General Conclusion and Outlook

The main aim of this study was to elucidate the structure formation processes occuring in protein rich, dispersed on a molecular level. The driving force that lead to the destabilization of the investigated model process cheese systems was hydrophobic interaction. Concerning the plots of the hydrophobic cluster analysis as shown in chapter 1 and the fact that the emulsifying salts enable especially alphaS1 and beta caseins to interact on a larger hydrophobic surface, the formation of insoluble structures during the processing of casein matrices is conclusive with the data presented within this work.

The differences in the apparent viscosity seen in the aluminium and steel are most likely to be due to a promoted autocatalytic reaction as it was presented for example in @Lenze2019 and @Cernikova2018a also referred to as the addition of rework. The samples proceesed in the aluminum cup showed faster processing times and lower values of viscosity in general. The latter is probably due to the increased heat transfer in the aluminum cup. The first effect however could be due to the far more porous surface of the aluminum cup, where special pre aggregated protein seeds are formed in the cracks of the cup, which then promote the autocatalytic reaction. This would also explain the remaining variance (~20%) in the process times, that could not be attributed to either compositional or process parameters. The modellation of a flow-curve or rheological profile

The formation of the kappa casein fibrils and the amorphous aggregates as seen in @Vollmer2021 could be further characterized in this study. The present study showed, that the secondary aggregates besides the kappa casein fibrils are dominantely made up out of beta and alphaS1 casein. The alphaS2 casein proved to play a secondary role in the formation of large aggregates, however the correlation analysis performed in the last section of this work gave insight towards alphaS2 playing a kind of transport function for hydrophobically associated caseins through the hydrophilc structures or domains. Interestingly, the alphaS caseins showed no affinity to each other in every one of the investigated phases. It can be concluded, that the protein-rich structures found during TEM imaging in @Vollmer2021 are amorphous aggregates from alphaS1 and beta casein, possibly connected by kappa casein fibrils or seeds therefrom. The areas that were depleted of protein are supposedly populated by the alphaS2 caseins and hydrophilicly interacting caseins. This theory could be supported by experimental data, when investigationg a colloidal system containing 3% total protein (w/w) of casein and an aliquout of melting salts. Chelation of the calcium phosphate nano clusters from the micelle up to a value of 70% is reported to lead to full dissociation of the micellar structure (@Fox2016). In @Vollmer2021a, a critical threshold value of PP of 1.2% is reported, to induce complete dissolution of CCP from the micelle. Hence it can be concluded, that the threshold value for complete micelle dissociation is reached within this study and the caseins are present, at least theoretically, in monomeric form. What cannot be estimated by the acid-base-titration as performed in @Vollmer2021a, previously stated by @WolfschoonAndlinger is the amount of proteinogenic bound calcium, in particular serine bound calcium. The cheeses investigated in @Vollmer2021a did not show a fibril formation as pronounced as in @Vollmer2021. The differences in the model matrices were a higher mass (~25% higher) that was processed and the abundance of TSC as a melting salt. TSC has been reported to be FINISH PARAGRAPH!!

Diffusing wave spectrometry (DWS) poses as another tool to follow aggregation processes, as in @Alexander2006, but also destabilization phenomena, as in @Hemar1999 and @Vogt2015. The latter used DWS and small amplitude oscillation rheology, on heated cheeses (Mozzarella, medium Cheddar, aged Cheddar) and determined a progressive increase of free water in the system as cheese is heated. This effect is also seen in the T2 relaxation data obtained in this study. Over the course of processing, a progressive increase in the mobile fraction of water (T2 ~120 ms) was apparent, whereas the T2 relaxation of the detected fat-phase decreased. Considering the effects of matrix separation, it can be concluded from @Vogt2015 that a matrix separation in heated cheeses is also indicated by a redistribution of water. Since the progressive loss of mobile water is detectable without apparent (or forced, as in @Dang2019) syneresis or water evaporation as indicated by a strongly increasing dry matter, this must mean that there are areas in the aggregated casein structure, that fulfil the function to hold the water in the system, whereas the other caseins participate in hydrophobic network formation.

@Vollmer2021 reported such a matrix separation in a model processed cheese system, that was processed using high heat (90 C) and approximately half the amount of stirring speed (~8 rpm) in a small processing

unit with a sample weight of ~40 g. The matrix separated into areas of high electron density, where the appearance of fibrillar or tubularily aggregated casein was detected and areas of lower protein density, where amorphous aggregates were displayed. When over-processing the cheese matrix, i.e. excessing the amount of energy and forced collision from shear that is needed to perform the 'creaming-reaction', a particulated, almost chrystal-like structure of the cheese becomes apparent, as it can be seen in Fig.xx (section 2). It has to be noted, however, that these samples were by design strongly overprocessed, in order to find an apperent "end" point of the creaming reaction, or a maximal aggregated product. This overprocessing also lead to a noteable increase in dry matter of ~10%, however, what could be seen were lighter and darker areas in the protein structure (Fig.xx).

Figure 1: Over-processed 'end-point' of the creaming reaction, appearance of the product. Incorporation of lighter globular areas (circle) into a darker matrix, containing fat (asterix)

Applying the general concept of imaging techniques that darker areas indicate areas of higher protein density, it can be readily concluded from the appearance of the samples at the "end point" of the creaming reaction, that a phase separation has taken place. From the investigation of the centrifugationally separated phases it was apparent, that the structures that were defined herein as 'hydrophobic-aggregates' were insoluble in water, but did show a stronger tendency to swell. The swelling behaviour was displayed in the pellet as well as in the cream phase (section 4 of this work). This shows, that the hydrophobic structures are formed under the exclusion of water at their core, but with hydrophilic structures, that are probably high in charge, at their surface. This also means, that the hydrophobic network formation might occur *in-situ* under the exclusion of water, without the necessety of water to leave the system, since it can be, in a way, compartementalized in thy system. This is supported by the fact that the samples investigated in @Vollmer2021 were not processed to the particulate "end-point" of the creaming reaction as described earlier, but were still elastic, probably due to the remaining water in the system. This behaviour of proteins is commonly known and one of the main reasons, why many proteins, such as whey proteins, appear in globular form.