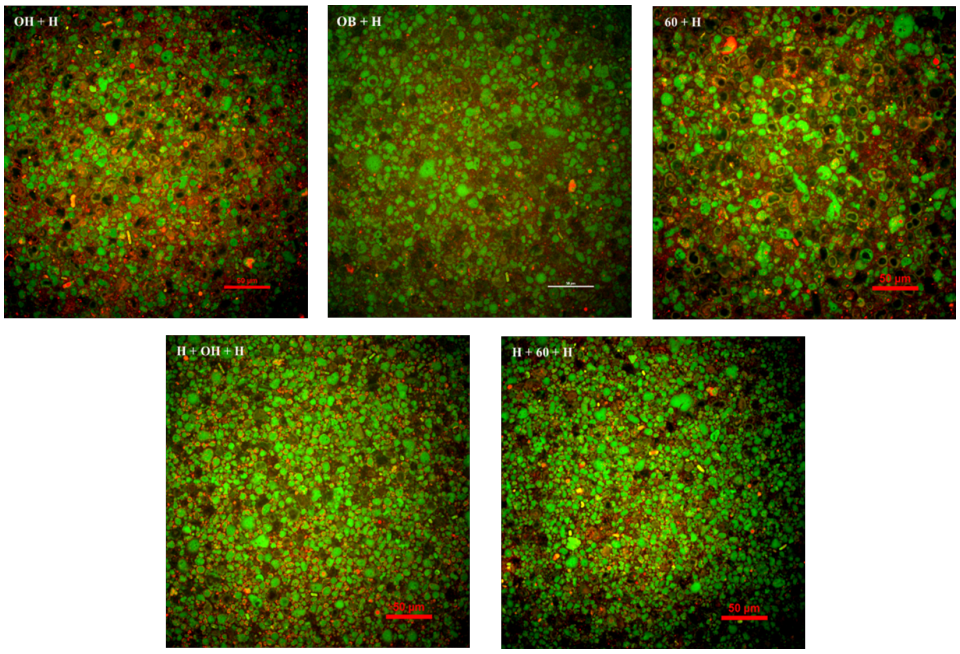

Fig. 4: Microstructure of cheese dispersions visualized by CLSM. Green coloring represents protein and orange represents fat. Representative images are chosen from a series of images available for each sample composition.

45.34 ± 0.28 % for OB + H), demonstrating a higher protein solubilization and fat emulsification. The H + OH + H sample presents the higher amount of fat in the sediment (17.27 ± 0.46 %) which can be related to the assumption that fat is interacting with insoluble protein, that ends up precipitating. To produce cheese powder, this type of sample could be mixed and spray-dried quickly, and once the fat is surrounded by protein it is protected against oxidation, but for other applications, as for solubilization of the powder, it would not be ideal. Therefore, the solubility of proteins still needs to be improved.

The solubility and aggregation state of proteins depend on a balance between temperature and pH. Therefore, we suggest complementing this present study with the variation of pH, with the aim of controlling the surface electric charge of proteins and aggregation process, so that we can benefit from both the presence of some aggregated particles, responsible for the increase in viscosity, and from non aggregated proteins that will act as fat emulsifiers. Casein micelles are stable at high temperatures, and moderate heating does not cause aggregation between micelles nor disruption of their internal structure. However, changing the pH can easily destabilise the micellar integrity. Proteins are usually insoluble at their isoelectric points, which is pH = 4.6 for caseins. Increasing the pH from the isoelectric point gives rise to more negatively charged micelles, which strengths the repulsive forces of the caseins chain, increasing solubility, and micelle size, due to producing loose and expanded structures in the micelles. Additionally, by acidifying the solution, the solubilisation of colloidal calcium phosphate from the micelles increases. Since this material is largely responsible for maintaining the integrity of the micelle, the loss of CCP may be possible accompanied by the dissociation of caseins from the micelles [38, 39], that may interact with fat droplets. The mineral balance is very dependent on pH and temperature, and so it would be interesting to look at the set effect of these two physicochemical parameters.

V. Conclusions

This master thesis opens a new knowledge platform regarding the effects of ohmic heating on cheese feed stability, hypothesizing that the use of this technology as a heat treatment to process the blend may change the protein, fat, and calcium balance, creating a stable emulsion.

The ohmic heating treatment was compared with the use of conventional heating and different parameters were used, such as temperature, 60ºC and 85ºC, and number of homogenizations. For both heating treatments, higher temperatures led to an increase in particle size, which we hypothesize to be due to aggregation of casein micelles and β-Lg. An extra homogenization prior to heat treatment was able to decrease the particle size, through disruption of protein agglomerates and fat droplets. In terms of rheologic behaviour, all conditions produced emulsions with Newtonian behaviour and with values of apparent viscosities appropriate for spray drying. Regarding the stability of the emulsions, all samples displayed instability mechanisms, through sedimentation and creaming. Samples processed with a two step homogenization led to a higher sedimentation intensities, which could be due to the presence of an excess of insoluble protein that ends up precipitating. The creaming layer was lower for these two samples, which we hypothesize to be related to fat droplets interacting with protein forming complexes that remain stable.

The positive impact of ohmic heating compared with conventional heating was seen, however, in the differences between the calcium balance and fat and protein contents, by comparing the results obtained for the two samples heated at 85ºC for 5 min with only one step homogenization (OH + H and OB + H). Despite the high temperature, which is known to hamper the diffusion of calcium, the use of moderate electric field led to a significant higher solubility of calcium, compared with the conventional heating technique. This is thought to be related with the disturbances on the charge or-

Table 3: Chemical analysis of the cheese powder dispersions. Fat, protein, and calcium content (%) based on the dry matter content (%) for each layer obtained after centrifugation. Values are mean ± SD (n=2).

Sample ID	Layers	Dry Matter %	Fat %	Protein %	Calcium %
OH + H	Top	13.69 ± 0.27	33.28 ± 0.74	5.99 ± 1.14	
	Water phase	62.18 ± 0.75	52.15 ± 1.57	58.50 ± 1.47	45.27 ± 9.18
	Sediment	24.13 ± 0.48	7.88 ± 0.83	35.51 ± 0.10	54.73 ± 9.18
H + OH + H	Top	13.76 ± 0.90	29.59 ± 1.84	10.65 ± 0.59	
	Water phase	59.07 ± 2.28	47.18 ± 2.51	53.32 ± 0.68	54.23 ± 5.50
	Sediment	27.17 ± 1.39	17.27 ± 0.46	36.02 ± 0.09	45.77 ± 5.50
OB + H	Top	15.96 ± 0.48	38.76 ± 1.13	6.07 ± 0.74	
	Water phase	55.52 ± 0.27	45.34 ± 0.28	52.06 ± 1.85	22.95 ± 9.29
	Sediment	28.52 ± 1.74	12.44 ± 0.87	41.87 ± 2.59	77.05 ± 9.29
60 + H	Top	16.23 ± 0.39	39.77 ± 0.69	9.71 ± 3.11	
	Water phase	60.88 ± 2.46	50.11 ± 1.27	55.94 ± 1.02	48.05 ± 16.23
	Sediment	22.89 ± 2.08	6.83 ± 0.14	34.35 ± 4.13	51.95 ± 16.23
H + 60 + H	Top	12.59 ± 0.29	30.22 ± 0.62	12.92 ± 1.94	
	Water phase	64.20 ± 3.19	57.69 ± 1.22	54.99 ± 2.42	37.16 ± 1.34
	Sediment	23.21 ± 3.59	9.74 ± 1.20	32.09 ± 4.37	62.84 ± 1.34

ganization within protein structure and its orientation in the direction of the applied electric field, which facilitated the diffusion of calcium. Fat emulsification and protein stabilization play equally important roles in keeping cheese emulsion stability, and so the higher values of soluble protein and fat when OH was used, also demonstrates the great potential this technology shows on emulsion stability.

Overall, ohmic heating is an exciting opportunity to improve the quality of food, and continued innovation will benefit both food manufacturers and consumers. Results obtained in the present research clearly show that ohmic heating technology is a powerful tool that mainly interferes with calcium solubilization, which consequently affects fat emulsification by proteins. However, there is still room for improvement, as the technology still demonstrated the appearance of instability mechanisms, such as sedimentation and creaming, and appeared to have an adverse effect on protein solubility, as fat is interacting with insoluble protein.

a. Future perspectives

This study is just the "Once upon a time" of a story where ohmic heating is the main character. The plot still needs to be written, and other parameters still need to be casted. It would be very interesting to investigate more about the effect of ohmic heating on caseins itself, by understanding the effect of this technology on protein charge, which can be done through the measurement of ζ-potential, and also by investigating which individual casein proteins are affected by OH, through SDS-PAGE. In this study, OH was used at 85ºC to investigate the effects of extreme conditions. Looking at the effect of a broader range of temperatures and voltages would be a way to find the ideal conditions for emulsion stability. In order to improve protein and calcium solubility, it would be appealing to combine the effect of temperature and electricity with pH variations once it is known to alter solubility of proteins and calcium phosphate. Furthermore, the use of OH should also be linked with the use of different cheese types and compositions, different ages, and different addition of ingredients once these seem to also have an effect on emulsion stability.

There are no studies regarding the effect of ohmic heating on cheese feed stability for cheese powder production, which makes this is an innovative investigation that opens doors to a whole new field where this new technology is used as processing condition and has a substantial potential to change and enhance the dairy industry. It still requires a great amount of research, and I would leave the suggestion for the next experiments to be carried out using natural cheese instead of cheese powder, as it would reproduce a more authentic and more studied cheese emulsion. Recreating cheese feed using cheese powder just forms a more complex system that may influence the results in a certain way. Furthermore, more than two replicas should be made for each sample, so that statistical analysis can be performed.

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