Rheological Profile of structure formation in model processed cheese

Introduction

The rheological profile or generally spoken the viscosity of dispersed systems in the food, pharmaceutical or cosmetic industry is an important parameter for the quality assurance of such products. Additionally, the rheological behavior under certain process conditions can give insight towards the structuring mechanisms occurring on a molecular level. Many studies have been performed on dispersed an highly concentrated food systems such as processed cheese. Most of them were compositional in nature, meaning that effects of certain educt components on the properties of the final product were investigated. @Salek2017 investigated the effects of special mixtures of emulsifying salts on the hardness of processed cheeses. Maximum hardness was achieved by a combination of DSP and TSPP. @Cunha2013 studied the effects of different types of fat (hardened and non hardened plant oil in comparison to butter) on the texture of processed cheese. Samples made with both plant oils showed lower fat globule size, higher viscosity and higher hardness than those made with butter.

The step-wise structure build-up has been described in detail by @Lenze2019. The aggregation process was identified as the formation of a continuously aggregated protein matrix wherein the fat is first emulsified and then de-emulsified prior to network formation of the proteins. To the authors knowledge, either other proteinogenic entities than predominantly casein were present during processing, or surface active ingredients or dairy cream was used as the fat phase, hence the structuring and simultaneous emulsifying properties of casein alone has not yet been determined. The TEM images obtained by @Vollmer2021 are from samples that were free of proteins or surfactants other than casein. The samples were prepared by the author, with reduced processing speed in order to get a detailed representation of the structure formation processes occurring. TEM imaging revealed the early presence of fibrillar structures, that hold the fat in the matrix and also form a dense network.

Step-wise structure formations were also detected in the works of @Noronha2008 and @Noronha2008c. Two peaks were detectable during processing of a model processed cheese in a blade cooker. The first peak or increase was attributed to water uptake and the formation of a hydrated casein matrix, since it was reported that 75% of the added water was absorbed. Subsequently, a fat emulsification took place. After sufficient emulsification of the dispersed phase, the fat particles were reported to be incorporated into the cheese matrix which lead to the formation of a cohesive matrix as indicated by a second peak.

It has already been shown by others that initially micellar casein sources such as native casein or rennet casein only perform the process known as “creaming reaction” under the presence of emulsifying salts. The micelle must be (at least partially) dissociated to form new structures. When using sodium caseinate as source material, no such dissociation is necessary or even possible, since the caseins are already present in monomeric form. In order to get an overview of the structure formation induced by casein overall, several model cheeses with varying protein sources are produced in this study: rennet casein, native casein and sodium caseinate. Salt composition is varied in samples made from sodium caseinate using HCl and citric acid in order to investigate potential structure formation processes that occur from caseins alone without the addition of melting salts. A further simplified model system was used, which contained 15% total protein for the structure formation and 20% fat (plant oil). The dry matter of the system is around 40%. In order to investigate structural changes during processing, the dairy matrix can be processed in a shear-stress rheometer, in which the structure build-up under controlled shear and heat can be followed as in @Lenze2019.

The aim was to find two distinctly different rheological profiles in order to further investigate (chapter 4) if the changes in rheological behavior can also be seen in distributional and compositional data of the matrix, analyzed at multiple steps of processing.

Material and Methods

Production of model process cheese premix
The composition of the processed cheese premix used corresponded to the model processed cheese recipe developed by @Lenze2019 as follows:

Table 1: Recipe for the model processed cheese investigated throughout this study

Ingredient	Source	Amount (w/w, %)
Protein	Casein (rennet, native or sodium)	18.42
Fat	Sunflower oil	19.59
Water	Milli-Q water	58.48
Emulsifying salt	Trisodium citrate, dibasic (Na3C6H5O7 · H2O)	0.44
—	Disodium phosphate dihydrate (Na2HPO4 · 2H2O)	0.44
—	Pentasodium triphosphate (Na5P3O10)	1.75
Acid for target pH=5.88	Citric acid monohydrate (C6H8O7 · H2O)	0.88

The emulsifying salts displayed in Table 2 stayed the same throughout this trial to ensure an equal dissociation of the caseins and therefore making the single analyses comparable with each other. In the subsequent sections of this work, the single emulsifying salts will be referred to as their abbreviations (i.e. TSC, DSP, PP) shown in Table 2. Also when speaking of "the emulsifying salts" or "the emulsifying salt mixture" or "the melting salts", the combination of these salts with citric acid as acidulent is meant. In Figures and plots within this work, the term "emS" is used as abbreviation for the emulsifying or melting salt combination.

The recipe displayed in Table 2 is based on the original product; yet, influencing factors such as the type of fat or the age of the cheese can be reduced in order to obtain a system that is as reproducible and comparable as possible. In the course of the present work, native (micellar) casein, rennet casein and sodium caseinate were used as protein sources. Sodium and rennet casein were commercially available products (ICL, Tel Aviv, Israel), the native casein was produced as it was described in detail in @Dumpler2018. Sunflower oil was used as fat. The pH value, which should be ~5.88, was adjusted using powdered citric acid. The melting salts were also weighed out as powder the dry matter.

The individual recipe components were weighed into a beaker on an analytical balance. The protein powder was weighed separately into a weighing dish. The protein-oil-water dispersion was prepared with the aid of the dispersing device "Ultra Turrax T25" at a speed of about 5,000-9,000 min-1. First, the salts, oil and water were mixed to form emulsion until the salts were dissolved. Then the protein powder was slowly mixed in to obtain a homogeneous processed cheese premix. The pH value was determined shortly before processing using a solid pH meter.

Production of the processed cheese and viscosity measurement
Thermal processing of the processed cheese premix and the simultaneous online viscosity measurement was carried out using the "AR Rheometer 1000" from TA Instruments. The rheometer is equipped with a peltier element and an external water bath to control the temperature in the sample cup. A stirrer blade with a length of 6 cm was used as the measuring geometry, which is attached to the drive shaft with a screw connection. This was used both for viscosity measurement and for mixing and shearing the cheese mass. To close off the sample cup and prevent water loss, a suitable lid was made and fitted until the stirring blade could rotate smoothly. The samples were processed at ~15 min^-1 at 90 C. In a later experimental set-up that was also used to produce the samples in @Vollmer2021 and @Vollmer2021a, the aluminum cup was replaced with a V4 stainless steel cup, to reduce friction of product, that adhered to the walls of the aluminum processing cup. The steel-cup was used on an "Anton Paar MCR-700" rheometer, temperature and speed settings remained the same for samples analyzed during this trial. An image of the experimental set-up can be seen in @Vollmer2021.

Shortly before the measurement, the process temperature was set. The premix was placed in the sample cup and the measurement started as soon as the desired temperature was reached. Flow curves were obtained as a function over time time and measurements were performed in triplicate.

Comparison of models tested during model development

The models tested in this section were derived from the model processed cheese samples as they were presented in @Lenze2019. The model was further developed, to include only, or mainly caseins as the proteinogenic phase in order to follow the structuring events happening only from caseins. The three different casein powders were compareable in their composition, with ~90% protein and 1% whey proteins. The manufactured native casein had a lactose content of ~1%, the lactose content in rennet casein was the highest with ~7% (w/w), sodium caseinate had ~5% of lactose. This was resembled by the darker color of the samples made from the latter two after processing, probably due to the formation of early Amadori products. Since lactose was used as a dry matter supplement in samples with reduced fat content in @Lenze2019, the gamma-lactosylation of lysine residues in the casein, which is responsible for the coloring, is not a reaction that hinders the structure formation in any way.

The varying parameters of the tested models are summarized in Table 3, an overview of their flow-curves is displayed in Fig.1.

Table 2: Composition and processing conditions of the samples tested during model development

casein	salt	fat	premix	cup-applied shear
native	emulsifying salt mixture (emS)	oil	yes	alu-200
native	emulsifying salt mixture (emS)	oil	yes	steel-200
rennet	emulsifying salt mixture (emS)	oil	yes	alu-200
rennet	emulsifying salt mixture (emS)	oil	yes	alu-100
rennet	emulsifying salt mixture (emS)	oil	yes	steel-200
rennet	emulsifying salt mixture (emS)	milkfat	no	alu-100
rennet	emulsifying salt mixture (emS)	milkfat	yes	alu-100
rennet	emulsifying salt mixture (emS)	oil	no	alu-100
sodium	citric acid (CitAc)	oil	yes	alu-200
sodium	emulsifying salt mixture (emS)	oil	yes	alu-200
sodium	Hydrochloric Acid (HCl)	oil	yes	alu-200

Fig.2 shows the viscosity increase of the tested models that were different in their oil composition. If the fat source was dairy cream, structure formation took place even without an premixing step (green). The aim of this work was, however, to investigate *inter alia* the emulsification properties and further potential aggregation of casein coated fat globules or particles, at later stages of processing. To do so, no pre-emulsified fat other than emulsified with caseins, should be present. Milk fat is pre-emulsified fat, The milk fat globule is stabilized by milkfat globule membrane proteins. Therefore, plant oil in the form of sunflower oil was used as the dispersed phase. However, without a pre emulsifying step which is furthered referred to herein as premixing was included in the sample preparation. The effect of premixing can be found in Fig.2 as well, the violet points show a non-premixed system, the red points are the same sample, processed after premixing at ~8000 rpm.

The overall rheological profile of samples made from native or rennet casein, differs only slightly. Native casein samples show a faster structure formation than rennet casein samples, as well as an overall higher viscosity. This can be due to the different initial particle sizes of the casein powders, since the rennet casein powder had a more granular consistency, whereas the native casein powder was powdery. @Dickinson2012 described the formation of stronger gels from inhomogenously hydrated or dispersed samples, due to faster bridging-flocculation of the fat particles. Bridging-flocculation was promoted by vast amounts of unadsorbed

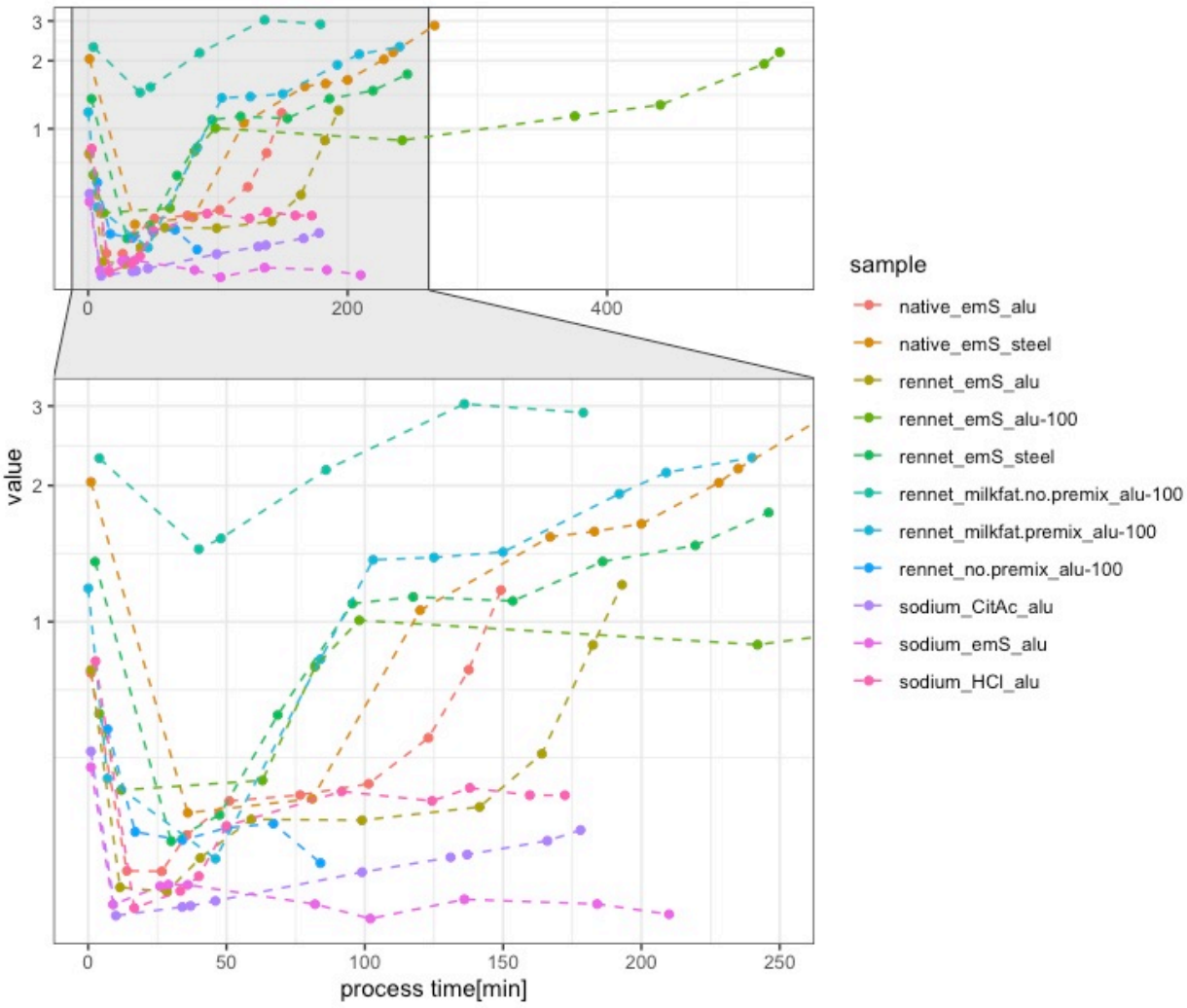

Figure 1: Overview of Viscosity Development during structure-formation reaction in model processed cheeses varying either in composition (native, rennet or sodium casein[ate] with the mixture of emulsifying salts used herin [emS] or sodium caseinate with either [HCl] or citric acid [CitAc] for pH adjustment), preparation (pre-homogenisation [premix, no.premix] and pre-emulsification of fat [milkfat, oil is the default]) or processing conditions (heat-transfer i.e. free Energy [alu, steel], or shear rate i.e. mixing/aggregation rate [alu-100, alu 200/s is the default])

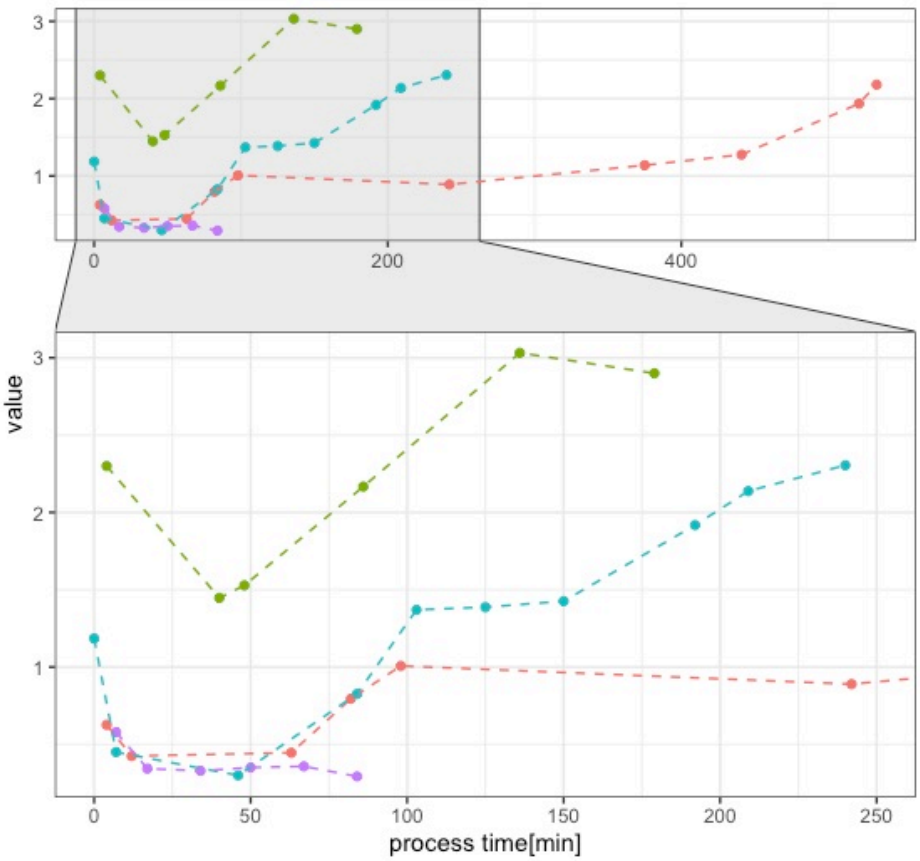

Figure 2: Comparison of Rheological profile during structure formation of pre-homogenised (red, blue) or non-homogenised (violet, green) samples and of pre-emulsified (green, blue) fat (in form of milkfat) or unemulsified (red, violet) fat (in form of oil). Note that the red and violet curves are identical in their composition but vary in terms of pre-homogenisation.

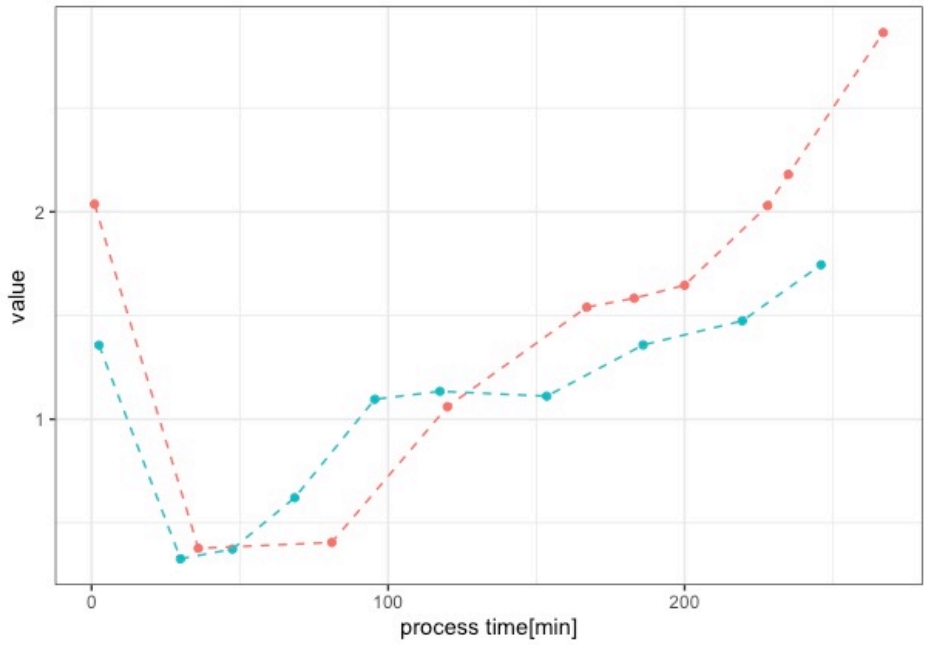

Figure 3: Rheological Profile of samples made from native (red) or rennet (blue) casein, processed in a steel cup

and also in parts unhydrated protein or casein particles. This also lead to a prefered formation of particulate fat globules and thus, for an emulsion filled gel to the formation of a particle gel. This is represented in Fig.4 by the faster processing of the native casein model cheeses, since a particle gel has a higher rigidity than an emulsion filled gel.

When comparing Fig.3 and Fig.4 it becomes apparent, that the aluminium cup leads to faster processing, which is due to a better heat transfer of the aluminum and the higher porosity in the aluminium cup, which might provoke an autocatalytic effect. Comparing the shear rates, we see that the process speed is dependent on the shear rate, which is in conclusion with rheological behaviour for non-Newtonian fluids, as well as with the faster structure formation (i.e. higher reaction rate), due to higher probability of collision of the particles. Faster processing by higher shear rates was also reported by @Fu2018.

The model testing led to the model composition of Table 1. For faster sample throughput, a processing speed of 200/s in the aluminum cup was chosen. The premixing step was made standard protocol for sample preparation and was set to not exceed 10.000 rpm.

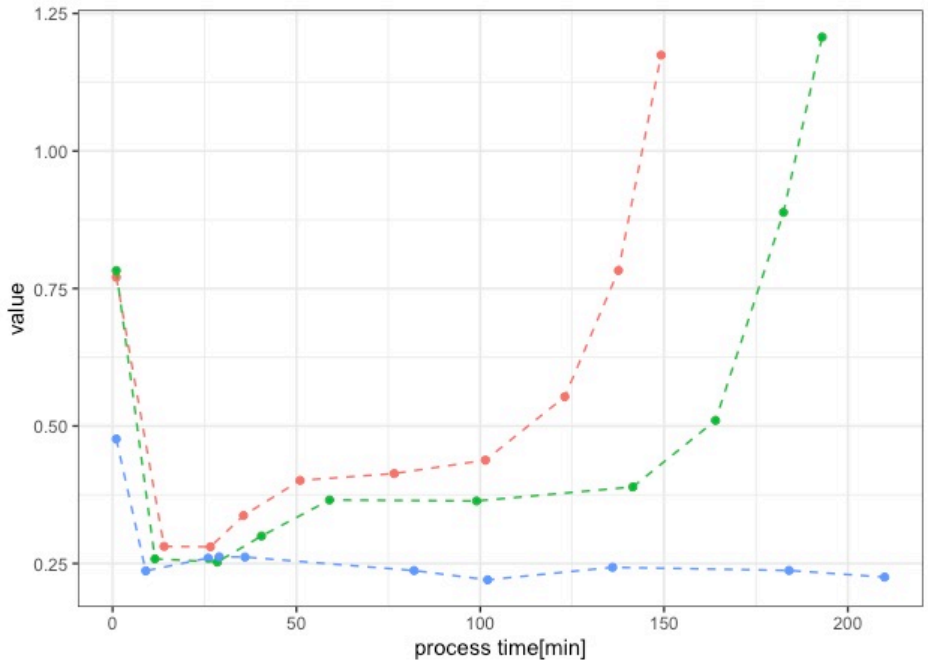

Figure 4: Rheological Profile of samples made from native (red), rennet (green) or sodium (blue) caseinate, processed with the mixture of emulsifying salts used herein in an aluminium cup. The processing conditions for these samples were set to the standard conditions for further analysis.

Results and discussion

Flow curves during processing of model processed cheeses

The composition of model process cheese (Table 2) lead to a dry mater of 40 % with protein concentrations from 15-17% TP, depending on the total protein content of the source material. Samples were pre-mixed prior to processing, since it was found that without an initial emulsification step, no stable educt (melt/sol) could be produced in the processing cup. It should be noted that @Lenze2019 used pre-emulsified fat (milk fat or oil + small molecule surfactants) in the process which led to the stable educt.

Fig.5 shows the flow curves of the processed sodium samples. When processed without melting salts but HCl as pH adjustment, we can see a structure development up to a first plateau (blue). After that, no further structure development could be recorded. The compared to the other samples rather high standard deviations represent a cohesiveness of the matrix at this point, represented by a strong tendency to pull strings, when sampled after 150 minutes. @Vollmer2021 reported that kappa casein fibrils in a similar model processed cheese made from native casein were the key element for structure formation in model processed cheeses, since their supposed building could be followed as well as their structuring of the matrix. Kappa casein is the only casein that is not affected by calcium, which also means that it is not influenced by the ion exchange process that is used for the production of sodium caseinate, as described in the next paragraph. Therefore kappa casein is present in its more or less native state, at least from the point of view of ionic substitutes. Hence, the detectable increase in apparent viscosity from a level of 0.2 to ~0.5 between 40 and 60 minutes of processing could be assigned to an adsorption of kappa casein fibrils to the interphase, possibly as the only larger aggregate present in the system.

In the green curve which was processed with emulsifying salts, no structure build-up could be detected. This might be due to the little amounts of calcium being available in the sodium caseinate samples, thus a far lesser concentration of Calcium Ions can be released into the serum. Sodium caseinate is generated by acid percipitation of caseins with subsequent alkalization and final neutralization; a process that forms single

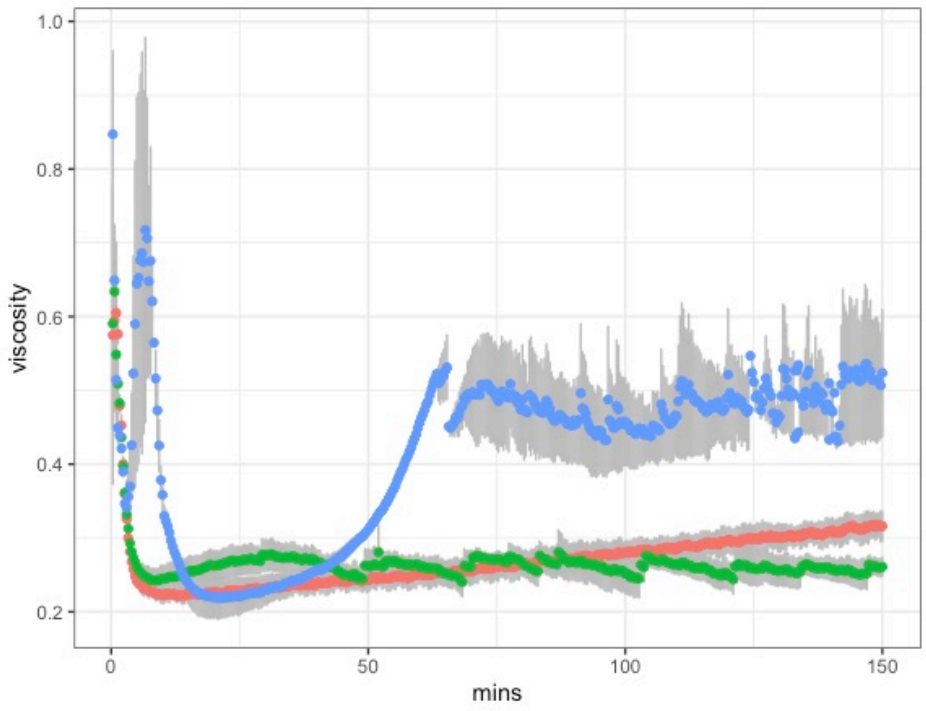

Figure 5: Detailed rheological profile of model processed cheese samples made with sodium caseinate as source material. Variation was in the type of salt used for pH adjustment to a value of 5.88: HCl (blue), Citric Acid (red), emulsifying salts (green). Measurements were performed in triplicate and plotted as mean, variation in viscosity indicated as grey shade.

caseinates, wherein the calcium ions are to some degree replaced with sodium ions, whereby the CCP are completely absent. This means that the remaining calcium ions in sodium caseinate are small in number and directly bound to phosphoserine residues. 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 shown, 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 in monomeric form.

It can be suggested that the initially chelated calcium ions in samples made from rennet or native casein are not immobile, or even inertly bound in their chelated complexes, but could participate in processes that lead to aggregation. This will be discussed in further detail during this work.

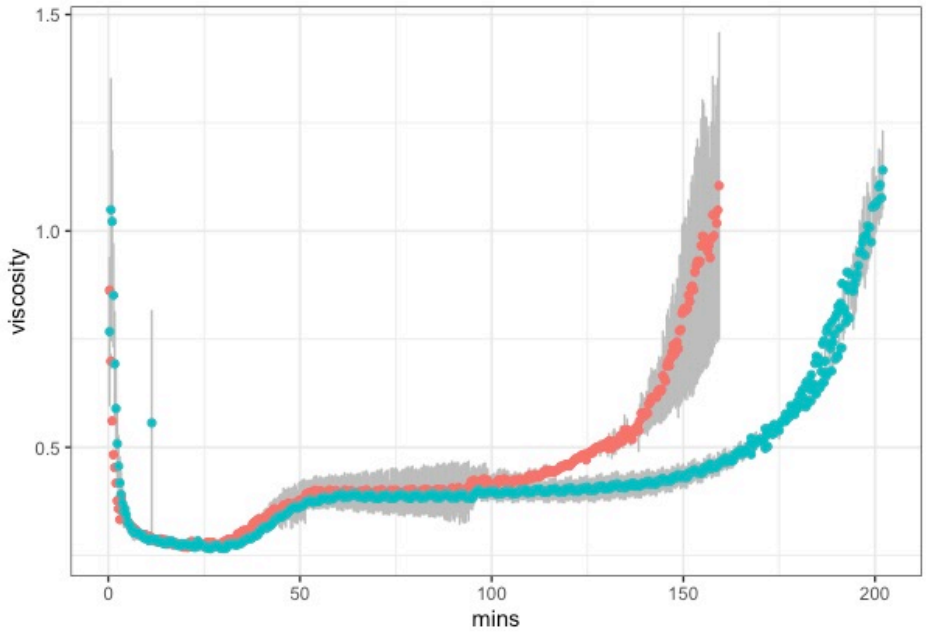

Figure 6: Detailed rheological profile of model processed cheese samples made with native casein (red), or rennet casein (blue) as source material. Measurements were performed in triplicate and plotted as mean; variation in viscosity indicated as grey shade.

Native casein samples build up their structure slightly faster than samples made from rennet casein (Fig.6). Also, native casein had a higher variance in total process duration. The first stages of structure formation up to a processing time of ~100 minutes however, show no difference concerning the source material. The start of the second phase of structure formation is up to 50 minutes earlier than in samples made from native casein. This could attributed to higher matrix inhomogenity of the native casein samples, due to smaller particle size of the powder. When pre-mixing the samples, it was observed that the smaller particle size of the Native Casein showed powdered clusters. It has been previously reported, that a higher matrix inhomogenity, like the appearance of such powdered clusters, leads to higher gel stabilization in emulsion filled gels due to a faster bridging stabilization of the oil droplets (@Oliver2016, @Dickinson2012). Such a bridging stabilzation was shown to be induced by excess unadsorbed protein (@Semenova2010). The rennet casein premixes showed a coarser structure, since the grain size was around 0.1 mm. Thus, the matrix hydrates more slowly, as indicated in the slightly later starting time of the first exponential phase.

Controls (Fig.7) show that native casein without any addition of emulsifying salts at native pH (6.57) shows a slow, if any, structure formation after about 125 min of processing. This is probably due to the slight heat induced dissociation of the casein micelle, and/or a possible re-aggregation of released caseins therefrom. Sodium caseinate shows a higher level of viscosity after melting (0.2) than the native control, which was measured at ~.03. The native control did not produce a stable emulsion or gel evenly during heat-processing;

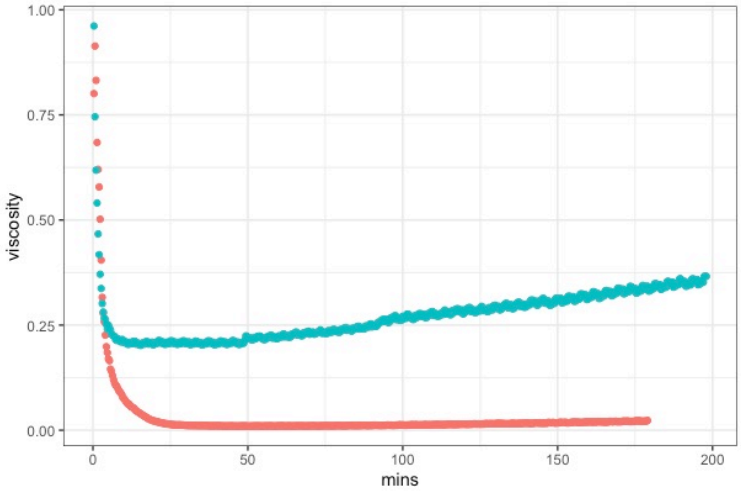

Figure 7: Rheological profile of control samples w/o any addition of salts: native casein (red) and sodium caseinate (blue)

a watery opaque liquid with free fat was apparent next to clumped, i.e. amorphously aggregated structure. Samples from sodium caseinate without the addition of salts did form a stable premix and also showed structure formation.

The comparison of the controls shows, that when processed without salt addition at native pH an overall increase of 0.11 a.u. in viscosity takes place, similar to the increase measured in models with sodium caseinate and citric acid. Slight step-wise increases in viscosity can be seen at 50 min and at 100 min. Those were also the process times, where exponential increases in viscosity were detected in the model samples. It can be concluded, thet the phosphate and citrate salts, maybe also in combination with their respective sodium cations are responsible for the abundance of a detectable viscosity increase in samples from sodium caseinate made with emulsifying salts.

pH values post-processing

The pH for all samples was set to 5.88 +/- 0.02, prior to processing and changed over the course of processing according to Fig.8. Samples from rennet casein and native casein showed an increase in pH over the course of the reaction (up to 6.17), samples from sodium caseinate showed a slight increase in pH for samples made with citric acid but no additional calcium chelators up to 5.86 +/- 0.01. The strongest increase in pH was apparent for samples made from sodium caseinate with emulsifying salts: pH of the processed sample rose to 6.48 +/- 0.02. Only the samples made of sodium caseinate and HCl (i.e. without any Calcium chelating agents) showed a decrease in pH to 5.22 +/- 0.01. The decrease might be due to over aciding as the matrix was strongly coagulated already during premixing, so the initial pH might have been indeed at a lower level to begin with, and expressed itself only after melting. These samples also showed a structure formation up to the first exponential and second log phase of processing. Due to the low pH, it could be also possible that this is not an effect induced by kappa casein (-fibrils), but due to beta casein being close to its IEP (5.20) and therefore emulsifying the fat phase. We can see that the emulsifying salts otherwise generally increase the pH of the samples and thus change the charge in the casein molecules more negatively. The strong pH increase detected in sodium caseinate samples processed with emulsifying salts could be due to certain degrees of dephosphorylation of the calcium sensitive caseinates (alphaS1, alphaS2 and beta casein). Since the hydroxy group of the corresponding Serin residue is a far weaker acid than the respective phosphate group, the pH of a casein containing matrix will increase with ongoing de-phosphorylation of the caseines.

Buffering the pH at the desired value is crucial for the properties of the final product, a lower processing pH (5.2 - 5.6) resulted in more coarse and particulate gels, whereas a pH value of 5.8 - 6.2 gave creamy products

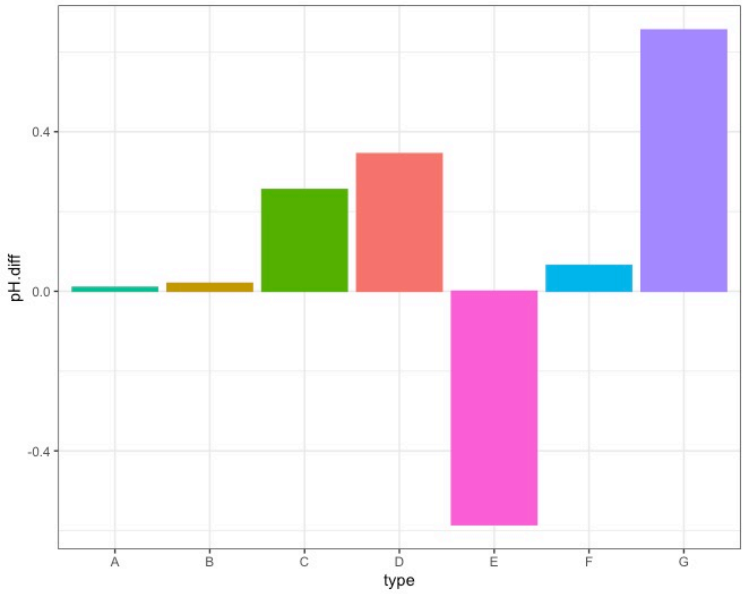

Figure 8: Difference in starting pH of the sample premix and pH of the sample product after processing: (A) sodium caseinate w/o emulsifying salts, (B) native casein w/o emulsifying salts, (C) rennet casein with emulsifying salts, (D) native casein with emulsifying salts, (E) sodium caseinate with HCl, (F) sodium caseinate with citric acid, (G) sodium caseinate with emulsifying salts

(@Barth2017). It is apparent, that the pH value in the model samples from native and rennet casein was buffered in the desired range, whereas the samples made from sodium caseinate couldn't be buffered at the target level.

Occurence of a third log phase and the fitting of a model flow curve

In another embodiment of the experimental set-up, rennet casein samples were processed in a steel cup, using the same temperature and speed settings. To the authors surprise, longer processing times were needed and the rheological profile appeared to be different than the samples processed in the aluminum cup. In total, the steel cup lead to ~30% longer processing times. However, in relation to the respective full process times, the time needed for the first and second exponential phases to start, displayed the same ratio. In Fig.9 the appearance of another plateau phase during the second exponential phase is visible between 12.000 and 15.000 seconds of processing. This plateau phase was also seen in the flow curves of the samples analyzed by @Vollmer2021. Since the samples in @Vollmer2021 were processed at half the processing speed, the occurence of the additional plateau phase seems to display an intermediate halt of structure formation. It is reported that the occurence of large fibrillar structures are very electron dense, i.e. high in protein, next to areas, where a low electron density is apparent. Before the display of the plateau phase, the casein fibrils appear in bundles, during the plateau phase, these bundles get broken down, which seems a reasonable explanation for this effect. Also, it is to be expected, that this part of the second log phase only displays itself in the set-up with an aluminum cup, due to the different physical properties of steel and aluminum.

Firstly, aluminum has an ~5 times higher heat transfer capacity than steel. Also, the aluminium cup displayed a higher friction of the samples due to adherence of the matrix to the walls of the cup. This puts a higher amount of shear stress on the sample, induced by the sample itself. Higher shear led to faster processing in general, as it was shown during model development. It is also possible, that an autocatalytic effect took place in the aluminum cup. The addition of pre-processed sample to a new matrix was shown to induce a rapid increase in structure development (@Fu2018, @Cernikova2018a, @Lenze2019). It is thinkable, that this effect took also place here to a certain degree, but in-situ by seed formation in pores or cracks of the aluminum cup.

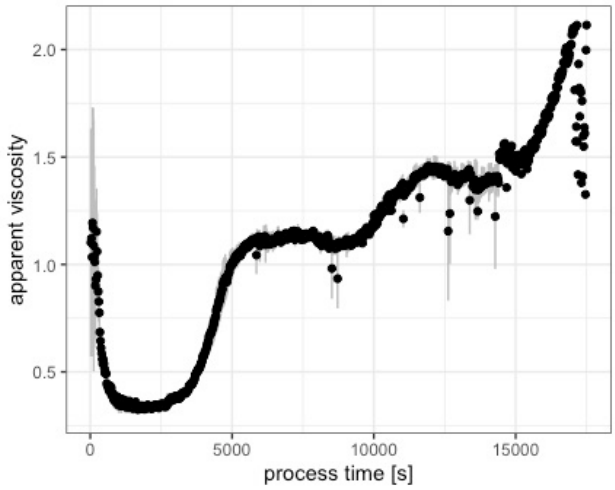

Figure 9: Plotted mean values of the measured apparent viscosity of model processed cheese samples produced from rennet casein prepared in a steel cup: a third lag phase, which represents an intermediate stabilization at an apparent viscosity level during the second exponential phase of structure formation, was observed.

In various other studies, model process cheese matrices similar and compareable to this system were processed by differently shaped means. The unity of all means of processing, either kneading type shearing using a Farinograph as in the works of @Noronha2008c, or processing at high speed using a rapid viscometer as in @Fu2018, a step-wise structure formation is reported. The different structure formation curves that showed a step-wise or two phased process as indicated by exponential increase in apparent viscosity were used to fit a general rheological profile for the model processed cheese, also for the use in later correlation analysis. The flow-curves of native as well as rennet casein were used for the modellation, since it was shown in @Rock2010 and also in this study, that the two protein species displayed no large differences in apparent viscosity.

In order to get the pronounced two step process but also a dynamic lag phase represented in a fitted viscosity model, not the model with the best fit was chosen, but with a good empirical estimation under consideration of the R^2 of the fit. By including the variance of a later or earlier occuring second exponential phase, i.e. the effect of matrix inhomogenity, either due to different fat globule size or powder particle size, which occured during the pre-mixing step, could be included in the model. The plotted mean curves with their respective variance can be found in the supplementary material. To also include the intermediate stabilization described earlier, the curves from the steel cup were also included in the fit. The flow curve was fitted using a generalized additive model. Such "gam" models with integrated smoothness estimation are impelemented within the R programming language, which was used to prepare this thesis. The 'gam' function for basic model fitting takes into account any quadratically penalized general linear model. This means that the regression of every data point or linear sets therefrom are considered within the model. To prevent from over-fitting, the degree of smoothness of model terms is estimated as part of fitting. In more detail, a generalized additive model of the form

$$g(\mu_i) = \mathrm{f2}(i\mathrm{x4}, i\mathrm{x5}) + \mathrm{b0} + i\mathrm{b1x1} + i\mathrm{b2x2} + f(i\mathrm{x3})$$

wherein the response variable, in our example the viscosity, is represented as an expectation μ_i withing a link function $g(x)$. The general additive model ("gam") of this formula would then be

$$y = x1 + x2 + s(x3) + s(x4, x5)$$

Per definition of "gam" within R, a maximum smoothing term is applied and the fitted

$$y(x) = g(s(x))$$

resulted in an $R^2 = 0.71$. As it is the case for many built-in numeric operations in R, the algorithm for the general additive model has an implemented, nested two-side ANOVA test. The fitted values are displayed in Fig.10.

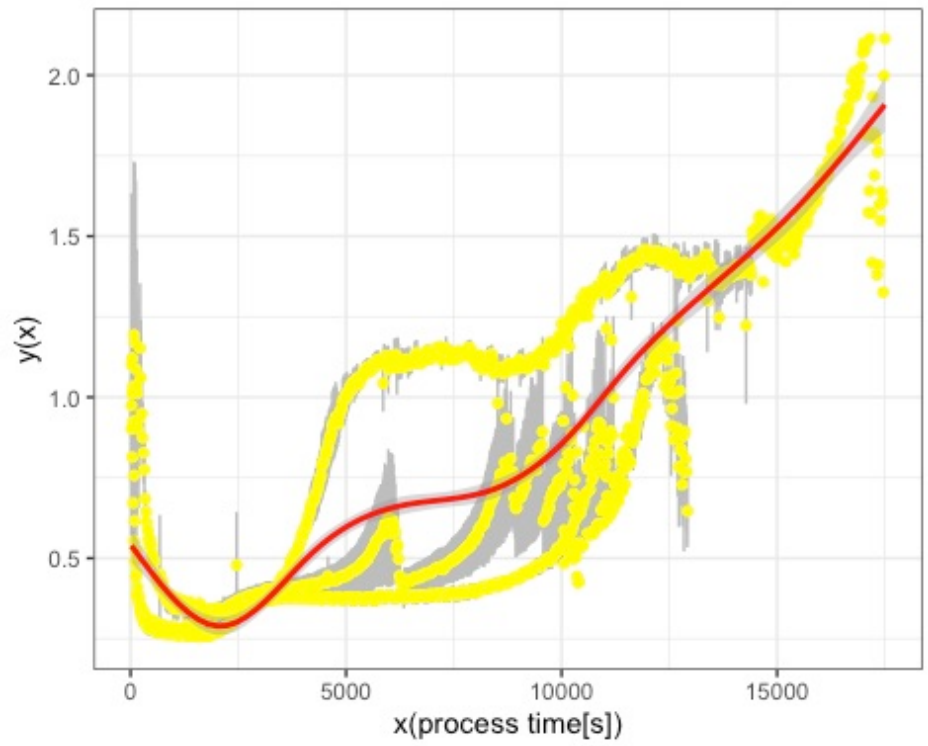

Figure 10: Fitted residuals from the function from the general additive model, applied with internal cubic spline smoothing term; yellow is the data, grey is thes standard deviation from the data and the red line represents the fitted values.

Summary and outlook

In this section, a multi-step structure formation for samples made from rennet and native casein could be reported. A "one-step" structure formation was found in samples made from sodium caseinate processed with HCl (to adjust the educt pH to the target value of 5.88) but no further use of buffering salts or agents. Thsi type of structure formation was attributed to soluble beta casein adsorbing to the o/w interphase and thereby decreasing the overall elasicity of the system. The samples made from rennet casein and native casein, displayed similar behaviour, variances were attributed to varying matrix inhomogenity. The targeted step-wise structure formation as it was displayed in @Lenze2019 could be reproduced with an improved model processed ceese system. Furthermore the indifference of native or rennet casein to be used to form the targeted structure could be confirmed.

Interesting here as well was the behaviour of sodium caseinate in relation to the degree of calcium deprivation. The rest of calcium ions in sodium caseinate is directly bound to phosphoserine residues. When processed without melting salts, we could see a structure development up to the first plateau. After that, no further structure development could be recorded. Combining the models suggests that the initially chelated calcium ions in samples made from native or rennet casein are not immobile or inert in the system within their chelated complex, but can - and will after time- initiate a second structure formation phase, i.e. a second growth phase. Samples made from sodium caseinate, that were still calcium deprived but had no pH buffering (i.e. the sodium caseinate model with HCl) showed an increase in viscosity. Thus the mobility of the caseins

to form self-assemblies like in the HCl model as well as the inhibition of structure formation through the lack of Calcium ions could be shown in this section.

For samples made from sodium caseinate, there seems to be no inner force or linking agent to form a secondary structure as seen in samples made from initially micellar (native or rennet) caseins. This is surprising since the levels of PP to induce complete dissociation of the micelle by chelation of CCP were above the reported threshold in similar models (@Vollmer2021a). Therefore, sodium and native models should present caseins in monomeric structure after melting. The caseins even have the same ionic substitute, since sodium salts were used. It can be suggested that the presence of large amounts of previously colloidal calcium is forcing the proteins to either deplete from the solved calcium ion or readily bind to it.

One aim of this study was to find. models with high similarity in composition (ionic environment and strength) but with very different structure formation properties. Native and Rennet Casein samples showed no significant difference in the overall shape of the structure formation, only the start of second exponential phase showed variance. This effect can be attributed to a differing matrix homogeneity due to differing powder-particle-sizes.

Since calcium is long known to be the factor stabilizing caseinate based emulsions (@Dickinson1998), the null model for the creaming process, i.e. the model system that had similar composition but showed no structure formation could be identified. This model was the sodium caseinate system, processed with emulsifying salts. As expected, calcium deprived emulsion gels didn't show structure formation. Therefore the two models that were investigated in their respective composition (see section 4) were chosen to be native casein and sodium caseinate, prepared with oil in a premixing step and processed with emulsifying salts.

The findings herein are in conclusion with findings of other works concerning the creaming reaction, but especially in the context of this work and the connected studies performed by @Lenze2019, @Vollmer2021 and @Vollmer2021a. The modellation of a characteristic flow curve was performed and will be used later within correlation analysis of the experimental data. The modelled flow curve resembled the shape of the flow curve presented in @Vollmer2021.